AVANÇOS NO TRATAMENTO SISTÉMICO DE DOENTES COM MELANOMA METASTIZADO ENTRE 2011 E 2019

TERESA MARIA SANTOS AMARAL

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Eberhard Karls University of Tuebingen
Center for Dermatooncology
Head: Professor Dr. C. Garbe
Department of Dermatology
Director: Professor Dr. M. Röcken

Progress in systemic therapy of advanced melanoma between 2011 and 2019

Thesis developed based on cumulative publications
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Teresa Amaral
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Dúbia é a vida, inconstante o que a governa.
O que esperamos nem sempre acontece
Nem nos falece sempre,
Nem há com que a alma uma ou outra cousa espere.

in "*Poemas de Ricardo Reis. Fernando Pessoa*

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Acknowledgments

“Those who pass by us, do not go alone, and do not leave us alone; they leave a bit of themselves, and take a little of us.”

Antoine de Saint-Exupéry

I have been very fortunate all my life. I had a wonderful childhood, my family loved me very much, and they always believed in me. Later on, when I pursued a medical career, and despite all the hurdles, I always had someone who truly believed in me.

I am now the result of all the people that passed by me, and left something of them. I treasure all the gifts I received. I am sure they also took part of me with them.

It is somehow unfair to only name some here. However, I will do so, since this is also the place to thank them for their presence and inspiration:

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- My Ph.D. mentor and supervisor, Claus Garbe, for his support and commitment with my professional growth. His leadership skills taught me more than I could expect.
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Finally, I want to thank my friends - you know who you are - for their unconditional support and love during this journey. I am fully committed to honor all the energy and thoughtfulness you dedicated to me, by staying true to myself.

1. Summary

1.1. English Summary

Background: Ten years ago, stage IV melanoma carried a dismal prognosis, with very short survival. The therapies available then, namely dacarbazine, temozolomide, and other chemotherapies, alone or in combination with surgery and/or radiotherapy, were unable to provide clinically significant benefit. In the last decade, the medical community testified a revolution in the treatment of advanced melanoma with the introduction of immune checkpoint inhibitors, and targeted therapy with mitogen-activated protein kinase inhibitors (MAPKi). Both therapies were able to provide sustainable overall survival benefits in stage IV melanoma. Here, we aimed to investigate whether the survival advantages seen in phase II, and III clinical trials investigating the afore mentioned therapies, were reproducible or not in a real-world setting.

Patients and methods: In this retrospective, multicentric analysis, we included stage IV melanoma patients, diagnosed between 2011 and 2018, treated in the skin cancer center in Tuebingen, or in other German centers, and prospectively documented in the German Central Malignant Melanoma Registry (CMMR).

The following patients' and tumor data were collected from the CMMR: year of birth, gender, date of primary tumor diagnosis, type of melanoma, localization, tumor thickness, Clark level, presence of ulceration, presence of regression, date of sentinel lymph node biopsy (if applicable), presence of lymph node metastases, stage at first diagnosis and date and localization of recurrence. The following variables were further included, based on patients' clinical chart review: date and type of local and systemic therapy in stage IV, date and type of best overall response, according to the Response Evaluation

Criteria in Solid Tumors version 1.1 (RECIST 1.1), date of progressive disease, date of last contact, cause of death, and death date. Patients included had a minimum follow-up of three months. Progression free survival (PFS) was defined as the time between date of stage IV diagnosis, start date of systemic therapy, or date of melanoma brain metastases (MBM) diagnosis, depending on the type of population evaluated, and date of progressive disease, or last contact or death, for the patients that didn't progress. Overall survival (OS) was defined as the time between stage IV diagnosis, start date of systemic therapy, or date of MBM diagnosis, depending on the type of population evaluated, and date of last contact or death.

Kaplan-Meier estimates were used for the calculation of PFS and OS. Differences between groups were assessed using the log-rank test. When applicable, hazard ratios (HR) with 95% confidence intervals (CI) were calculated to quantify the impact on survival. Multivariate logistic regression models were used to examine associations between variables, when appropriate. Results were reported as two-sided *p* values with 95% CIs. All *p*-values presented are two-sided tests of statistical significance at 0.05. For the indirect comparison between MAPKi, the Bucher method was used. All analyses were submitted to and approved by the local Ethics Committee.

Results: Six publications reporting data from 3143 stage IV melanoma patients were included in this thesis. The 3-years (3-y) OS rate for patients treated with first-line chemotherapy, the most used systemic therapy in 2011-2014 was 15.9% (95% CI: 8.8-23). For patients treated with first-line immunotherapy in the same period, the 3-y OS rate was 37.4% (95% CI: 16.6-58.2). In the period of 2015-2018, the 3-y OS rate for patients treated with first-line immunotherapy almost duplicated compared to the 2011-2014 period, and was 64.6% (95% CI: 53.2–76) for patients achieving a complete response (CR),

partial response (PR) or stable disease (SD). For patients achieving a CR, the 3-y OS rate was 87.7% (95% CI: 70.8–100).

In patients harboring a BRAFV600 mutation, and with presence of worse prognostic factors, namely elevated baseline lactate dehydrogenase (LDH), worse Eastern Cooperative Oncology Group Performance Status (ECOG PS) and higher tumor volume, the combination of vemurafenib plus cobimetinib showed a non-significant lower risk for progression or death, compared to dabrafenib plus trametinib, and encorafenib plus binimetinib.

Compared to cutaneous melanoma, patients with uveal melanoma derived less benefit from systemic therapy, even when treated with combined immunotherapy. The median OS (mOS) was only 16.1 months (95% CI: 12.9–19.3), but still higher than those reported for both chemotherapy and programmed cell death protein 1 (PD-1) monotherapy in this sub-group.

The benefit observed in intracerebral disease was similar to the benefit in extracerebral disease, when combined immunotherapy (nivolumab plus ipilimumab) was used. The mOS in patients with MBM was 19 months (95% CI: 15.9-22.0). For the whole collective of patients receiving nivolumab plus ipilimumab, the 3-y OS rate was 30.1% (95% CI: 22.2-37.9). For patients achieving a CR, the 2-y OS rate was 85.6% (95%CI: 69.3-100), very similar to other stage IV patients. Patients receiving combined immunotherapy and local therapy (surgery or stereotactic radiotherapy) had a better outcome when compared to patients that didn't receive local therapy – the 2-y OS rate was 49.5% versus 40.9 % (95%CI: 40.9-58.1 and 26.6-55.2). This benefit was observed regardless the timing of local therapy, i.e., before or after starting nivolumab plus ipilimumab.

Conclusions: Our real-world data confirmed the improvement of survival outcomes in stage IV melanoma patients with the introduction of immune checkpoint inhibitors, and

MAPKi in the last decade. Results show that, currently, PD-1 based immunotherapy should be offered as first-line therapy in stage IV melanoma. In patients with BRAFV600 mutation and worse prognostic features, first-line systemic therapy with MAPKi could be considered. In patients with MBM, combination of systemic immunotherapy and local therapy should be offered, when feasible.

Key words: melanoma, stage IV, systemic therapy, overall survival

1.2. Portuguese summary

Contexto prévio: Há cerca de dez anos, os doentes diagnosticados com melanoma em estadio IV tinham um prognóstico reservado e uma sobrevivência muito curta. As terapêuticas então disponíveis, nomeadamente dacarbazina, temozolomida e outros citotóxicos, isoladamente ou em combinação com cirurgia e/ou radioterapia, não proporcionavam benefícios clínicos significativos. Na última década, a comunidade médica e científica testemunhou uma revolução no tratamento do melanoma em estadio IV com a introdução da imunoterapia, particularmente dos inibidores de checkpoint, e das terapêuticas alvo, nomeadamente dos inibidores da via da *mitogen-activated protein cinase* (MAPKi). Ambas as terapêuticas demonstraram benefícios sustentados em termos de sobrevivência global em doentes com melanoma metastizado. Nesta análise, pretendemos investigar se o aumento de sobrevivência global observado nos ensaios clínicos de fase II e III que levaram à aprovação destas terapêuticas, podem ou não ser reproduzidos em contexto de prática clínica diária.

População incluída: Nesta análise retrospectiva, multicêntrica, incluímos doentes diagnosticados com melanoma estadio IV entre 2011 e 2018, e tratados no centro oncológico de Tuebingen, ou noutros centros da Alemanha, e documentados prospectivamente no Registo Central de Melanoma Maligno (CMMR) alemão.

Os seguintes dados foram obtidos através do CMMR: ano de nascimento, sexo, data de diagnóstico do tumor primário, tipo histológico, localização anatómica, espessura, nível de clark, presença de ulceração, presença de regressão, data da biópsia do gânglio sentinela (se aplicável), presença de metástases no gânglio sentinela, estadio à data do primeiro diagnóstico, e data e localização da recidiva. Outros dados foram posteriormente recolhidos através da consulta do processo clínico: tipo de terapêutica local e

sistémica em estadio IV e respectivas datas de início e fim, melhor resposta à terapêutica e respectiva data, de acordo com os critérios RECIST 1.1, data de progressão da doença, data do último contacto, e causa e data da morte. Todos os doentes incluídos foram seguidos durante, pelo menos, três meses.

Análise estatística: A sobrevivência livre de progressão (PFS) foi definida como o tempo entre a data de diagnóstico em estadio IV, data de início da terapia sistémica, ou data do diagnóstico de metástases cerebrais, dependendo do tipo de população avaliada, e data de progressão da doença, data do último contacto ou data de óbito no caso dos doentes que não tiveram progressão. A sobrevivência global (OS) foi definida como o tempo entre a data do diagnóstico em estadio IV, data de início da terapia sistémica, ou data do diagnóstico de metástases cerebrais, dependendo do tipo de população avaliada, e data do último contacto ou data de óbito.

Usámos estimativas de Kaplan-Meier para o cálculo da PFS e OS. As diferenças entre grupos foram avaliadas utilizando o teste de *log-rank*. Quando aplicável, foram calculados *hazard ratios* (HR) com intervalos de confiança de 95% (95% IC) para quantificar o impacto na sobrevivência. Quando aplicável, foram também utilizados modelos de regressão logística multivariada para avaliar associações entre variáveis. As associações entre variáveis foram consideradas estatisticamente significativas para valores de $p < 0.05$. Para a comparação indirecta entre os MAPKi, foi usado o método de Bucher. Todas as análises foram submetidas e aprovadas pelas Comissões de Ética locais.

Resultados: Seis publicações incluindo dados de 3143 doentes diagnosticados com melanoma em estadio IV foram compiladas para a elaboração da presente tese. A taxa de OS aos 3 anos (*3-y OS rate*) para doentes tratados com quimioterapia em primeira linha, a terapêutica sistémica mais utilizada em 2011-2014 foi de 15.9% (95% CI: 8.8-

23); para doentes tratados com imunoterapia em primeira linha no mesmo período, a *3-y OS rate* foi de 37.4% (95% CI: 16.6-58.2). No período entre 2015-2018, a *3-y OS rate* para doentes tratados com imunoterapia em primeira linha, quase que duplicou em comparação com o período de 2011-2014, e foi de 64.6% (95% CI: 53.2-76) para doentes com resposta completa, resposta parcial ou doença estável. Para doentes com resposta completa, a *3-y OS rate* foi de 87.7% (95%CI: 70.8-100).

Em doentes com mutação BRAFV600 e presença de factores de pior prognóstico, nomeadamente valores elevados de LDH à data de início de tratamento, ECOG PS reduzido e maior volume tumoral, a combinação vemurafenib/cobimetinib demostrou uma redução não significativa do risco de progressão ou morte em comparação com dabrafenib/trametinib, e encorafenib/binimetinib.

Em comparação com o melanoma cutâneo, os doentes com melanoma ocular beneficiaram menos da terapia sistémica, mesmo quando tratados com nivolumab/ipilimumab. A sobrevivência global média (mOS) foi de apenas 16.1 meses (95% CI: 12.9-19.3), ainda assim mais elevada do que a reportada em doentes tratados com quimioterapia ou monoterapia com anti-PD-1.

Em termos de sobrevivência global, o benefício da imunoterapia e das terapêuticas alvo na doença intracerebral foi semelhante ao observado na doença extracerebral. Isto foi particularmente verdade em doentes tratados com nivolumab/ipilimumab. A mOS foi de 19 meses (95% CI: 15.9-22.0) e a *3-y OS rate* foi de 30.1% (95% CI: 22.2-37.9) Para os doentes com resposta completa, a *2-y OS rate* foi de 85.6% (95% CI: 69.3-100), muito semelhante às taxas de sobrevivência observadas em outros doentes em estadio IV, sem metastização cerebral. Os doentes que receberam nivolumab/ipilimumab e terapêutica local (radiocirugia ou cirurgia) beneficiaram mais em comparação com os doentes que não receberam terapêutica local - a *2-y OS rate* foi de 49.5% versus 40.9% (95% CI: 40.9-58.1 e 26.6-55.2). Este benefício foi observado independentemente do *timing*

em que os doentes receberam terapêutica local, i.e., antes ou depois de iniciar nivolumab/ipilimumab.

Conclusões: Os nossos dados da prática clínica diária confirmam uma melhoria em termos de sobrevivência global em doentes com melanoma em estadio IV, diagnosticados e tratados entre 2011-2019. Estes benefícios deveram-se à introdução de novas opções terapêuticas, nomeadamente imunoterapia com inibidores de checkpoint e terapêuticas alvo com MAKi. Os nossos resultados mostraram que actualmente, a imunoterapia com inibidores de checkpoint, deve ser oferecida em primeira linha em todos os doentes com melanoma em estadio IV. Em doentes com mutação BRAFV600 e com presença de factores de pior prognóstico, terapêutica com MAPKi pode igualmente ser considerada em primeira linha metastática. Em doentes com metastização cerebral, a combinação de terapia sistémica e local deve ser discutida e ponderada, em primeira linha ou posteriormente, sempre que viável.

Palavras chave: Melanoma, estadio IV, terapêutica sistémica, sobrevivência global

1.3. Publications included in the thesis

1) Forschner A, Eichner F, **Amaral T**, Keim U, Garbe C, Eigentler TK. Improvement of overall survival in stage IV melanoma patients during 2011-2014: analysis of real-world data in 441 patients of the German Central Malignant Melanoma Registry (CMMR). Journal of cancer research and clinical oncology. 2017; 143: 533-40. **(IF=3.656)**

2) **Amaral T**, Seeber O, Mersi E, Sanchez S, Thomas I, Meiwes A, Forschner A, Leiter U, Eigentler T, Keim U, Garbe C. Primary Resistance to PD-1-Based Immunotherapy-A Study in 319 Patients with Stage IV Melanoma. Cancers. 2020; 12. **(IF=6.102)**

3) Glutsch V, **Amaral T**, Garbe C, Thoms KM, Mohr P, Hauschild A, Schilling B. Indirect Comparison of Combined BRAF and MEK Inhibition in Melanoma Patients with Elevated Baseline Lactate Dehydrogenase. Acta dermato-venereologica. 2020; 100: adv00174. **(IF=4.016)**

4) Heppt MV, **Amaral T**, Kähler KC, Heinzerling L, Hassel JC, Meissner M, Kreuzberg N, Loquai C, Reinhardt L, Utikal J, Dabrowski E, Gesierich A, Pföhler C, Terheyden P, Thoms K-M, Zimmer L, Eigentler TK, Kirchberger MC, Stege HM, Meier F, Schlaak M, Berking C. Combined immune checkpoint blockade for metastatic uveal melanoma: a retrospective, multi-center study. Journal for immunotherapy of cancer. 2019; 7: 299. **(IF=9.913)**

5) **Amaral T**, Tampouri I, Eigentler T, Keim U, Klumpp B, Heinrich V, Zips D, Paulsen F, Gepfner-Tuma I, Skardelly M, Tatagiba M, Tabatabai G, Garbe C, Forschner A. Immunotherapy plus surgery/radiosurgery is associated with favorable survival in patients with melanoma brain metastasis. Immunotherapy. 2019; 11: 297-309. **(IF=2.964)**

6) **Amaral T**, Kiecker F, Schaefer S, Stege H, Kaehler K, Terheyden P, Gesierich A, Gutzmer R, Haferkamp S, Uttikal J, Berking C, Rafei-Shamsabadi D, Reinhardt L, Meier F, Karoglan A, Posch C, Gambichler T, Pfoehler C, Thoms K, Tietze J, Debus D, Herbst R, Emmert S, Loquai C, Hassel JC, Meiss F, Tueting T, Heinrich V, Eigentler T, Garbe C, Zimmer L. Combined immunotherapy with nivolumab and ipilimumab with and without local therapy in patients with melanoma brain metastasis: a DeCOG* study in 380 patients. Journal for immunotherapy of cancer. 2020; 8. **(IF=9.913)**

Total impact of the publications included in the current thesis, according to the Portuguese current legislation - *Diário da República, 2.a série — N.o 153 — 7 de agosto de 2015; Regulamento n.o 519/2015, Capítulo II, artigo 20º, ii - Trabalhos científicos alternativos à Tese*:

- Impact factor as first author – 37.96
- Impact factor as not first author – 12.63
- **Total impact factor – 50.59**

1.4. Abbreviation list

AE	Adverse Events
AJCC	American Joint Committee on Cancer
ALM	Acral Lentigo Melanoma
APC	Antigen Presenting Cells
ASCO	American Society of Clinical Oncology
BRAF	B-Raf Proto-Oncogene
	v-Raf murine Sarcoma Viral Oncogene Homolog B
irAE	Immune Related Adverse Events
IARC	International Agency of Research on Cancer
CI	Confidence Interval
CMMR	German Central Malignant Melanoma Registry
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CR	Complete Response
DCR	Disease Control Rate
EMA	European Medicines Agency
ECOG PS	Eastern Cooperative Oncology Group Performance Status
EORTC	European Organisation for Research and Treatment of Cancer
ESMO	European Society of Medical Oncology
FDA	Food and Drug Administration
HLA	Human Leukocyte Antigen
HR	Hazard Ratio
ICI	Immune Checkpoint Inhibitors
LDH	Lactate Dehydrogenase
LMM	Lentigo Melanoma
MAPKi	Mitogen-activated Protein Kinase Kinase inhibitors
MBM	Melanoma Brain Metastases
MEK	Mitogen-activated Protein Kinase Kinase
NM	Nodular Melanoma
ORR	Overall Response Rate
OS	Overall Survival
mOS	Median Overall Survival
PD-1	Programmed Cell Death Protein 1

PD-L1	Programmed Cell Death Ligand 1
PD	Progressive Disease
PFS	Progression Free Survival
PR	Partial Response
RECIST 1.1	Response Evaluation Criteria in Solid Tumors version 1.1
SD	Stable Disease
SIRT	Selective Internal Radiotherapy
SOC	Standard of Care
SPO	Sociedade Portuguesa de Oncologia
SSM	Superficial Spreading Melanoma
TACE	Trans-arterial Chemoembolization
TCR	T-cell receptors
ULN	Upper Limit Normal
UV	Ultraviolet
WG	Working Group
y	years

2. Introduction

2.1. Incidence and prevalence of melanoma

In recent years, there was a worldwide increase of melanoma incidence. [1] By 2020, the number of newly diagnosed melanomas worldwide is expected to reach 279,938, and it is estimated that about 67,809 people will die from this disease. The numbers are from the International Agency for Research on Cancer (IARC). [2] Other national reports provided similar data, showing that the increased incidence rate of melanoma is a global issue. [3-5] The incidence rate of cutaneous melanoma is higher in white populations compared to Spanish, Afro-American, Indian and Asian. [6] The mean annually age-adjusted incidence of melanoma in whites per 100 000 persons is 18.4 and in Spanish, Afro-Americans, Indians and Asians it is 2.3, 0.8, 1.6 and 1.0, respectively.

In most European countries, the melanoma incidence rate almost doubled between 1990 and 2005, increasing between + 2% and + 10% annually. [7] In 2012, melanoma was the fifth most frequent solid tumor entity in Germany. The incidence rate according to the age-standardization rose from 12.4/100,000 to 19.2/100,000 between 1999 and 2012, representing an increase of approximately 55%. [3] In this publication we reported an increase in incidence of melanoma from 12.7/100,000 to 19.2/100,000 in men and from 12.1/100,000 to 19.2/100,000 in women, which represents an average annual increase of +3.1% and +3.5% in men and women, respectively. There was a marked annual increase of 10.9% for both sexes between 2006 and 2009, that coincided with the period when the nationwide skin cancer screening program was introduced. [3] An extrapolation of the data until 2030 shows that the age-standardized incidence rates will continue to increase. In Germany, the expected incidence rates are 31/100,000 and 30/100,000 for men and women, corresponding to a relative increase of about 60% for both sexes. [3]

The last IARC report shows that in 2018 in Portugal, the age-standardized incidence rate (World) per 100,000 for both sexes, all ages and for all cancers was 259,5. [8] For melanoma, the rate was 6.4 compared to 21.6 in Germany, in the same report.

2.2. Biology of melanoma

2.2.1. Melanoma subtypes

2.2.1.1. Cutaneous Melanoma

The two most common subtypes of cutaneous melanoma concern the 1) superficial spreading melanoma (SSM) in about 57% of cases, and the 2) nodular melanoma (NM) in about 21% of cases. Other less frequent melanoma subtypes are 3) the lentigo maligna melanoma (LMM) in about 9% of cases, occurring mainly in chronically sun-exposed skin of older patients, and the 4) acral-lentiginous melanoma (ALM) in about 4% of cases, which can be located in fingers, toes, palms and soles, and is associated with a poorer prognosis. [9-12]

There is robust evidence that cutaneous melanoma is associated with the intermittent exposure to ultraviolet (UV) radiation and history of sunburns early in life, namely throughout childhood and adolescence; however, the risk appears to be present regardless the age group. [13-15] The change in leisure and holiday patterns in the later decades, and also the change on the type of protective clothes used while sunbathing, resulted in significantly increase to UV exposure. This is the main reason for the global increase of this tumor entity. [16,17]

Approximately 6% of all diagnosed melanomas occur in body regions that have little exposure to UV radiation. Contrary, the majority of melanomas (94%) are located in body

regions frequently or intermittently exposed to UV radiation, such as the face, chest, back, arms and legs. [9,18]

The development of melanomas as its correlation with UV exposure has been shown previously by several groups. In 2010 the whole spectrum of somatic mutations in the entire genome of a melanoma metastasis was catalogued for the first time. [19] It was shown that about 70% of the detected single base substitutions were of the type C-T and also around 70% of the dinucleotide substitutions were of the type CC-TT. It is largely known that these are "signature mutations" for exposure to UV radiation, and therefore, this finding represents an important proof of the connection between the development of melanoma and UV radiation exposure.

Due to this relation between UV radiation exposure and cutaneous melanoma, this subtype of melanoma belongs to the human malignancies with the highest tumor mutational burden. [20] The high tumor mutational burden has been associated with response to immunotherapy in melanoma and other solid tumors. [21,22]

2.2.1.2. Mucosal Melanoma

About 55% of the mucosal melanomas are diagnosed in the head and neck area, 24% the anorectal region and 21% the genital tract. [23-27] This type of melanoma is usually diagnosed in later stages, which might contribute to the less favorable prognosis. The lower expression of the programmed death ligand 1 (PD-L1) and overall lower tumor mutational burden, may also contribute to the lower response rates to immune checkpoint inhibitors. [28-31]

2.2.1.3. Uveal Melanoma

Uveal melanomas have a different metastatic pattern from cutaneous melanomas. Since the eye has no lymphatic system, almost all uveal melanoma metastases are found directly in the liver via hematogenic spread. This particular pattern of metastatic spread turns these patients into perfect candidates for liver directed therapeutic approaches including surgical resection, trans-arterial chemoembolization (TACE), [32-35] and selective internal radiotherapy (SIRT). [36-38] Both methods, TACE and SIRT, achieve good response rates of up to 57% and 63%, respectively. Similar to mucosal melanoma, the response rates to immune checkpoint inhibitors are considerably lower than for cutaneous melanoma. [39-42] However, combined immunotherapy has been shown to be a safe and more effective therapeutic option for these patients, when compared to chemotherapy or PD-1 monotherapy. [43]

2.3. The 8th edition of the AJCC classification

Melanoma, like other solid tumors, is staged according to the TNM classification. The new TNM classification was published in 2017 by the American Joint Committee on Cancer (AJCC). [44] The changes introduced affected mostly the classification of stage III melanoma with a new sub-stage IIID. Considering that adjuvant therapy has become available and approved in stage III as well, a very short discussion on the implications of these new classification is of importance.

The 8th edition of the AJCC melanoma classification displays what can be defined as a very favorable outcome for stage III patients. In fact, with these outcomes, the discussion arose whether there was the need to treat all stage III patients. It was particularly striking that stage IIC had worse prognosis than stage IIIA. Several authors addressed this aspect, and our group did that as well. [45-47] By evaluating three different datasets of

untreated stage III patients, i.e., patients from the placebo arm of the European Organisation for Research and Treatment of Cancer (EORTC) 18991 and 18071 trials, and from a Tuebingen cohort, we have discussed and pointed out that, the survival rates presented in the new AJCC classification might be overrated. [48] This has implications in both trials' design and in indication for systemic treatment. We advocated that all stage IIIB and IIIC patients should receive systemic therapy, and that for stage IIIA patients, the decision should be individualized. There were not many stage IIIA patients included in the clinical trials evaluating systemic therapy, and the prognosis is considerably favorable.

As for stage IV, a new substage - M1d - was introduced for patients with brain metastases, recognizing that these patients, when left untreated, have a different and worse prognosis compared with other stage IV patients. The M1d substage includes patients with brain metastases with or without metastases in other organs. The M1c category no longer includes patients with brain metastases, and elevated lactate dehydrogenase (LDH) no longer defines M1c category. LDH level is defined by using a suffix next to the M category: normal LDH is identified as 0 (zero) and elevated LDH is identified as 1 (one). No suffix is used if the LDH value is not recorded or is unspecified. The particular subgroup of M1d patients is one of the focus of this thesis (Manuscripts 5 and 6). **Table 1** displays a very simplified summary of the TNM classification for cutaneous melanoma.

Table 1: Overview of the TNM classification for cutaneous melanoma

Stage	Criteria
I	Tumor thickness up to 2mm without ulceration no metastases
II	Tumor thickness > 1mm up to 2mm with ulceration Any tumor with a tumor thickness of more than 2mm no metastases
III	Any tumor thickness with metastases in nearby skin areas or lymph nodes
IV	Any tumor thickness with metastases in distant skin areas, lymph nodes or organs (e.g. liver, lung, brain, ...)

2.4. Systemic therapy of advanced melanoma

The landscape of the systemic therapy in melanoma has changed considerably in the last 10 years. For decades, different combinations of chemotherapy have been investigated in advanced melanoma, but these therapies produced very marginal benefits. [49] Until the introduction of immune checkpoint inhibitors (ipilimumab, nivolumab and pembrolizumab), and targeted therapies with BRAF and MEK inhibitors (vemurafenib plus cobimetinib, dabrafenib plus trametinib and encorafenib plus binimetinib) in the therapeutic arsenal, there was no effective therapy for patients diagnosed with advanced melanoma. Targeted therapies can be offered to patients whose melanomas carry a BRAFV600 mutation, and immune checkpoint inhibitors can be used to treated both BRAFV600 mutated and BRAF wild-type melanomas.

Figure 1 depict a simplified timeline of the systemic therapies currently available and their timing of re-imbursement in the last decade in Portugal and Germany.

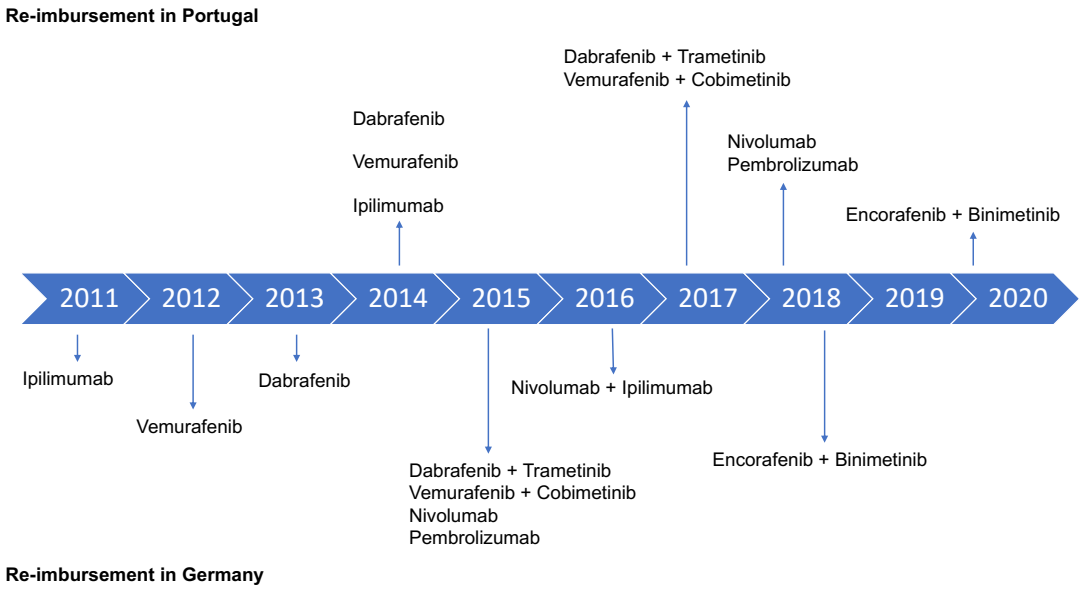

Figure 1: Timing of re-imbursement for systemic therapies in stage IV melanoma, in Portugal (top) and Germany (bottom)

2.4.1. Immunotherapy with CTLA-4 and PD1/PDL-1 inhibitors

Cancer immunotherapies aim to harness the anti-cancer response of the immune system to selectively destroy cancer cells while leaving normal tissues unharmed. In the last years, immune checkpoint inhibitors have been established as a potent novel cancer immunotherapy showing unparalleled anti-tumor effects in previously difficult to treat malignancies like metastatic melanoma and lung cancer, among others. Importantly, immune checkpoint inhibitors were primarily investigated in patients with cutaneous melanoma, due to its high immunogenicity and immune responsiveness. [50-52] As previously mentioned, it is now widely accepted that a high tumor mutational burden, observed in tumors like cutaneous melanoma, is associated with a large number of potent neoepitopes being presented on the cancer cell surface, and being directly responsible for the immune recognition of such tumors and efficacy of immune checkpoint inhibitors. [53,54]

Ipilimumab, an immune checkpoint inhibitor targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) was the first developed, and was followed by the programmed cell death-1 (PD-1) inhibitors, nivolumab and pembrolizumab, and PD-Ligand 1 (PD-L1) antibodies (e.g. atezolizumab). [55] These antibodies block the interaction between the inhibitory T lymphocyte receptors CTLA-4 or PD-1 with their natural ligands CD80/CD86 or PD-L1/PD-L2, respectively. In a normal setting, ligand binding leads to a deactivation of T cells limiting the immune response to a stimulus, and thereby protecting from an overshooting immune response. However, some tumors express PD-L1 on their surface leading to PD-1 mediated deactivation of tumor-infiltrating T cells and immune evasion. Antibodies blocking the interaction of PD-1 on T cells with its ligand PD-L1 on tumor cells lead to the suppression release of tumor-specific T cells. These T-cells are now enabled to efficiently recognize and kill tumor cells. Importantly, efficacy of PD-1/PD-L1 blocking antibodies relies on pre-existing tumor-reactive T cells recognizing tumor-specific epitopes like neoepitopes on the tumor cell surface.

On the other hand, blockade of CTLA-4 on T cells primarily supports their activation (priming). Key for T cell priming are antigen presenting cells (APC) taking up extracellular antigens from pathogens or tumors, and presenting these via HLA class I and II molecules on their surface. These complexes of antigens, and either HLA class I or II molecules can be recognized by matching T-cell receptors (TCR) of CD8+ or CD4+ T cells, respectively, leading to the activation of antigen-specific lymphocytes only. However, for efficient T cell activation, APC also need to display co-stimulatory factors like CD80 and CD86 being recognized by the activating receptor CD28 on T cells. Upon stimulation, T cells express the inhibitory receptor CTLA-4, which has a higher affinity to the APC surface molecules CD80/CD86 than CD28 competing for their binding. Upon ligand binding CTLA-4 can also directly inhibit TCR-driven intracellular signaling. [56] As a net effect CTLA-4 activation abrogates T cell activation via a negative feedback loop. CTLA-4

blocking antibodies therefore enhance priming and effector activity of antigen-specific T cells and inhibit immune suppression mediated by a specific group of CD4+ T cells, the regulatory T cells (Tregs).

Figure *2* and

Figure *3* display a simplified model of mechanism of action of both CTLA-1 and PD1 checkpoint inhibitors.

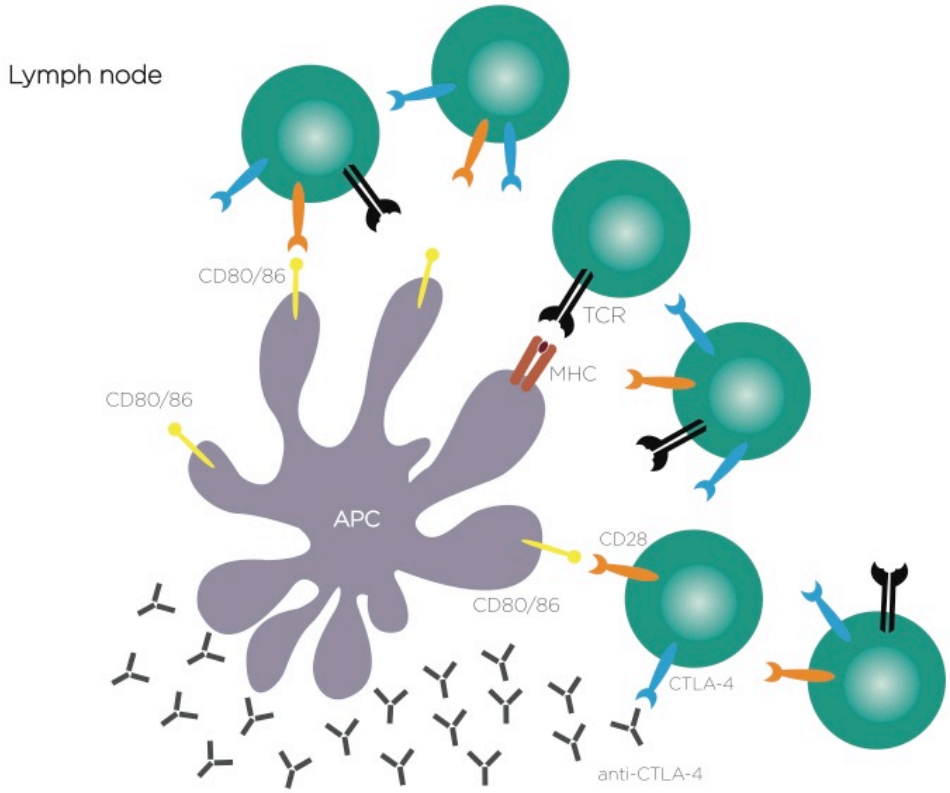

Figure 2: The priming phase and CTLA-4 blockade

Antigen presenting cells (APC) activate naïve T-cells using two different ligands: MHC-I<>TCR and CD80/86 <> CD28. After this initial priming and activation, CTLA-4 is upregulated and expressed in the T-cell membrane. CTLA-4 has a higher affinity to CD80/86 than CD28 and displaces the initial ligation. When CTLA-4 binds to CD80/86, it blocks further T-cell activation, and limits the T-cell activation induced by APC's. CTLA-4 can effectively be targeted using CTLA-4 inhibitors such as ipilimumab and tremelimumab, which stop the

inhibitory signals, prolonging T-cell activation. APC: antigen presenting cells; MHC-I: major histocompatibility complex one; CTLA-4: cytotoxic T-lymphocyte-associated protein 4. From *Amaral et al.* [57]

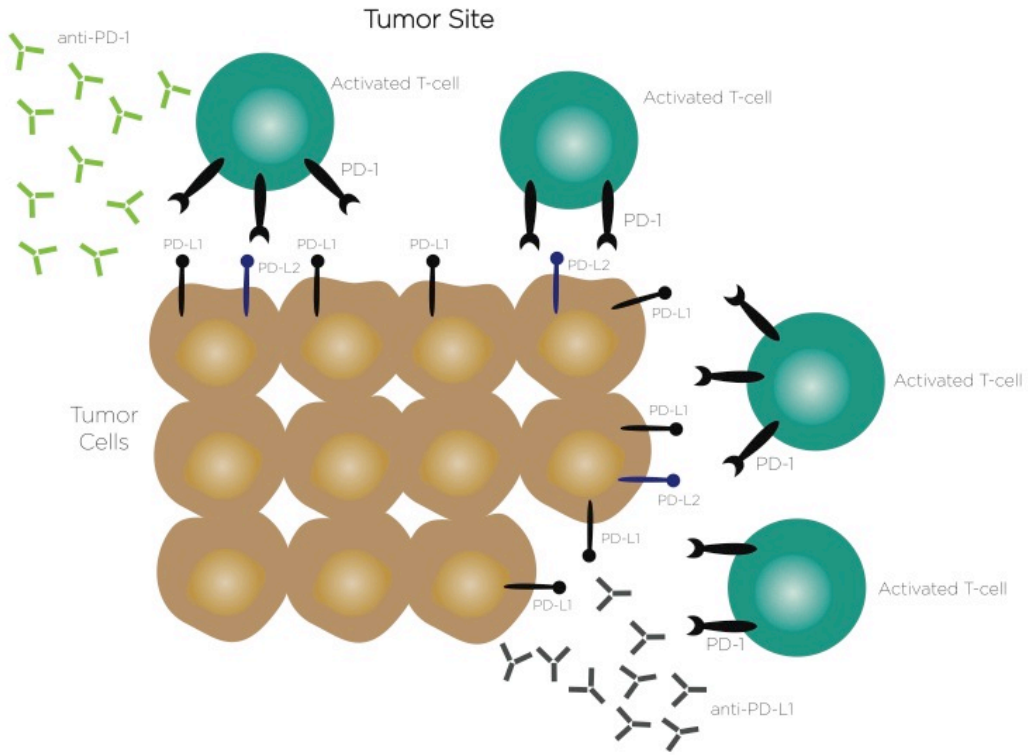

Figure 3: The effector phase and the PD-1/PD-L1/2 blockade

PD-1 is expressed by T-cells that acts as an inhibitory molecule when binding to the two identified ligands: PD-L1 and PD-L2. Binding of PD-1 to PD-L1/2 inhibits tumor cell apoptosis, promotes T-cell exhaustion and prevents an active antitumor response. Therapy with PD-1 inhibitors (pembrolizumab and nivolumab) will stop the negative regulation, and preclude T-cell exhaustion and deactivation, reestablishing antitumor immune response. From *Amaral et al.* [57]

2.4.1.1. Anti- CTLA4 – ipilimumab

The antibody ipilimumab was the first one to be investigated in the treatment of metastatic melanoma patients. The first trials evaluating ipilimumab in stage IV melanoma did so by comparing ipilimumab 3 mg/kg, plus a gp100 peptide vaccine, ipilimumab plus gp100 placebo, and gp100 plus ipilimumab placebo, all administered once every 3 weeks for four treatments, in patients that have already received and progressed under systemic therapy. [52] Results showed that ipilimumab, with or without a gp100 peptide vaccine, as compared with gp100 alone, improved overall survival (OS) in this population of patients.

The results of these first trials, led to further investigation comparing ipilimumab with the standard of care (SOC) at that time for stage IV melanoma, which was chemotherapy. A phase III trial evaluated the combination of ipilimumab 10mg/kg plus dacarbazine versus dacarbazine alone in treatment naïve patients. [58] The results showed that ipilimumab in combination with dacarbazine, as compared with dacarbazine plus placebo, improved OS in this population.

Further studies evaluating ipilimumab monotherapy in previously treated and treatment naïve patients showed that, for the first time in advanced melanoma, systemic therapy was able to induce long-term responses in this population. [59-63] In 2015, a pooled analysis of long-term survival data from phase II and III trials of ipilimumab in unresectable or metastatic melanoma was published. [64] With a follow-up of up to 10 years, the authors showed that a plateau in the survival curve can be recognized at approximately 3 years. This plateau was independent of prior therapy or ipilimumab dose investigated. These data once again confirmed that with ipilimumab monotherapy, long-term responses can be achieved and are durable in 20-26% of the patients.

Based on the previously results, ipilimumab was approved by the FDA and the EMA for the treatment of advanced melanoma. [65] Patients are treated with four cycles of ipilimumab 3 mg/kg, every three weeks.

2.4.1.2. Anti-PD-1 – nivolumab and pembrolizumab

The investigation of immunotherapy for the treatment of advanced melanoma advanced with further research on anti-PD-1 inhibitors, namely nivolumab and pembrolizumab. The mechanism of action of these two molecules is slightly different from the mechanism of action of ipilimumab, but the basic principle is the same, that is to harness the immune system allowing for identification and elimination of tumor cells. PD-1 is a crucial immune-checkpoint receptor that is mostly expressed by activated T cells, mediating immunosuppression, and is mostly activated in peripheral tissues. Here, the T cells can be exposed to immunosuppressive PD-1 ligands PD-L1 (B7-H1) and PD-L2 (B7-DC) expressed by tumor cells, stromal cells, or both. [66-69] Anti-PD-1 inhibitors such as nivolumab and pembrolizumab block the interaction between PD-1 and PD-L1 and this enhances T-cell responses. [70,71]

PD-1 inhibitors were initially evaluated in tumors that are known to be immunogenic, and, currently, also known for having a high tumor mutational load, namely melanoma and lung cancer. [72] Results showed that anti–PD-1 antibodies produced objective responses in approximately one in four to one in five patients with non–small-cell lung cancer, melanoma, or renal-cell cancer. Further trials investigating PD-1 based immunotherapy in melanoma showed that this therapy was also able to induce higher response rates compared to the SOC chemotherapy, in both treatment naïve patients, and patients who progressed under ipilimumab therapy. [73-85] These results were seen regardless BRAF mutation status, and are summarized in **Table 2**.

Based on the results from these trials, nivolumab and pembrolizumab were approved by the FDA and the EMA for the treatment of inoperable metastatic melanoma. [86,87]

Over the years, the dosing regimen of nivolumab and pembrolizumab has been simplified, and instead of a weight-based dose, similar to chemotherapy, a flat dose of 240mg every 2 weeks or 480mg every 4 weeks is now used for nivolumab. Pembrolizumab can be administered every 3 weeks (200 mg) or every 6 weeks (400mg). The results achieved with flat dose and longer intervals were similar to the weight-based dose and shorter intervals. [88,89]

Table 2: Summary of results from the trials investigating PD-1 based immunotherapy in advanced melanoma

Study	**Immune checkpoint inhibitors**									
	CHECKMATE				KEYNOTE					
	067		037	066	001		002		006	
Agent(s)	Nivo + Ipi	Nivo	Nivo	Nivo	Pembro		Pembro		Pembro	
					(all pts)	(tx naive)	(2mg/kg)	(10mg/kg)	(Q2W)	(Q3W)
Patients, n (study arm)	314	316	272	210	655	151	180	181	279	277
BRAF mutant, %	32	31	22	0	24	---	24.4	22.1	35	35
ECOG ≥ 1, %	26	25	40	28.6	---	---	44.4	44.8	30	32
M1c, %	58	58	75	61	8	---	82.2	82.3	64	68
LDH > ULN, %	48	47	52	37.6	38	---	43.3	39.8	29	35
Follow up, months	>60	>60	24	38.4	55	55	28	28	45.9	
Median OS, months	NR	36.9	15.7	37.5	23.8	38.6	13.4	14.7	32.7	
1-yr OS, %	73	74	58.9	---	---	---	53.7	55.6	---	
2-yr OS, %	64	59	38.7	---	---	---	35.9	38.2	55.2	
3-yr OS, %	58	52	---	31.2	---	---	---	---	48.1	
4-yr OS, %	53	46	---	---	38	48	---	---	42.3	
5-yr OS, %	52	44	---	---	34	41	---	---	38.7	

Median PFS, months	11.5	6.9	3.1	5.1	8.3	16.9	2.9	3.0	8.4	
1-yr PFS, %	50	43	---	---	---	---	22.1	27.9	---	
2-yr PFS, %	41	37	---	---	---	---	16	22	32.7	
3-yr PFS, %	39	32	---	32.2	---	---	---	---	28.8	
4-yr PFS, %	37	31	---	---	25	35	---	---	23	
5-yr PFS, %	36	29	---	---	21	29	---	---	---	
ORR, %	58	44.6	27	42.9	41	52	22	28	42	
CR/PR, %	22/36	19/26	27	40/50	16/25	25/27	---	---	13/29	
Median DOR, months	NR	NR	31.9	NR	---	NR	22.8	NR	NR	
Related AEs, %	96	87	77	77.7	86	---	56.7	59.2	79	
Discontinuation due to AE %	31	8	15	8.7	8	---	13.5	16.2	10	
CTCAE grade 3/4 AEs, %	59	23	11	15	17	---	3.3	6.1	17	17

2 NR= not reached

2.4.1.3. Nivolumab plus ipilimumab

Combination of nivolumab and ipilimumab is the current SOC for stage IV melanoma. This combination was investigated in a prospective, randomized phase 3 study, the CheckMate 067 study, recently updated with survival data after 5 years of follow-up. [78,90] This study compared nivolumab plus ipilimumab with nivolumab monotherapy and ipilimumab monotherapy in treatment-naïve patients. Although this trial was not powered to detect a difference between the combination therapy and the monotherapies, the consecutive survival updates have shown that the difference in terms of OS rates between nivolumab plus ipilimumab and the monotherapy arms have been consistently increasing. The 2-y, 3-y, 4-y, and 5-y OS rates difference between nivolumab plus ipilimumab and nivolumab alone are 5%, 6%, 7% and 8%, respectively. [78,91,92] In the subgroup of patients with BRAFV600 mutation receiving combined immunotherapy or nivolumab monotherapy, the difference in the 5-y OS rates is even more notorious (60% versus 46%).

The authors of the Checkmate 067 also evaluated the subsequent therapies received by the patients included. The time until receiving another therapy is an important aspect, not only in terms of survival but also in terms of quality of life. The median time from trial inclusion to subsequent systemic therapy was more than 60.0 months (median not reached) in the nivolumab plus ipilimumab group, 25.2 months in the nivolumab group, and 8.0 months in the ipilimumab group. Besides the time to subsequent systemic therapy, another aspect evaluated was the median treatment-free interval. Here, patients treated with combined immunotherapy also derived greater benefit compared to those receiving nivolumab or ipilimumab monotherapy; 18.1 months versus 1.8 months and 1.9 months, respectively. This was mirrored in the percentage of patients who were not

receiving subsequent systemic at the time of the 5-y follow-up survival analysis: 74% in the combination arm, 58% in the nivolumab arm, and 45% in the ipilimumab arm. [78]

The superior results of the combination therapy were, however, associated with a higher toxicity. Common Terminology Criteria for Adverse Events (CTACE) grade 3-4 treatment-related adverse events were seen in 59% of the patients receiving nivolumab plus ipilimumab, in 22% of the patients receiving nivolumab and in 28% of those treated with ipilimumab. [92] Taking that into consideration, the Checkmate 511 trial investigated whether a lower dose of ipilimumab (1mg/kg) combined with a higher dose of nivolumab (3mg/kg) could derive the same efficacy benefits as the standard dose, while resulting in lower toxicity rates. [93] With a lower dose of ipilimumab, the CTCAE grade 3-4 toxicity was reduced by half with a comparable efficacy. The follow-up in this study is however shorter than in the Checkmate 067 (18 months versus 60 months), and longer follow-up is required to confirm these results. [93]

2.4.2. Targeted therapy with BRAF and MEK inhibitors

Approximately 40 to 60% of cutaneous melanomas harbor mutations in BRAF that lead to constitutive activation of downstream signaling through the MAPK pathway. [94] Approximately 90% of these mutations result in the substitution of glutamic acid for valine at codon 600 (BRAFV600E), although other activating mutations are known (e.g., BRAFV600K and BRAFV600R). Vemurafenib, was the first selective BRAFV600 inhibitor to be investigated in the treatment of advanced melanoma. [95] The BRIM-3 trial was a phase 3 randomized clinical trial that compared vemurafenib with the SOC dacarbazine in patients with previously untreated, metastatic melanoma with the BRAFV600E

mutation. The results showed that vemurafenib was superior to dacarbazine in terms of prolonging PFS and OS.

Dabrafenib is another BRAF inhibitor investigated at the same time as vemurafenib with similar results in terms of efficacy. [96] The difference between the both BRAF inhibitors is mainly related with the toxicity profile. Dabrafenib induces almost no photosensitivity compared to vemurafenib (41%), fewer keratoacanthomas and squamous cell carcinomas (7% versus 20-30%). Arthralgia (56%), fatigue (46%) and rash (41%) were commonly reported with vemurafenib treatment. [95] On the other hand, pyrexia is the most common adverse event associated with dabrafenib treatment - almost 50% of the patients reported pyrexia that led to treatment interruption.

Treatment with BRAF inhibitors monotherapy induces high response rates but resistance supersedes shortly after. [95,97,98] Combination with another MAPK inhibitor, in this case a MEK inhibitor, is one of the ways to overcome the resistance and short duration of response of monotherapy with BRAF inhibitors. [99]

Three different BRAF/MEK combinations are currently available for patients with advanced melanoma. These combinations were investigated in randomized phase III trials, and compared with BRAF inhibitors monotherapy showing improved survival outcomes in BRAFV600 mutated melanoma. The combination of vemurafenib plus cobimetinib was investigated in the coBRIM trial, dabrafenib plus trametinib was investigated in the COMBI-d and COMBI-v study, and encorafenib plus binimetinib was investigated in the COLUMBUS study. [100-103]

Recently, a pooled analysis evaluating the survival of BRAFV600 mutated patients treated with BRAF/MEK inhibitors in the COMBI-d and COMBI-v trials showed that, with a median follow-up of 5-y, the OS rate was 34%. A complete response was observed in

19% of the patients, and in this subgroup the 5-y OS rate was 71% (95% CI, 62 to 79). [104] These results show that if targeted therapy is chosen to treat patients with BRAFV600 mutated melanoma, combined targeted therapy and not monotherapy should be used. Since the efficacy and survival outcomes are very similar in with the three combinations of BRAF/MEK inhibitors, the combination chosen is mostly related with the safety profile that differs between them. Our recently published indirect analysis showed a non-significant risk reduction for progression and death in the subgroup with elevated baseline LDH receiving vemurafenib plus cobimetinib, compared with dabrafenib plus trametinib and encorafenib plus binimetinib. Therefore, in this subgroup of patients, combination of vemurafenib plus cobimetinib might be considered. [105]

Table 3 provides a summary of the survival outcomes in trials investigating targeted therapy in BRAFV600 mutated melanoma patients.

Table 3: Summary of results from the trials investigating targeted therapy in advanced melanoma

Study	**Combination targeted therapy**				
	COMBI-d	COMBI-v	CoBRIM	COLUMBUS	
Agent(s)	D + T	D + T	V + C	E + B	
Patients, n (study arm)	211	352	247	577 (Part1)	258 (Part2)
ECOG ≥ 1, %	27	29	24	29	27
M1c, %	67	63	59	64	67
LDH > ULN, %	36	34	46	29	31
Follow up, months	≥ 36.0	23	21.2	36.8	
Median OS, months	25.1	26.1	22.5	33.6	
1-yr OS, %	74	73	74.5	76	
2-yr OS, %	52	53	49.0	58	

3-yr OS, %	44	45	38.5	47	
4-yr OS, %	37		34.7	39	
5-yr OS, %	34		---	---	
Median PFS, months	11.0	12.1	12.3	14.9	12.9
1-yr PFS, %	---	---	---	56	
2-yr PFS, %	30	30	---	37	
3-yr PFS, %	22	24	---	29	
4-yr PFS, %	21		---	25	
5-yr PFS, %	19		---	---	
ORR, %	69	67	70	64	66
CR/PR, %	16/53	19/48	16/54	13/51	8/58
Median DOR, months	12	13.8	13.0	18.6	12.7
Related AEs, %	97	99	99	98	98
Discontinuation due to AE %	14	16	13	15	12
CTCAE grade 3/4 AEs, %	48	57	77	64	47

D+T= Dabrafenib + Trametinib; V+C= Vemurafenib + Cobimetinib; E+B= Encorafenib + Binimetinib.

2.5. Systemic therapies in melanoma brain metastasis

Patients with MB pose a particular therapeutic challenge, and have a worse prognosis compared to stage other IV patients. This has been acknowledged in the new AJCC classification, that included patients with MBM in a particular subgroup – M1d. [44] This particular subgroup of patients and their outcomes to systemic therapy have been evaluated separately in this thesis (Manuscripts 5 and 6).

The studies evaluating systemic therapy in stage IV melanoma patients have systematically excluded patients with brain metastases. In fact, the presence of active MBM is an

exclusion criterion for the great majority of phase III clinical trials, regardless the tumor entity.

Trials specifically investigating immunotherapy and targeted therapy in patients with MBM have shown that these therapies are also effective intracranially, and that the intracranial response rate is similar to the extracranial response. [106-109] Currently, there is evidence that PD-1-based immunotherapy, and particularly combined immunotherapy with nivolumab and ipilimumab might be more effective than BRAF/MEK inhibitors. [110,111]

For patients with MBM, the combination of local and systemic therapies has long been investigated. Retrospective data show that the patients receiving a combination of local therapy and systemic therapy have better outcomes when compared to patients who do not receive local therapy. [112-119] The timing of the local therapy, i.e., up-front or later in the course of the disease, doesn't seem to be statistically significant. However, local therapies given up-front seem to derive better outcomes. [120-123] There is still ongoing debate whether some patients might be better served with systemic therapy alone, considering the very positive outcomes seen in clinical trials. Not applying local therapy reduces local complications, potential cognitive impairment, and might be particularly adequate for patients with a low number of asymptomatic MBM. This question along with the best sequence regarding local and systemic therapy will be addressed in ongoing/planed clinical trials. [124,125]

2.6. Access to systemic therapies approved in the advanced setting in Portugal and Germany

Despite the positive results of systemic therapy in advanced melanoma patients, these therapies are not available for all patients at the same time. In Germany, therapies that

are approved by EMA become available and reimbursed in the clinical practice immediately after their approval. In other countries, namely in Portugal, the process is different (see **Figure 1**).

The asymmetric re-imbursement process precludes access to effective therapy. Particularly in melanoma, this asymmetric access to systemic therapies was investigated in 2017. The authors of this work reported that, at the time, more than 5000 patients with metastatic melanoma per year in Europe did not have access to recommended first-line innovative treatments. This obviously translates into pourer survival outcomes in patients from countries that do not have access to innovative therapies.

In Europe, the European Society of Medical Oncology Magnitude of Clinical Benefit Scale working group (ESMO-MCBS WG), of which I am current member, has addressed this topic. The evolving classification has been available for the last five years, and using it one can easily identify and define whether the therapies available in a defined setting should or shouldn't be reimbursed, based in criteria such as improved survival outcomes, toxicity, and quality of life. [126-128] The progress in systemic therapy of advanced melanoma, which is the topic of this thesis, is only important in the extent that it is available for all patients who could benefit from it. [129]

3. Objectives

Aim 1) To determine long-term outcomes in patients with stage IV melanoma treated with targeted and immunotherapy between 2011-2019

Work project 1 – this work used data available from the German Central Malignant Melanoma Registry (CMMR) database in combination with data from other local databases. The manuscripts generated with this work project were manuscript 1, 2, 3 and 4.

Aim 2) To evaluate the impact in survival outcomes of targeted and immunotherapy, and their combination with local therapies, in patients with melanoma brain metastases

Work project 2 – this work used data available from the CMMR database in combination with data from other local databases. The manuscripts generated with this work project were manuscript 5 and 6.

4. Patients and methods

The datasets used for the publications presented here were: 1) one registry-based dataset, the German CMMR, 2) data published from 3 randomized clinical trials, and 3) the German CMMR combined with local registry datasets, respectively:

- The German CMMR (Manuscript 1, 2, 5)
- The data publicly available from trials co-BRIM, COMBI-v and COLUMBUS part 1 (Manuscript 3)
- The German CMMR in combination with local registry datasets (Manuscripts 4 and 6)

This section provides a brief description of the methods used to evaluate data from each dataset. A detailed description of the methodology used in each work project is provided in the respective publication.

4.1. The German CMMR (Manuscript 1, 2, 5)

4.1.1. Study design and Data Source

The German CMMR is a prospective database, active since April 1963. Sixty German centers have been contributing with data from patients diagnosed with primary melanoma. Currently, these centers report data only for defined variables, and are not fully active. The participation is not mandatory and there is no reimbursement.

In manuscripts 1, 2, and 5, only patients treated and followed in the skin cancer center of Tuebingen were included. Patients includes had at least 3 months follow-up.

4.1.2. Key Variables

The German CMMR contains information on birthyear, gender, date of primary tumor diagnosis, type of melanoma, tumor localization, tumor thickness, clark level, presence of ulceration, presence of regression, date of sentinel lymph node biopsy (if applicable), presence of lymph node metastases, stage at first diagnosis, and date and localization of recurrence.

The following variables were further collected based on patients' clinical chart review: date and type of local and systemic therapy in advanced stages, toxicity, date and type of best overall response, date of progressive disease, last contact date, cause of death and death date. PFS was defined as the time between date of stage IV diagnosis, and date of progressive disease, or last contact or death, for patients who didn't progress. OS was defined as the time between stage IV diagnosis and date of last contact or death.

4.1.3. Statistical Analyses

Descriptive statistics were used to characterize the patients' population and treatment patterns. Estimates of survival rates were calculated among subgroups using Kaplan Meier estimates for PFS and OS. When appropriate, multivariate logistic regression models were used to examine associations between variables. All *p*-values presented were two-sided tests of statistical significance at 0.05. All statistical analyses were performed using SPSS v.25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

4.2. The co-BRIM, COMBI-v and COLUMBUS study database (Manuscript 3)

4.2.1. Study design and Data Source

Manuscript number 3 was based on the publicly available dataset from the studies co-BRIM, COMBI-v and COLUMBUS. [130-132]

All data used has been previously published, therefore, there was no dedicated informed consent and ethical approval.

4.2.2. Key Variables

coBRIM, COMBI-v and COLUMBUS part 1 were randomized, double-blind phase 3 trials comparing oral vemurafenib, 960 mg twice daily, plus cobimetinib, 60 mg once daily, for 21 days with placebo and vemurafenib (coBRIM), oral dabrafenib, 150 mg twice daily, plus trametinib, 2 mg once daily, with vemurafenib, 960 mg twice daily, (COMBI-v) or oral encorafenib, 450 mg once daily, plus binimetinib, 45 mg twice daily, with vemurafenib, 960 mg twice daily, or encorafenib, 300 mg once daily, (COLUMBUS part 1). The primary endpoint of coBRIM and COLUMBUS trial was PFS. Primary endpoint of COMBI-v was OS. Key inclusion criteria were comparable across the studies. Patients with untreated brain metastases were not eligible.

In this analysis the subgroups with normal and elevated LDH have been statistically analyzed using a model for making indirect comparisons of the magnitude of treatment effects without losing the power of randomization (Bucher analysis). The aim of this analysis was the indirect comparison of PFS and OS as well as ORR in the subgroups with elevated LDH levels using the Bucher method.

4.2.3. Statistical Analyses

Due to data availability, the PFS analysis comparing coBRIM with COMBI-v was done using local assessment data, while the comparison with COLUMBUS part 1 used data from the independent central review. As defined by the particular study protocol, all enrolled patients were included in the analysis. The Bucher analysis was based on the assessments of benefit of the Federal Joint Committee (G-BA) for vemurafenib plus cobimetinib (module 5), dabrafenib plus trametinib (module 4) and encorafenib plus binimetinib (module 4) as well as data from the COLUMBUS-part 1. Median OS and PFS were calculated using the Kaplan–Meier method.

4.3. The multicentric studies – German CMMR in combination with local databases (Manuscript 4,6)

4.3.1. Study design and Data Source

The centers included are detailed in the respective publications, and all adhered to the data collection procedures developed by the CMMR. The participating centers were all Certified German Cancer Centers, which means that regular inspections are performed by the Company OnkoZert, which is financed by the German Cancer Society.

For each manuscript, an ethics committee approval was requested, and the numbers of the approval are included in the respective manuscripts. In each study, we used specific pseudo-anonymized forms to document patients' data. All participating centers received the mentioned pseudo-anonymized forms including the prespecified information to be collected. Data were extracted from patients' medical records, either by medical doctors or by clinical research documentation professionals, depending on the site.

4.3.2. Key Variables

For manuscript 4, besides the data already mentioned from the German CMMR, the following information was collected: demographic data, ECOG PS, available information on the genotype, number of organs with metastases, and previous therapies. As potential serum biomarkers, LDH, C-reactive protein, and the relative counts of lymphocytes, neutrophils, and eosinophils were also collected.

For manuscript 6, besides the data already mentioned from the German CMMR, the following information was collected: BRAF mutation status, number of melanoma brain metastases, ECOG PS, LDH level and protein S100B level, treatment with corticosteroids at the time of starting combined immunotherapy, date and type of local and systemic therapy in advanced stages, toxicity, date and type of best overall response, date of progressive disease, last contact date, cause of death and death date.

4.3.3. Statistical Analyses

Descriptive statistics were used to characterize the patients' population and treatment patterns. For manuscript 4, OS and PFS were calculated as the time from the initiation of the first cycle of combined checkpoint blockade until melanoma-specific or treatment-related death and disease progression, respectively. Time-to-event analyses were calculated where death or progression were considered as events. If neither occurred or if patients were lost to follow-up, the date of the last documented presentation was used as a censored observation. The survival and progression probabilities were calculated using the Kaplan-Meier method for censored failure time data assuming proportional hazards. When appropriate, multivariate logistic regression models were used to examine associations. Hazard ratios (HR) with 95% confidence intervals (CI) were calculated to quantify the impact on survival. The association of treatment response as a categorical variable with clinical characteristics or serum biomarkers was investigated with the Chi-

square test and logistic regression, as appropriate. Results were reported as two-sided p values with 95% CIs. All *p*-values presented are two-sided tests of statistical significance at 0.05. All analyses were carried out with SPSS statistics version 23.0 (IBM) or GraphPad Prism version 5.01 (GraphPad Software).

For manuscript 6, estimates of survival rates, OS and follow-up time were calculated considering the date of melanoma brain metastases diagnosis, and last patient contact or death. Kaplan-Meier estimates were used for the calculation of OS. Differences between groups were assessed using the log-rank test. When appropriate, multivariate logistic regression models were used to examine associations. Results were reported as two-sided *p* values with 95% CIs. All *p*-values presented are two-sided tests of statistical significance at 0.05. All statistical analyses were performed using SPSS v.25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

5. Results

For the elaboration of this thesis, 6 original manuscripts were included, and will be presented in dedicated separate sections.

To evaluate the long-term outcomes in patients with stage IV melanoma treated with immunotherapy and targeted therapy between 2011-2019 (*Work project 1*), the following manuscripts were considered:

- **Improvement of overall survival in stage IV melanoma patients during 2011-2014: analysis of real-world data in 441 patients of the German Central Malignant Melanoma Registry (CMMR)**

- **Primary Resistance to PD-1-Based Immunotherapy - A Study in 319 Patients with Stage IV Melanoma**

- **Indirect Comparison of Combined BRAF and MEK Inhibition in Melanoma Patients with Elevated Baseline Lactate Dehydrogenase**

- **Combined immune checkpoint blockade for metastatic uveal melanoma: a retrospective, multi-center study**

For evaluating the impact in survival outcomes of immunotherapy and targeted therapy, and their combination with local therapies in patients with melanoma brain metastases (*Work project 2*), the following manuscripts were considered:

- **Immunotherapy plus surgery/radiosurgery is associated with favorable survival in patients with melanoma brain metastasis**

- **Combined immunotherapy with nivolumab and ipilimumab with and without local therapy in patients with melanoma brain metastasis: a DeCOG* study in 380 patients**

For all work projects as first author, Teresa Amaral, led the design of the study, performed the data analyses, interacting with biostatisticians when needed, performing data analysis, interpreting results, and writing all the manuscripts that are presented in this thesis.

For all the work projects as co-author, Teresa Amaral was involved in the design of the study and in the data analysis, interpreted and wrote the results, and co-wrote the manuscript.

6. Manuscripts

1. **"Improvement of overall survival in stage IV melanoma patients during 2011-2014: analysis of real-world data in 441 patients of the German Central Malignant Melanoma Registry (CMMR)."** Forschner, A., F. Eichner, **T. Amaral**, U. Keim, C. Garbe and T. K. Eigentler (2017). J Cancer Res Clin Oncol 143(3): 533-540.

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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

Improvement of overall survival in stage IV melanoma patients during 2011–2014: analysis of real-world data in 441 patients of the German Central Malignant Melanoma Registry (CMMR)

Andrea Forschner[1] · Felizitas Eichner[1,2] · Teresa Amaral[1,3] · Ulrike Keim[1] · Claus Garbe[1] · Thomas Kurt Eigentler[1]

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Abstract
Background During 2011 and 2014, new treatment modalities like tyrosine kinase inhibitors and checkpoint inhibitors were introduced into the therapy of metastatic melanoma. This study addresses the question whether overall survival (OS) of metastatic melanoma patients has already been improved in 441 patients diagnosed with metastatic melanoma between 2011 and 2014 in the real-world setting at the University Hospital Tuebingen.
Methods All patients were documented with their different therapies by the CMMR and followed up until March 2016. Survival probabilities were calculated by Kaplan–Meier estimators, and log-rank tests were used to evaluate significances. Hazard ratios were estimated by Cox regression analysis for survival probabilities and prognostic factors in stage IV melanoma.
Results Best OS was observed in patients ($n = 93$) treated by metastasectomy as primary treatment with the intention to completely excise all metastases (3-year OS 61%). OS for patients with first-line systemic treatment ($n = 258$) was unfavorable in general (3-year OS 23%). Of those, the most favorable outcome was observed in patients without brain metastasis and treated with immunotherapy (mostly ipilimumab), as first-line treatment (median OS 35 months, 3-year OS 43%). In case of brain metastases, patients with targeted therapy had a better OS (median 14 months) than patients with ipilimumab treatment (median 7 months). Among all patients with first-line systemic treatment, outcome of patients diagnosed in the years 2013/2014, compared to 2011 and 2012, showed an improved survival. Three-year OS for patients that entered stage IV in 2013/2014 was 37% compared to those that entered stage IV in 2011 (18%) and 2012 (20%).
Conclusion The analysis of real-world data of treatment of metastatic melanoma showed an improvement of OS with both immunotherapy and targeted therapy. In case of cerebral metastasis, patients treated with targeted therapy showed a longer median OS than patients treated with ipilimumab.

Keywords Melanoma · Survival · Checkpoint inhibitors · Targeted therapy · Chemotherapy · Pembrolizumab · Nivolumab · Ipilimumab · Brain metastasis

Introduction

Systemic treatment of metastasized melanoma has been disappointing for decades. Chemotherapy with dacarbazine or carboplatin and paclitaxel resulted in median survival times of 7–9 months, and for a long time, no other treatment regime was found to prolong survival in advanced metastatic melanoma patients (Dummer et al. 2012; Eigentler et al. 2003; Pflugfelder et al. 2011; Tsao et al. 2004). At that time, one-year overall survival (OS) of stage IV melanoma patients was about 25–30% (Balch et al. 2009).

It is impressive to see that in the last 5 years 1-year OS for stage IV patients reported in clinical trials has risen to over 70%. This dramatic improvement is due to the

✉ Andrea Forschner
andrea.forschner@med.uni-tuebingen.de

[1] Department of Dermatology, University Medical Center Tübingen, Liebermeisterstr. 25, 72076 Tübingen, Germany

[2] Graduate School of Life Sciences, Utrecht University, Utrecht, Netherlands

[3] Portuguese Air Force Health Direction, Paço do Lumiar, 1649-020 Lisbon, Portugal

availability of multiple new drugs. So, it has become possible to inhibit the mitogen-activated protein kinase (MAPK) pathway in BRAF V600-mutant melanoma selectively by using BRAF/MEK inhibitors. Furthermore, checkpoint inhibitors enable an increase in host T cell response against tumor cells.

Monotherapy with BRAF inhibitors led to an increase in the median progression-free survival (PFS) of about 5 months and 1-year OS reached 68% (Chapman et al. 2011; Hauschild et al. 2012). Combinations of BRAF and MEK inhibitors prolonged median PFS to even ~10 months and improved 1-year OS to 74% (Larkin et al. 2014; Long et al. 2014). Concerning checkpoint inhibitors, treatment with ipilimumab resulted in a 1-year OS of 46% (Robert et al. 2011), whereas treatment with PD-1 inhibitors showed a one-year OS of ~70% (Robert et al. 2015a, b).

These new treatment options have significantly improved the prognosis of metastasized melanoma patients—but mainly in clinical trials with selected cohorts. For patient care, it is even more important to assess their efficacy in the real-world setting. To determine whether these new drugs can provide a survival benefit for patients in a real-world setting also, we performed an OS analysis of our patients suffering from advanced melanoma who entered stage IV between 2011 and 2014 and received treatment at the University Hospital Tuebingen.

Methods

Our analysis was performed on prospectively collected data of stage IV melanoma patients entered into the Central Malignant Melanoma Registry (CMMR). Routinely, all melanoma patients of our hospital are registered in the CMMR. Informed consent was obtained from all patients included in this study. Captured data include general information like date of birth, sex, origin and date of death, if applicable. In addition, the CMMR provides melanoma-specific variables such as localization, size, histological type, Breslow's tumor thickness and Clark level. For stage IV patients, 61 additional variables are documented including localization of metastases, BRAF, KIT and NRAS mutation status, treatment lines, period of treatment, treatment regimens, best response, dose modifications and type of outcome. Adverse events or serious adverse events are not captured. Data are entered into the database each time the patient visits the hospital for a treatment cycle, surgery or radiotherapy. Phone contact with the patient, the family or external treating practitioners is included into the patient file if the patient had no contact with hospital for an extended amount of time.

Statistical analysis was performed using the statistical program for social sciences SPSS version 23 (IBM, New York, USA). Survival probabilities and median survival with 95% confidence intervals (CI) were estimated according to the Kaplan–Meier method with the time depicted in months. A log-rank test was performed to reveal possible differences between the groups, and p-values < 0.05 were considered statistically significant. Afterward, a Cox regression model was fitted to obtain hazard ratios (HR) and their 95% confidence intervals. Follow-up time was defined from the date entering stage IV to the date of last known contact or death. This study was approved by the local ethics committee of the University of Tuebingen (reference number 676/2016BO2).

Results

In September 2016, the CMMR comprised a total number of over 13,900 patients with invasive melanomas born between 1884 and 2007, who were treated at the University Hospital Tuebingen. The selected patient cohort for these analyzed consisted of 187 women and 254 men. Follow-up ranged from 10 days to 61 months with a median follow-up time of 14 months. The median age at time point of advanced disease was 59 years [19Y–96Y, interquartile range 48Y–72Y]. When entering stage IV, 105 patients (23.8%) had cerebral metastases, 256 patients (58%) lung metastases and 138 patients (30%) liver metastases.

OS between patients with metastases only to distant skin or lymph nodes (M1a, $n = 53$), metastases of the lung (M1b, $n = 75$) and metastases of other organs or increased LDH (M1c, $n = 313$) showed a significant survival difference ($p < 0.0001$, Fig. 1). One-year OS was best for M1a patients with 86.6%, followed by M1b patients with 74.3% and M1c patients with 51.6% (Table 1).

In the first-line situation, patients treated surgically ($n = 93$) had a significantly improved OS over patients treated systemically ($n = 258$) ($p < 0.0001$, Fig. 2). Furthermore, patients whose metastases could be removed completely by surgery had the most favorable prognosis and a 1-year OS of 76.8% compared to 57.5% of systemically treated patients (Table 1).

Of the 258 patients who received first-line systemic treatment, 47% were BRAF wild type and 41% were BRAF mutated. In 12% of the cases, the mutation status was not determined. Of them, 37% suffered from uveal melanoma and the others were diagnosed with stage IV in 2011 when BRAF inhibitors were not available outside of studies in Germany.

Figure 3 shows a comparison of OS for the different systemic treatment options. Sixty-eight patients received targeted therapy, 52 patients immunotherapy and 132 patients chemotherapy. Six patients were excluded from this analysis because they were treated in

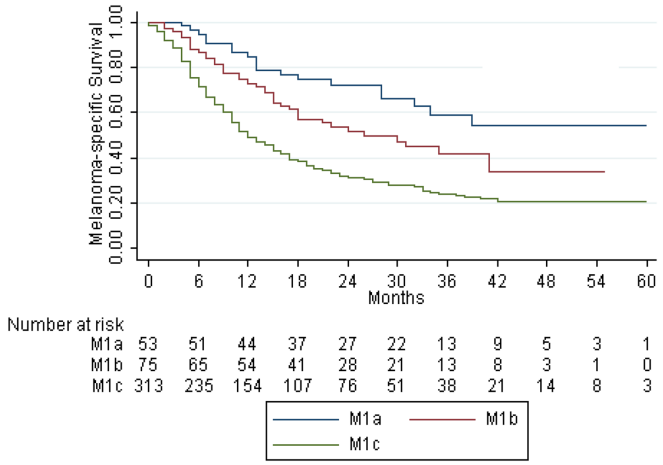

Fig. 1 Kaplan–Meier survival curves by stage M1a, M1b and M1c, $p < 0.0001$

Table 1 Overall survival in months of subgroups of stage IV melanoma patients

Subgroup	Median OS [months] (95% CI)	One-year OS [%] (95% CI)	Two-year OS [%] (95% CI)	Three-year OS [%] (95% CI)	HR (95% CI)
Stage M1a ($n = 53$)	Not reached	86.6 (77.4–95.8)	72.1 (59.6–84.6)	59 (43.3–74.7)	1
Stage M1b ($n = 75$)	26 (14.4–37.6)	74.3 (64.3–84.3)	53.6 (41.8–65.4)	41.7 (28.6–54.8)	1.75 (1.02–3.03)
Stage M1c ($n = 313$)	12 (9,8–14.2)	51.6 (46.1–57.1)	31.9 (26.6–37.2)	24 (18.7–29.3)	3.05 (1.91–4.87)
First-line surgery ($n = 93$)	Not reached	76.8 (68.3–85.5)	66.8 (56.8–76.8)	61.1 (50.1–72.1)	0.36 (0.25–0.53)
First-line systemic therapy ($n = 258$)	15 (12.1–18)	57.5 (51.4–63.6)	35.8 (29.7–41.9)	23.4 (17.1–29.7)	1
First-line targeted therapy ($n = 68$)	16 (10.6–21.4)	64.7 (53.3–76.1)	36.3 (24.5–48.1)	27.6 (15.8–39.4)	0.73 (0.52–1.03)
First-line immunotherapy ($n = 52$)	33 (21.7–44.3)	67.1 (54.4–79.8)	60.3 (46.6–74.0)	37.4 (16.6–58.2)	0.5 (0.32–0.77)
First-line chemotherapy ($n = 132$)	11 (7.6–14.4)	49.6 (41–58.2)	26.9 (19.1–34.7)	15.9 (8.8–23)	1
Targeted therapy + brain metastases ($n = 24$)	14 (5.4–22.6)	58.3 (38.5–78.1)	28.6 (10.2–47)	15.9 (17.2–33.1)	1
Targeted therapy no brain metastases ($n = 44$)	17 (12.1–21.9)	68.2 (54.5–82)	40.8 (25.7–55.9)	34 (18.7–49.3)	0.75 (0.42–1.35)
Immunotherapy + brain metastases ($n = 10$)	7 (0–17.9)	50 (19–81)	40 (9.6–70.4)		1
Immunotherapy no brain metastases ($n = 42$)	35 (25.5–44.5)	71.2 (71.1–71.3)	65.4 (65.3–65.6)	43.1 (43.3–42.9)	0.38 (0.15–0.94)
Chemotherapy + brain metastases ($n = 23$)	9 (4.3–13.7)	26.1 (26–26.3)			1
Chemotherapy no brain metastases ($n = 109$)	14 (10.3–17.7)	54.7 (54.6–54.8)	32.9 (32.8–33)	19.4 (19.3–19.5)	0.45 (0.28–0.72)
2011 stage IV diagnosis ($n = 68$)	12 (8.3–15.7)	51.5 (51.4–51.6)	30.9 (30.8–31.0)	17.6 (17.5–17.7)	1
2012 stage IV diagnosis ($n = 70$)	13 (8.6–17.4)	58.2 (58.1–58.3)	32.2 (32.1–32.3)	19.7 (19.6–19.8)	0.82 (0.61–1.11)
2013/2014 stage IV diagnosis ($n = 114$)	17 (12.8–21.2)	60.2 (60.1–60.3)	41.6 (41.5–41.7)	37.2 (37.1–37.3)	0.79 (0.6–1.04)

a blinded clinical trial. Twelve of the 68 patients (17.6%) with targeted therapy were treated by a combination of BRAF and MEK inhibitor, seven (10.3%) received MEK inhibitor only and 49 (72%) received BRAF inhibitor monotherapy.

Forty-two of the 52 (80.8%) patients with immunotherapy received ipilimumab and five (9.6%) patients PD-1 antibodies. The five remaining patients were treated with the bispecific antibody L19 IL2 in clinical trials, three of them in combination with dacarbazine.

We calculated a significant difference between the treatments options ($p = 0.003$). Best OS was detected in patients treated with immunotherapy. For these patients, median OS was 33 months, compared to 16 months for patients receiving targeted therapy and 11 months for patients in the chemotherapy group (Fig. 3; Table 1).

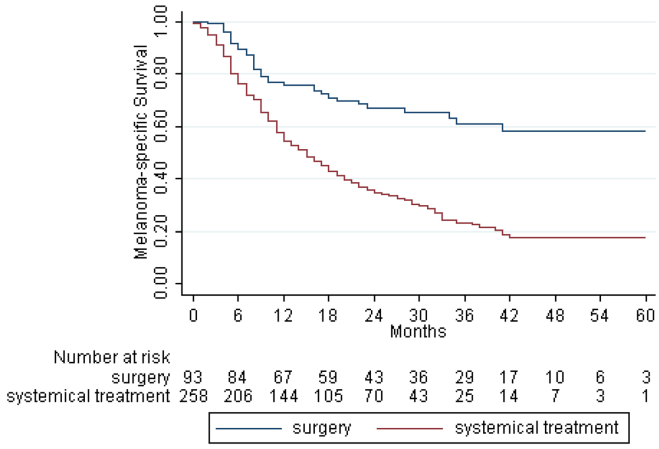

Fig. 2 Kaplan–Meier survival curves by first-line surgical vs. systemic treatment, $p < 0.0001$

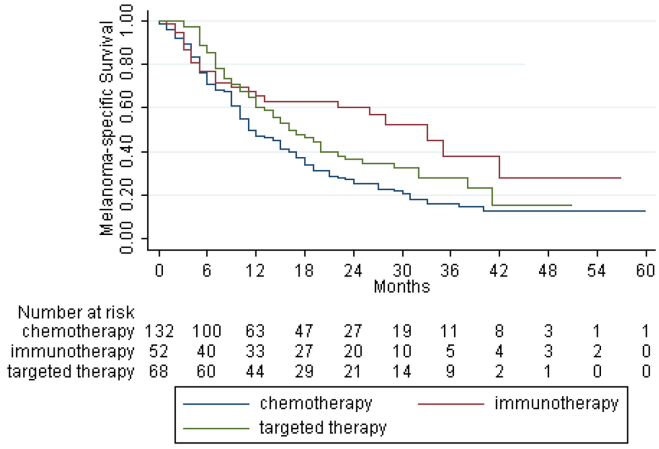

Fig. 3 Kaplan–Meier survival curves by first-line systemic treatment type, $p = 0.003$

In the group of patients receiving targeted therapy, 24 of 68 (35.3%) had brain metastases, but only ten of 52 patients (19.2%) were in the immunotherapy group and 23 of 132 patients (17.4%) were in the chemotherapy group.

For patients treated with targeted therapy, median OS was 14 months for patients with brain metastases compared to 17 months for patients without brain metastases (Fig. 4; Table 1). Patients who received immunotherapy as first-line treatment had a median OS of 7 months in case of brain metastases compared to 35 months for patients without brain metastases (Fig. 5; Table 1). For more details, please refer to Table 1. OS was significantly different between patients with and without brain metastases who were treated with first-line chemotherapy ($p = 0.001$; median OS 9 months for patients with cerebral metastases vs. 14 months for patients without brain metastases; Table 1).

An illustration of the survival curves of patients treated systemically as first option depending on the year of entering stage IV melanoma is provided in Fig. 6. In 2011, 70.1% of the patients were treated with chemotherapy, 11.8% with immunotherapy and 16.2% with targeted therapy. In 2012, 32.9% of the systemically treated patients

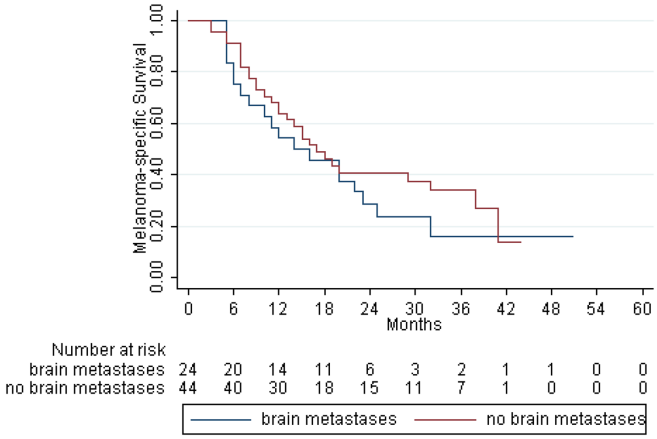

Fig. 4 Kaplan–Meier survival curves by first-line targeted therapy and cerebral status, $p = 0.329$

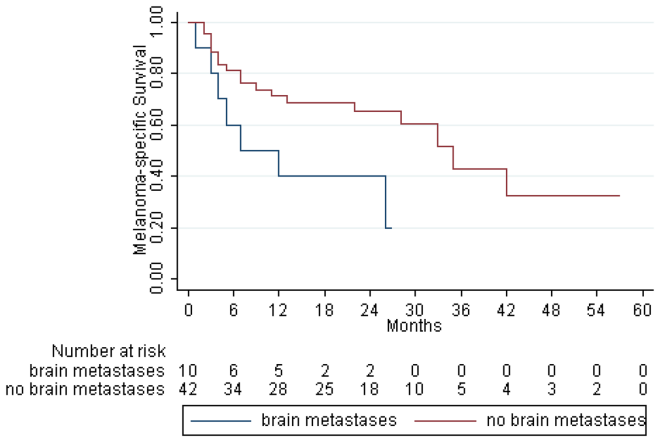

Fig. 5 Kaplan–Meier survival curves by first-line immunotherapy and cerebral status, $p = 0.027$

received targeted therapy first line, 11.4% immunotherapy and 55.7% chemotherapy, whereas in the years 2013/2014, 32.8% of the patients were treated with targeted therapy, 31.6% with immunotherapy and only 38.6% with chemotherapy. Median OS for patients entering stage IV in 2011 was 12 months, in 2012 13 months and in 2013/2014 17 months. The difference in survival probability between 2012 and 2013/2014 was rather small after 1 year, but increased during longer follow-up. Three-year OS was 19.7% for the 2012 group and 37.2% for the 2013/2014 group (Table 1).

Discussion

In general, our analyses of real-world treated melanoma patients confirmed findings of clinical trials of novel drugs to improve OS. Not surprisingly, the improvement was less extensive compared to clinical trials, as these normally include selected patients, only. In contrast, in our study all real-life patients were included, who, e.g., suffered from brain metastases, had comorbidities or had a decreased performance status. This approach is important as these are the patients in the daily clinical practice.

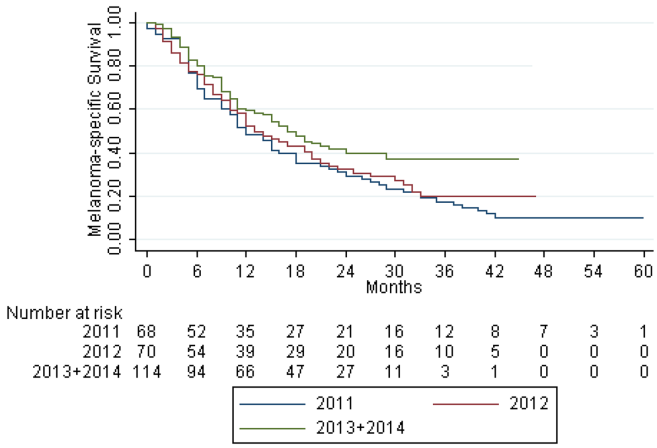

Fig. 6 Kaplan–Meier survival curves by year entry stage IV in systemically treated patients, $p = 0.081$

Compared to OS data of the time period without novel drugs in systemic melanoma therapy, survival for M1a, M1b and even M1c patients improved over the last years steadily: 1-year OS for M1a patients has increased from 62%, reported by Balch et al. in 2009, to 85% in our cohort. Likewise, 1-year OS for M1b patients has improved from 53 to 74% and for M1c patients from 33 to 52% (Balch et al. 2009). As the indication, when to start systemic treatment and to perform surgery has not changed over the years, the improvement of OS in each M stage must be due to the availability of new, more effective drugs.

Regarding the year of entering advanced disease, we detected an improved OS for patients starting systemic treatment in the years 2013/2014 compared to those of the years 2012 and 2011. The reason for this improvement lies in the approval and availability of novel drugs. The BRAF inhibitor vemurafenib was approved in 2012 as was dabrafenib in 2013. Until 2013, chemotherapy pretreatment was requested before initiating ipilimumab. Ipilimumab was approved in Germany for first-line treatment not until 2013. For these reasons, the proportion of patients treated first line by immunotherapy or targeted therapy increased markedly in 2011 and 2013/2014.

Patients that started systemic therapy in the years 2013/2014 had a 1-year OS of 60% and 2-year OS of 42%. These results are even more favorable than those reported by Leeneman et al. (2015) from the Netherlands, comparing OS in "real-world" systemically treated patients up to 2011 with those treated in the years 2012–2015. They reported a 1-year OS of 40% and a 2-year OS of 20% in the 2012–2015 group. The increased survival probability in our patients might be due to the close melanoma follow-up program in Germany that compromises radiologic staging in metastasized patients every 6 months permitting an early stage IV diagnosis and therefore an early time point to start systemic treatment. This so-called lead-time bias might explain the OS differences between our patients and the patients of Leeneman et al.

Comparing first-line systemic therapy to first-line surgical therapy, our data revealed that surgically treated patients still had an improved OS despite the availability of novel drugs. This benefit is at least partly due to a "selection bias": Only patients with limited tumor burden are usually considered for complete metastasectomy, whereas widespread metastases lead to the initiation of systemic treatment. Our findings are consistent with the current literature. In 2012, Weide et al. reported a 5-year OS of 37% in case of complete metastasectomy in stage IV patients, compared to 10% for patients receiving other treatment modalities (Weide et al. 2012). In our cohort, 5-year OS of first-line surgically treated patients was even 58%. This improvement of OS in surgically treated patients is probably due to better detection of metastases (a) by PET-CT scans with a better selection of patients and (b) by the newly established systemic treatment options which are now regularly applied second line in case of further disease progression.

Overall, our results confirm the necessity to consider a complete metastasectomy before initiating systemic treatment as it is recommended in the malignant melanoma guidelines (Pflugfelder et al. 2013).

Concerning systemic treatment types, immunotherapy and targeted therapy seem to be more or less equally effective in the first year: One-year OS was 67% in the immunotherapy group and 65% in the targeted therapy group.

However, we detected an impressive difference in the 2-year OS with 60% for patients receiving immunotherapy and 36% for patients receiving targeted therapy. Patients without cerebral metastases receiving immunotherapy as first-line treatment had a better median OS (35 months) than patients with targeted therapy (17 months). These data are consistent with the literature, also. Median OS in treatment naïve patients treated with ipilimumab was about 30 months (Thompson et al. 2012), and patients treated with dabrafenib achieved a median OS of 19 months (Long et al. 2015).

On the one hand, this difference could be explained by the fact that most of the patients with targeted therapy develop drug resistance over time, while patients responding to immunotherapy seem to have a long-lasting response. However, in selected patients acquired resistance to PD-1 antibodies after initial response has been described (Wong and Ribas 2016) (Niezgoda et al. 2015; Zaretsky et al. 2016). On the other hand, patients with extensive tumor masses and/or clinical symptoms were predominantly treated by BRAF/MEK inhibitors to achieve a fast response to get relief for the patient.

The development of resistance in patients treated by targeted therapy might be delayed by supplementing a MEK inhibitor with the BRAF inhibitor. The proportion of patients receiving such a combination treatment was only 18%. This rather small number is due to the fact that in Germany MEK inhibitors were only available outside clinical studies in the frame of an early access program from the end of 2014 on. The combination of BRAF and MEK inhibitors was approved in Germany in 2015.

Furthermore, the difference in OS might be due to imbalances in the presence of brain metastases throughout the treatment cohorts (35.3% in the group treated with targeted therapy vs. 19.2% in the immunotherapy group vs. 17.4% in the chemotherapy group). In case of cerebral metastasis, we detected a clear improvement of median OS for targeted treated patients (14 months) vs. patients treated by immunotherapy (7 months) or chemotherapy (9 months). Our data for patients with brain metastases treated by targeted therapy are even more favorable than published in the current literature. Long et al. reported a median OS of 8.3 months in treatment naïve cerebral metastasized patients that received a therapy with dabrafenib (Long et al. 2012). For vemurafenib, median OS of 5.3 months in symptomatic cerebral metastasized patients was reported by Dummer et al. (Dummer et al. 2014). Regarding the intracranial activity of ipilimumab, our findings are consistent with a paper by Margolin and colleagues. In their cohort, asymptomatic cerebral metastasized patients reached a median OS of 7 months under a treatment with ipilimumab (Margolin 2012).

This study has several limitations: It has to be considered that the number of patients with brain metastasis in each of our treatment groups was rather small. Therefore, our results have to be treated with caution and have to be confirmed in larger patient collectives. Furthermore, this study evaluated only first-line treatments. It would also be important to analyze the subsequent treatment lines, of course. For BRAF-mutant patients, however, our evaluation indicates a long-term survival benefit for patients treated by immunotherapy as first treatment option, despite all methodological limitations. Due to the fact that these real-world data compromise all patients with different extensions of the disease requiring different treatment procedures we were not able to power our analyses to detect a difference between the treatment groups, adequately.

We also have to consider that less than 10% of the first-line immunotherapy patients received PD-1 antibodies. In Germany, PD-1 antibodies were not approved until 2015 for the first-line treatment of advanced melanoma followed by the approval of combined nivolumab and ipilimumab in 2016. Likewise, first-line targeted therapy in 2016 consists in a combination of BRAF and MEK inhibition, whereas less than 20% of the first-line targeted treated patients received such a combination treatment in our study.

In summary, our analysis of real-world data of treatment schedules for metastatic melanoma during the years 2011–2014 showed an improvement of OS by immunotherapy as well as targeted therapy. In patients with brain metastasis, targeted therapy seems to prolong OS over ipilimumab. Current studies evaluate whether the combination of checkpoint and kinase inhibitors will furthermore prolong OS in metastatic melanoma patients.

Authors' contributions AF contributed to literature search, figures, data collection, data analysis, data interpretation and writing; FE and TKE were involved in data collection, data analysis, data interpretation and writing; TA was involved in data collection, data analysis and data interpretation; UK contributed to data analysis and figures; CG was involved in data interpretation and writing.

Compliance with ethical standards

Conflict of interest Andrea Forschner reports personal fees from BMS, MSD, Novartis, Roche, outside the submitted work. Claus Garbe reports personal fees from Amgen, LEO, MSD, Philogen, and grants and personal fees from BMS, Novartis, Roche, outside the submitted work. Thomas Kurt Eigentler reports personal fees from Amgen, BMS, MSD, Novartis, Roche, outside the submitted work. Felicitas Eichner, Teresa Amaral and Ulrike Keim have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the local ethics committee of the University of Tuebingen (Reference Number 676/2016BO2).

Informed consent Informed consent was obtained from all patients included in this study.

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2. **"Primary Resistance to PD-1-Based Immunotherapy - A Study in 319 Patients with Stage IV Melanoma." Amaral, T.,** O. Seeber, E. Mersi, S. Sanchez, I. Thomas, A. Meiwes, A. Forschner, U. Leiter, T. Eigentler, U. Keim and C. Garbe (2020). Cancers 12(4): 1027.

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Primary Resistance to PD-1-Based Immunotherapy—A Study in 319 Patients with Stage IV Melanoma

Teresa Amaral [1,2], Olivia Seeber [1], Edgar Mersi [1], Stephanie Sanchez [1], Ioannis Thomas [1], Andreas Meiwes [1], Andrea Forschner [1], Ulrike Leiter [1], Thomas Eigentler [1], Ulrike Keim [1] and Claus Garbe [1,*]

[1] Center for Dermatooncology, Department of Dermatology, Eberhard Karls University of Tuebingen, 72076 Tuebingen, Germany; teresa.amaral@med.uni-tuebingen.de
[2] Portuguese Air Force—Health Care Direction, 1649-020 Lisbon, Portugal
* Correspondence: Claus.garbe@med.uni-tuebingen.de; Tel.: +49-7071-298-87110; Fax: +49-7071-29-51-87

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Abstract: Background: Primary resistance to immunotherapy can be observed in approximately 40–65% of the stage IV melanoma patients treated with immune checkpoint inhibitors. A minority of the patients receive a second-line therapy, and the clinical benefit is small. Patients and methods: Stage IV melanoma patients treated with first-line PD-1-based immunotherapy between January 2015 and December 2018 were investigated. Primary resistance was defined as progressive disease (PD) at the time of the first tumor assessment after starting immunotherapy. Patients with complete response, partial response, and stable disease were classified as having disease control (DC). Overall survival (OS) and progression-free survival (PFS) were evaluated by Kaplan–Meier estimator. Univariate and multivariate logistic regression analyses were performed to determine prognostic factors associated with OS. Results: Three hundred and nineteen patients were included, and 40% had primary resistance to immunotherapy. The median follow-up time was 22 months. Patients with primary resistance had 1-, 2-, and 3-year OS rates of 41%, 15%, and 10%, respectively, compared to 91%, 81%, and 65% for the patients who achieved DC. The following independently significant prognostic factors for OS were identified: protein S100B level and primary tumor localization. There was a statistically significant difference for OS ($p < 0.0001$) but not for PFS ($p = 0.230$) when analyzing risk groups formed with a combination of these two variables (low-, intermediate-, and high-risk subgroups). Conclusions: Melanoma patients with primary resistance to immunotherapy have a dismal prognosis. Response at the first tumor assessment after starting immunotherapy is a stronger prognostic factor for the further course of the disease than pretreatment risk factors.

Keywords: metastatic melanoma; primary resistance; checkpoint-inhibitors; combined immunotherapy; pseudoprogression

1. Introduction

Immunotherapy with checkpoint inhibitors is currently the most effective therapy for metastatic melanoma, achieving high remission rates and long-term survival [1]. These therapies include ipilimumab, a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibody, nivolumab and pembrolizumab, both programmed cell death protein 1 (PD-1) antibodies, and the dual combination of anti-PD-1 and anti-CTLA-4 antibodies nivolumab plus ipilimumab. Results from studies investigating these therapies in melanoma patients are available [2–4]. Long-term survival data have also been published, specifically the 5- and 10-year overall survival (OS) rates for ipilimumab monotherapy, and 5-year OS rates for nivolumab, and the combination of nivolumab with ipilimumab [4–6]. The latest

update showed that the 5-year OS survival rate for nivolumab and ipilimumab was 52%, for nivolumab monotherapy was 44%, and for ipilimumab monotherapy was 26% [4].

Despite improvement in survival outcomes compared with the past (e.g., chemotherapy), primary resistance to checkpoint inhibitor therapy exists, and a considerable number of patients still do not derive benefit from these therapies [7,8]. Primary resistance is typically assumed in the clinical practice if tumor progression is observed at the first tumor assessment after therapy starts, which in our center takes place around week 12 (+/−5 days). It is observed in a rather high percentage of patients, estimated to be between 40% and 65%, depending on whether patients receive first-line immunotherapy or immunotherapy after progression under other systemic therapies [3,9,10]. Higher percentages were observed when patients were treated with ipilimumab monotherapy [2,11].

Resistance to immunotherapy can be classified as primary (or innate) and secondary or acquired [12–15]. Some authors also refer to an intermediate phenotype that is adaptive resistance [16]. Clinically, resistance mechanisms to immunotherapy can be grouped into those that always preclude response to immunotherapy and those that appear later, allowing tumor escape and progression after an initial benefit. However, the molecular mechanisms of resistance to immunotherapy involving the host, the tumor, and the tumor microenvironment can overlap and be present at different timepoints of the course of the disease.

Regarding primary resistance to immunotherapy, which is the focus of our analysis, the following resistance mechanisms have been described: (a) diminished sensitivity to the INF-signaling pathway [8,16,17]; (b) insufficient T-cell activation or absence of T-cells in the tumor microenvironment [18–20]; (c) increased infiltration of T-regulatory cells [21–23]; (d) upregulation of immunosuppressive markers [24,25]; (e) insufficient antigen presentation and/or antigen recognition, due to, for example, low tumor mutation burden, loss of MHC class I and β-2 microglobulin, or absence of neo-antigen presentation [17,26–29].

In the present study, we focus on the clinical outcomes of stage IV melanoma patients with primary resistance to first-line PD-1-based immunotherapy, specifically pembrolizumab, nivolumab, and nivolumab plus ipilimumab. We evaluate the course of the disease in patients prospectively registered in the Central Malignant Melanoma Registry (CMMR) of the German Dermatological Society, and treated between January 2015 and December 2018 at the University Hospital Tübingen. We addressed the following questions: (1) Which factors are associated with the development of primary resistance? (2) How does survival of patients with primary resistance compare to those with disease control (complete response (CR), partial response (PR), and stable disease (SD))? (3) Did the patients with primary resistance to PD-1-based immunotherapy receive further therapies, and if so, which therapies were offered and what was the outcome?

2. Patients and Methods

2.1. Patients Cohort

Three hundred and nineteen patients with stage IV melanoma treated with first-line anti-PD-1 antibodies immunotherapy were included. These patients had available data on the type of response at the time of the first tumor assessment after starting immunotherapy, and also data that allowed us to identify the best overall response to immunotherapy. The Ethics Committee of the Medical Faculty of the University of Tübingen approved this study (approval number 676/2016BO2).

All patients included signed the patients' informed consent and were prospectively recorded by the German Central Malignant Melanoma Registry (CMMR). Clinical data were obtained from the clinical records from the University Hospital Tübingen, documented in an open source database, Epi Info™, and later merged into a final SPSS® file. The following variables were recorded: gender, date of birth, date of stage IV diagnosis, stage at initial diagnosis according to the American Joint Committee on Cancer (AJCC) version 8 [30], localization and histopathological characteristics of the primary tumor, *BRAF* mutation status, protein S100B level, lactate dehydrogenase (LDH) level at the

time of stage IV diagnosis, localization and number of metastatic organs, type of systemic therapy for stage IV disease and respective start and end dates, response at the first tumor assessment after systemic therapy start, best overall response to systemic therapy, and time of last follow-up or death from any cause.

Primary resistance was defined as progressive disease (PD) at the time of first tumor assessment after immunotherapy start. In our center, this is performed after 12 weeks (+/−5 days). This evaluation was performed using RECIST 1.1 [31]. Patients with CR, PR, and SD were considered to have disease control (DC). Best overall response to first-line immunotherapy was defined as the best response—intracranial and extracranial—that patients achieved during the time they were treated. Taking that into consideration, patients for whom the best overall response was PD were, by definition, patients with primary resistance. These patients did not continue to receive immunotherapy, since the clinical evaluation also determined that they were not deriving benefit from the ongoing therapy.

Pseudoprogression was considered for patients who were classified as having PD by RECIST 1.1 [31] at the time of first assessment after immunotherapy start but, due to clinical benefit, continued receiving immunotherapy and had a response later in the course of their disease. These patients were not considered as primary resistant and were included in the group of disease control.

2.2. Statistical Analysis

Statistical analysis was performed using the statistical program for social sciences SPSS Version 25 (IBM, New York, NY, USA). STATA® v15 (StataCorp LLC, College Station, TX, USA) was used to generate the final version of the Kaplan–Meier survival curves.

Descriptive statistical analyses, frequency tables, and chi-square tables were used to characterize the patients' population. Variables with missing information were excluded from the respective analysis. Follow-up time was defined as the time between the date of stage IV diagnosis and the date of the last follow-up or death from any cause. Survival analyses were performed according to the Kaplan–Meier method. In addition, the 1-, 2-, and 3-year survival rates were calculated with a 95% confidence interval. Factors that were significant in the univariate analysis were included into the multivariate logistic regression analysis. The level of significance was 0.05 (two-sided) in all analyses. The cut-off date for data analysis was March 2019.

3. Results

3.1. Univariate and Multivariate Analysis

Table 1 shows characteristics of the study population: 192 patients (60%) had disease control (SD, PR, CR) and 127 (40%) patients had primary resistance. The median age of the patients at the time of stage IV melanoma diagnosis was 68 years; interquartile range (IQR) (56–77). Age was not associated with primary resistance. Thirty-five patients (11%) had more than 3 organs with metastases at the time of immunotherapy beginning and 292 patients (89%) had 1–3 organs with metastases. Sixty-three (19%) patients had brain metastases and 118 (36%) patients had liver metastases. The number of organs involved, the presence of brain metastases, and the presence of liver metastases were not associated with primary resistance in our analysis.

Table 1. Patients characteristics, univariate and multivariate analysis for the whole cohort, according to best overall response to first-line PD-1-based immunotherapy.

Characteristics	ICI Cohort *n* = 319 *n* (%)	*n* (%)		Univariate Analysis χ^2 Test ♣	Multivariate Logistic Regression Analysis
		Primary Resistance *n* = 127 (40)	DC (CR, PR, SD) *n* = 192 (60)		
Age Distribution					
Median (years [IQR])	68 (56–77)	65 (55–78)	68 (56–77)		
<60y	101 (32)	37 (29)	64 (33)	0.732	
60y–75y	114 (36)	47 (37)	67 (35)		
>75y	104 (32)	43 (34)	61 (32)		
Sex					
Male	192 (60)	68 (54)	124 (65)	**0.049**	0.822
Female	127 (40)	59 (46)	68 (35)		
Tumor localization *					
Head and neck	54 (22)	12 (13)	42 (27)		
Trunk	73 (29)	18 (20)	55 (34)	**0.000**	**0.001**
Extremity	109 (43)	54 (59)	55 (34)		
Other	15 (6)	7 (8)	8 (5)		
Histological subtype *					
SSM	76 (32)	31(37)	45 (29)		
NM	72 (30)	18(21)	54 (35)		
LMM	13 (6)	0	13 (9)	**0.007**	0.452
ALM	30 (12)	15 (18)	15 (10)		
Mucosal	15 (6)	7 (8)	8 (5)		
Other	32 (14)	14 (16)	18 (12)		
Stage at initial diagnosis *					
I	48 (17)	19 (18)	29 (17)		
II	84 (31)	25 (23)	59 (35)	0.114	
III	95 (35)	38 (37)	57 (34)		
IV	47 (17)	24 (22)	23 (14)		
Number of organs with metastases					
1-3	285 (89)	109 (86)	176 (92)	0.098	0.470
>3	34 (11)	18 (14)	16 (8)		
Brain metastases					
No	258 (81)	101 (79)	157 (82)	0.618	
Yes	61 (19)	26 (21)	35 (18)		
Liver metastases					
No	204 (64)	75 (59)	129 (67)	0.139	
Yes	115 (36)	52 (41)	63 (33)		
***BRAF* mutation** *					
*BRAF*mut	88 (45)	32 (44)	56 (46)	0.844	
*BRAF*wt	106 (56)	40 (56)	66 (54)		
LDH level *					
Normal	190 (68)	67 (60)	123 (73)	**0.029**	0.532
Elevated	90 (32)	44 (40)	46 (27)		
S100B level *					
Normal	157 (56)	44 (40)	113 (65)	**0.000**	**0.008**
Elevated	125 (44)	65 (60)	60 (35)		

* patients with no information available were excluded in the respective analysis; IQR = interquartile range; ♣ Chi-square test performed between the two groups—primary resistance and disease control; ICI = immune-checkpoint inhibitors cohort—145 patients received first-line treatment with nivolumab plus ipilimumab and 174 received antiPD-1 antibodies monotherapy (nivolumab *n* = 46 and pembrolizumab *n* = 128).; y = years; SSM = superficial spreading melanoma; NM = nodular melanoma; LMM = lentigo malignant melanoma; ALM = acral lentiginous melanoma; *BRAF*mut = presence of *BRAFV600E/K* mutation; *BRAF*wt = *BRAF* wild-type; LDH = lactate dehydrogenase; S100B = tumor marker protein S100B. *p*-values that are statistically significant are noted in bold.

In our cohort we had slightly more men (60%) than women (40%). In the univariate analysis, sex was a statistically significant factor associated with primary resistance, with male patients having better outcomes than female patients. In the multivariate logistic regression analysis, sex was not a statistically significant factor.

Tumor localization was significantly associated with primary resistance. Tumors of the extremities, including acral melanomas, showed significantly increased primary resistance. Tumor localization remained a significant factor in multivariate logistic regression analysis. The histological subtype was

also associated with primary resistance. Primary resistance was found especially in acral lentiginous melanoma, mucosal melanoma, and other melanomas. In the multivariate logistic regression analysis, however, the histological subtype was not a significant factor.

Another significant factor in the univariate analysis was an elevated level of the tumor marker protein S-100B, which was associated with a significantly increased primary resistance, both in the univariate and in the multivariate logistic regression analysis. An elevated LDH level was also significantly associated with increased primary resistance in the univariate analysis, but was not significant in the multivariate logistic regression analysis. The following variables were not significant either in univariate analysis or in multivariate analysis: stage at initial diagnosis, number of metastatic organs, presence of brain metastases, presence of liver metastases, and *BRAF* mutation status.

Table S1 shows characteristics of the study population where the primary resistance group includes patients with PD at the first evaluation after starting immunotherapy and patients with SD with a duration of less than 6 months (n = 169), and the DC group includes patients with CR, PR, and SD with a duration of more than 6 months (n = 190). The results are similar to the ones described above, except that the number of organs with metastases is a statistically significant factor in the univariate analysis and LDH level is no longer statistically significant.

3.2. Survival Analysis

The median overall survival (OS) and the 1-year, 2-year, and 3-year OS rates are summarized according to the best response in Table 2. The 2-year OS rates were 96% for patients with CR, 84% for patients with PR, and 64% for patients with SD. Patients with primary PD had a 2-year OS rate of 15%. The corresponding progression-free survival (PFS) rates are summarized in Table 3. Here, 2-year PFS was 81% for CR, 63% for PR, 22% for SD, and 3% for PD. The 2-year OS rate in the disease control (SD + PR + CR) group was 81% versus 15% in patients with primary resistance. As for the 2-year PFS rate it was 56% versus 3%, respectively.

Table 2. Median overall survival and overall survival rates for patients receiving first-line PD-1-based immunotherapy according to best overall response and type of immunotherapy.

Best Response	Median OS (Months; 95% CI)	OS (%; 95% CI)		
		1-Year	2-Year	3-Year
CR n = 50 (15.7%)	not reached	100%	95.7 (87.3–100)	87.7 (70.8–100)
PR n = 80 (25.1%)	not reached	89.5 (82.1–96.9)	84.4 (74.4–94.4)	84.4 (74.4–94.4)
SD n = 62 (19.4%)	28 (22.9–33.1)	86.3 (77.5–95.1)	63.8 (47.7–79.9)	24.6 (2.6–46.5)
PD n = 127 (39.8%)	11 (9.0–13.0)	41.3 (31.9–50.7)	14.7 (7.4–22.0)	10.1 (3.4–16.8)
DC n = 192 (60.2%)	not reached	91.3 (87.0–95.6)	81.0 (73.7–88.3)	64.6 (53.2–76)
PD-1 monotherapy n = 174 (66.2%)	26 (19.7–32.3)	71.1 (64.0–78.2)	53.3 (45.1–61.5)	41.3 (32.1–50.5)
PD-1 + CTLA4 n = 145 (54.6%)	31 (17.2–44.8)	72.8 (65–80.6)	54.5 (42.9–66.1)	42.5 (24.1–60.9)

OS = overall survival; CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease; DC = disease control (CR + PR + SD); PD-1 monotherapy = nivolumab or pembrolizumab; PD-1 + CTLA4 = nivolumab plus ipilimumab.

Table 3. Median progression-free survival and progression-free survival rates for patients receiving first-line PD-1-based immunotherapy according to best overall response and type of immunotherapy.

Best Response	Median PFS (Months; 95% CI)	PFS (%; 95% CI)		
		1-Year	2-Year	3-Year
CR n = 50 (15.7%)	Not reached	87.6 (78.4–96.8)	81.2 (68.9–93.5)	72.2 (52.2–92.2)
PR n = 80 (25.1%)	37 (14.97–59.03)	74.4 (64.2–85.0)	62.7 (50.0–75.4)	62.7 (50.0–75.4)
SD n = 62 (19.4%)	12 (8.97–15.03)	43.0 (29.3–56.7)	21.8 (6.3–37.3)	-
PD n = 127 (39.8%)	4 (3.56–4.44)	8.7 (3.8–13.6)	3.2 (0–6.5)	1.1 (0–3.1)
DC n = 192(60.2%)	33 (20.4–45.6)	68.1 (61.0–75.2)	56.2 (51.8–64.8)	48.7 (37.7–59.7)
PD-1 monotherapy n = 174 (66.2%)	8 (5.5–10.5)	40.3 (32.7–47.9)	30.5 (23.1–37.9)	24.1 (16.3–31.9)
PD-1 + CTLA4 n = 145 (54.6%)	9 (1.8–16.2)	48.5 (40.1–56.9)	39 (29.2–78.8)	-

PFS = progression-free survival; CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease; DC = disease control (CR + PR + SD); PD-1 monotherapy = nivolumab or pembrolizumab; PD-1 + CTLA4 = nivolumab plus ipilimumab.

A statistically significant difference can be seen in OS when patients are classified as having primary resistance or disease control at the time of first tumor assessment after starting immunotherapy (Figure 1A; $p < 0.0001$). The same is true for PFS (Figure 1B; $p < 0.0001$). After three years, a plateau was formed for the group with disease control at a level of 65%, while the PFS rate for primary resistance decreased to 10%. After three years, a certain plateau formation around a 45% PFS rate was also visible in patients with disease control, while in patients with primary resistance, the PFS rate dropped to 1%.

The OS curves according to Kaplan and Meier show that in cases of CR and PR, the survival remained largely stable after two years. This was not the case in patients that achieved SD, where there was a relatively steep drop in the survival curve after 18 months, leading to OS rates very close to those in patients with PD (Figure 1C; $p < 0.0001$). In the PFS analysis, there were even clearer differences between CR and PR. After the first year, there was a clear drop in PFS rates for PR compared to CR. After three years, patients with SD had approximately the same survival rates as patients with PD (Figure 1D; $p < 0.0001$).

Table S2 shows the patients characteristics for the whole cohort, considering the type of first-line immunotherapy received. One hundred and seventy-four patients received monotherapy with anti-PD-1 (nivolumab or pembrolizumab), while 145 patients were treated with the combination of nivolumab plus ipilimumab. The survival curves for OS overlapped completely, and in our cohort, there was no apparent benefit for the combination treatment (Figure 1E; $p = 0.993$). The survival curves for PFS separated approximately eight months after the start of treatment with a slightly more favorable course for the combined regimen, but this difference was not statistically significant (Figure 1F; $p = 0.216$).

In order to evaluate whether primary resistance can be predicted based on pre-existing risk factors, three subgroups were defined, considering the two factors that were significant in the multivariate regression analysis (i.e., primary tumor localization and protein S-100B level) (Figure S1). The subgroups were defined as follows: no risk factor (low-risk), one risk factor (intermediate-risk) and two risk factors (high-risk). The survival analysis showed that OS overlapped for the low and intermediate subgroups, while a significantly less favorable survival was observed for high-risk patients (Figure S1A; $p < 0.0001$). There was no significant difference in terms of PFS (Figure S1B; $p = 0.230$).

Finally, the analysis where the primary resistance group included patients with PD and SD for less than six months and the DC group included patients with CR, PR, and SD for more than six months

(Figure S2A,B) showed that the difference in terms of OS and PFS remained statistically significant ($p < 0.0001$).

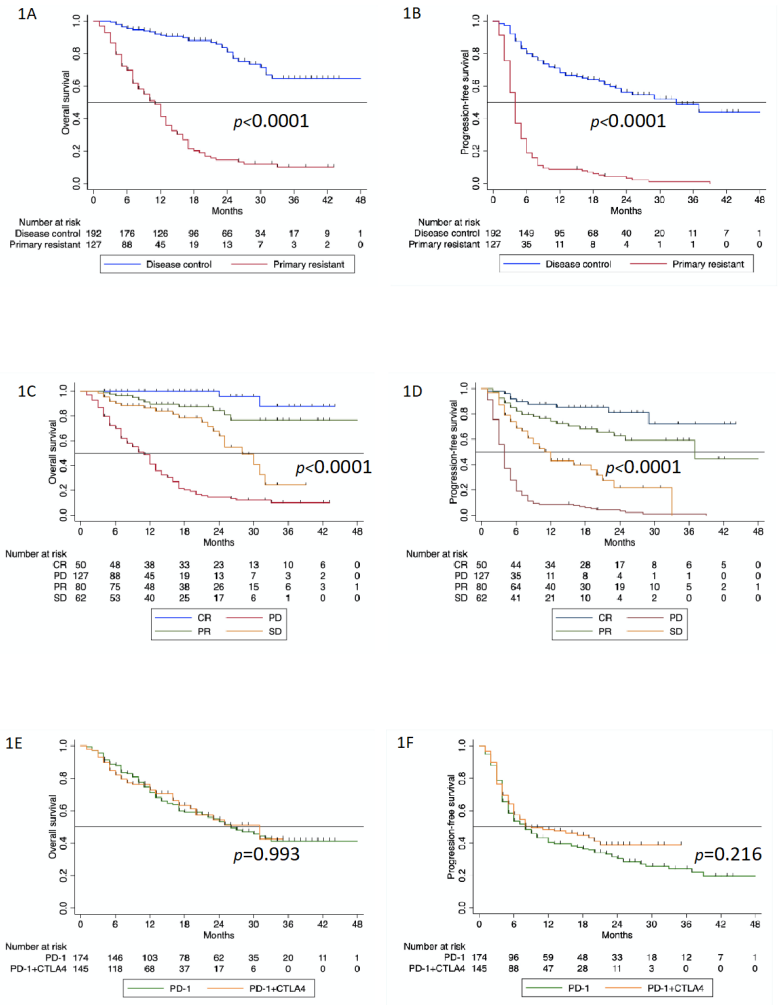

Figure 1. (**A**). Overall survival according to response to first-line PD-1-based immunotherapy ($p < 0.0001$); (**B**). progression-free survival according to response to first-line PD-1-based immunotherapy ($p < 0.0001$); (**C**). overall survival according to best overall response to first-line PD-1-based immunotherapy ($p < 0.0001$); (**D**). progression-free survival according to best overall response to first-line PD-1-based immunotherapy ($p < 0.0001$); (**E**). overall survival according to the type of first-line PD-1-based immunotherapy ($p = 0.993$); (**F**). progression-free survival according to the type of first-line PD-1-based immunotherapy ($p = 0.216$).

3.3. Second-Line Therapies and Outcomes

Tables S3 and S4 show the type of second-line therapy in patients with primary resistance, and also the best overall response achieved, according to the *BRAF* mutation status. Approximately 50% ($n = 63$) of the patients with primary resistance to immunotherapy received a second-line therapy. Sixty-four patients did not receive further systemic therapies. Twenty-one patients had tumors harboring a *BRAFV600E/K* mutation, 20 patients had *BRAF* wild-type tumors, and in 22 patients, there was no information regarding *BRAF* mutation status. The majority of the patients with *BRAFV600E/K* mutation (17/21) received targeted therapy with *BRAF* plus MEK inhibitors, three received immunotherapy, and one patient chemotherapy. Patients with *BRAF* wild-type tumors received in equal number immunotherapy (10/20) and chemotherapy (10/20).

Information on the best overall response for the second-line systemic therapy was available for 58 patients. In five patients, this information was not available. Patients with tumors harboring a *BRAFV600E/K* mutation received predominantly targeted therapy which resulted in a high response rate (CR or PR) of 63%. In patients with *BRAF* wild-type tumors treated either with second-line immunotherapy or chemotherapy, the response rate was only 11% (Table S3).

3.4. Pseudoprogression

In our cohort ($n = 319$), we identified six patients with pseudoprogression. Of these six patients, five showed initial PD but later achieved SD as best overall response to immunotherapy, and in one patient after initial PD, the best overall response to immunotherapy was CR.

4. Discussion

Our study shows that patients with primary resistance and tumor progression at the time of first tumor assessment after starting immunotherapy have a highly significantly unfavorable survival rate as compared to those who achieve disease control. Response at the time of first tumor assessment after starting immunotherapy is a better predictive factor for survival than other pretreatment risk factors for the development of primary resistance. Achieving an objective remission (CR or PR) is decisive for favorable OS. The median OS for patients with SD is significantly better than for patients with primary resistance (28 months versus 11 months). After three years, however, the survival curves converge strongly at an unfavorable level. This convergence is even more pronounced for PFS.

In the multivariate logistic regression analysis, only two significant risk factors for primary resistance to immunotherapy were identified. These were primary tumor localization and an elevated level of protein S-100B. In the univariate analysis, an elevated level of LDH was also a significant factor, but this did not remain significant in the multivariate analysis. The decisive factor here may be that the LDH value and the protein S-100B value usually increase in parallel, and that the S-100B value increases earlier and in more patients.

In the univariate analysis, the histological subtype was also a significant factor. Here there is an overlap with tumor localization, since ALM is more commonly seen in the extremities, and mucosal melanomas were classified in the other localizations group. The higher discriminatory power was observed for the tumor localization. There is also a relationship between tumor localization and sex, as melanomas in the extremities occur more frequently in females, and females have a less favorable response to immunotherapy than males [32–34]. Accordingly, sex was a significant risk factor in the univariate analysis but not in the multivariate analysis.

The definition of risk groups considering the two risk factors that remained significant in the multivariate analysis (i.e., primary tumor localization and protein S-100B) showed a relatively low predictive value. We observed a statistically significant difference in terms of OS between high- and intermediate- and low-risk groups, whereas this difference was not observed in PFS.

The three-year PFS and three-year OS rates reported here for PD-1 monotherapy and the combination of nivolumab plus ipilimumab are lower than those reported in the CheckMate 067

trial [35]. This might be partially explained by the selection of the patients included in that study compared to the unselected population in our cohort. As an example, patients with (active) brain metastases are typically excluded from clinical trials. In fact, only 3.6% of the patients included in the CheckMate 067 trial had brain metastases compared to 19% in our study. In our cohort, 36% of the patients also had liver metastases, which is associated with worse response to immunotherapy [36,37]. The percentage of patients that had elevated LDH is similar in both reports (32% in our cohort vs. 36% in the CheckMate 067 trial). In the CheckMate 067 trial, the S100B levels, which are a known prognostic factor [38,39], were not reported; in our study, 44% of the patients had elevated S100B. Together, these aspects define a collective of patients that probably had a worse prognosis compared to the patients included in the clinical trial.

In our cohort, there was no difference in terms of OS between patients treated with PD-1 monotherapy and those receiving nivolumab plus ipilimumab. This might be related to the median follow-up time of only 22 months, shorter than the last update from the CheckMate 067 trial, where the difference between combined immunotherapy and monotherapy was clearer with a five-year follow-up [4]. In our cohort, a significantly higher proportion of patients with *BRAFV600E/K* mutation received combined immunotherapy ($p = 0.003$). This subgroup seemed to respond better to combined immunotherapy compared to PD-1 monotherapy [4,40], and this might explain why we started to see a separation of the PFS curves. Possibly with a longer follow-up, a difference in OS can be expected.

The absence of difference in terms of OS in these two subgroups is probably also linked to the fact that they were not homogenous, with a selection bias regarding the type of immunotherapy. Older patients, who seem to respond better to immunotherapy [41], received predominantly PD-1 monotherapy ($p = 0.007$), patients with more than three metastatic organs received preferably combined immunotherapy ($p = 0.043$), and a significantly higher proportion of patients with brain metastasis were also treated with nivolumab plus ipilimumab ($p = 0.018$).

Only 50% of the patients with primary resistance to immunotherapy received a second systemic therapy, similar to the percentage of patients reported in other series receiving a second-line therapy [42]. In our cohort, patients with *BRAFV600E/K* mutation received predominantly targeted therapy, and in this subgroup, 63% of patients had a response (CR or PR). This was slightly higher than previously published [43], but in our cohort, only 21 patients with *BRAFV600E/K* mutation received second-line therapy, and therefore the outcomes need to be interpreted cautiously. Nevertheless, our group and others have already demonstrated that patients with *BRAFV600E/K* first-line immunotherapy followed by targeted therapy, similar to what patients in this cohort received, seem to have better outcomes than the inverse sequence [44,45]. The high response rate in our cohort might be explained by this favorable therapy sequencing.

On the other hand, for patients with *BRAF* wild-type tumors, only one patient responded to second-line therapy. Again, the number of patients was low ($n = 20$), but these results show that a second-line therapy in the *BRAF* wild-type cohort is not possible in a high number of patients and, when possible, still has a small impact on survival.

Strengths of this investigation are the fact that the data included was from a German certified skin cancer center with high standards for data quality. Three hundred and nineteen patients were analyzed, which is a large cohort of patients with stage IV melanoma managed with PD-1-based immunotherapy in a routine clinical setting. This high number of patients allowed us to perform subgroup analyses, with results of reasonable sensitivity. Further, this study provides follow-up data covering a period of up to 22 months.

The study limitations are related to its retrospective and monocentric design. Patients included were those receiving first-line immunotherapy for stage IV melanoma and for whom a response to therapy was documented. Since no other selection criteria were applied, the heterogeneity of the study population might have contributed to the differences observed in survival. Another limitation is the absence of histological confirmation of progressive disease in all patients with primary resistance.

This approach is currently changing and, in the future, it would certainly be of value to include other factors in the definition of primary resistance.

5. Conclusions

Patients with progressive disease at the first tumor assessment after starting first-line PD-1-based immunotherapy have a very unfavorable prognosis. Predicting primary resistance based on pre-existing risk characteristics is possible only to a limited extent. Response at time of first tumor assessment after starting immunotherapy is a stronger predictive factor. In future analysis, other factors, namely histological and molecular characterization of the progressive lesions, should be included in the definition of primary resistance.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6694/12/4/1027/s1, Figure S1: (**A**) Overall survival according to risk groups (low, intermediate, and high) ($p < 0.0001$). The factors used were primary tumor localization and protein S-100B level. The subgroups were defined as follows: no risk factor (low), one risk factor (intermediate), and two risk factors (high). (**B**) Progression-free survival according to risk groups (low, intermediate, and high) ($p = 0.230$). The factors used were primary tumor localization and protein S-100B level. The subgroups were defined as follows: no risk factor (low), one risk factor (intermediate), and two risk factors (high); Figure S2: (**A**) Overall survival for the disease control group (complete response, partial response, and stable disease for more than 6 months) and primary resistance (progressive disease and stable disease for less than 6 months). (**B**) Progression-free survival for the disease control group (complete response, partial response, and stable disease for more than 6 months) and primary resistance (progressive disease and stable disease for less than 6 months); Table S1: Patients characteristics and univariate analysis for the whole cohort. In this analysis, the primary resistance group includes progressive disease at the time of first tumor response evaluation after immunotherapy plus stable disease for less than 6 months. The disease control group includes complete response, partial response, and stable disease for longer than 6 months; Table S2: Patients characteristics and univariate analysis for the whole cohort according to type of first-line immunotherapy; Table S3: Second-line therapies in patients with primary resistance considering *BRAF* mutation status; Table S4: Best overall response to second-line therapies in patients with primary resistance considering *BRAF* mutation status.

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3. **"Indirect Comparison of Combined BRAF and MEK Inhibition in Melanoma Patients with Elevated Baseline Lactate Dehydrogenase."** Glutsch, V., **T. Amaral**, C. Garbe, K.-M. Thoms, P. Mohr, A. Hauschild and B. Schilling (2020). Acta dermato-venereologica.

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CLINICAL REPORT

Indirect Comparison of Combined BRAF and MEK Inhibition in Melanoma Patients with Elevated Baseline Lactate Dehydrogenase

Valerie GLUTSCH[1], Teresa AMARAL[2,3], Claus GARBE[2], Kai-Martin THOMS[4], Peter MOHR[5], Axel HAUSCHILD[6] and Bastian SCHILLING[1]

[1]Department of Dermatology, Venereology and Allergology, University Hospital Würzburg, Würzburg, Germany, [2]Center for Dermatooncology, Department of Dermatology, University Medical Center Tübingen, Tübingen, Germany, [3]Health Care Direction, Portuguese Air Force, Lisbon, Portugal, [4]Department of Dermatology, University Medical Center Göttingen, Göttingen, Germany, [5]Department of Dermatology, Skin Cancer Center, Elbekliniken Buxtehude, Buxtehude, Germany, and [6]Department of Dermatology, University Hospital Schleswig-Holstein, Kiel, Germany

The approval of BRAF and MEK inhibitors has significantly improved treatment outcomes for patients with BRAF-mutated metastatic melanoma. The 3 first-line targeted therapy trials have provided similar results, and thus the identification of predictive biomarkers may generate a more precise basis for clinical decision-making. Elevated baseline lactate dehydrogenase (LDH) has already been determined as a strong prognostic factor. Therefore, this indirect analysis compared subgroups with elevated baseline LDH across the pivotal targeted therapy trials co-BRIM, COMBI-v and COLUMBUS part 1. The Bucher method was used to compare progression-free survival, objective response rate and overall survival indirectly. The results show a non-significant risk reduction for progression in the subgroup with elevated baseline LDH receiving vemurafenib plus cobimetinib compared with dabrafenib plus trametinib and encorafenib plus binimetinib. Although an indirect comparison, these data might provide some guidance for treatment recommendations in melanoma patients with elevated LDH.

Key words: melanoma; BRAF; lactate dehydrogenase.

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Corr: Bastian Schilling, Department of Dermatology, Venereology and Allergology, University Hospital Würzburg, Josef-Schneider-Str. 2, DE-97080 Würzburg, Germany. E-mail: schilling_b@ukw.de

SIGNIFICANCE

Targeted therapy has significantly improved the prognosis of patients with BRAF-mutated metastatic melanoma. Since there are 3 different targeted therapy regimes available, the identification of predictive factors may generate a more precise basis for clinical decision-making. An increased level of lactate dehydrogenase has been determined as a strong prognostic factor. Therefore, the aim of this study was to determine if approved targeted therapy regimes differ significantly in terms of efficacy in patients with elevated lactate dehydrogenase. The study used the Bucher method to indirectly compare the outcome of melanoma patients with elevated lactate dehydrogenase across the pivotal trials co-BRIM, COMBI-v and COLUMBUS part 1.

Small molecule BRAF and MEK inhibitors have clearly improved the prognosis for patients with BRAF mutant metastatic melanoma (1–3). In the coBRIM (NCT01271803) and COMBI-v trials (NCT01597908), combined BRAF and MEK inhibition with vemurafenib plus cobimetinib (coBRIM) or dabrafenib plus trametinib (COMBI-v) improved progression-free survival (PFS), overall response rate (ORR) and overall survival (OS) compared with vemurafenib monotherapy in BRAF-V600-mutated metastatic melanoma. A third trial (COLUMBUS part 1, NCT01909453) compared combined encorafenib plus binimetinib with vemurafenib monotherapy, and also demonstrated an advantage for PFS and ORR in the combination arm (3). While these 3 trials have provided similar results in terms of efficacy in treatment-naive patients with BRAF-V600-mutated metastatic melanoma, the identification of predictive biomarkers may generate a more precise basis for clinical decision-making and patient management.

Well-accepted prognostic factors in patients with metastatic melanoma include disease stage, baseline Eastern Cooperative Oncology Group performance status (ECOG PS) and baseline lactate dehydrogenase levels (LDH) (4, 5). In particular, an elevated baseline level of LDH has been determined as a strong negative prognostic factor in patients with advanced melanoma (6–8), now incorporated into the AJCC staging system as an independent factor (9). LDH is a ubiquitous enzyme that plays a key role in cell metabolism and growth. By catalysing the reduction of pyruvate to lactate, the so-called Warburg effect, LDH creates an acidic milieu that is favourable for tumour angiogenesis and suppression of anti-tumour immune responses (10). In prospective clinical trials evaluating dual MAPKi, an elevated LDH (defined as > local upper limit of normal (ULN)) predicted inferior outcome compared with patients without elevated LDH. The extent of this association was different in coBRIM, COMBI-v and COLUMBUS part 1. However, the clinical significance of these differences has not been analysed comprehensively. To this end, this study conducted an indirect analysis (1) to compare PFS, ORR and OS in the subgroups with elevated baseline LDH from the clinical trials coBRIM, COMBI-v and COLUMBUS part 1 and consequently (2) to interrogate if a particular

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regime in this particular subgroup might provide greater benefit to patients.

MATERIALS AND METHODS

Detailed methods of the particular trials have already been reported (1–3). Briefly, coBRIM, COMBI-v and COLUMBUS part 1 were randomized, double-blind phase 3 trials comparing oral vemurafenib, 960 mg twice daily, plus cobimetinib, 60 mg once daily, for 21 days with placebo and vemurafenib (coBRIM), oral dabrafenib, 150 mg twice daily, plus trametinib, 2 mg once daily, with vemurafenib, 960 mg twice daily, (COMBI-v) or oral encorafenib, 450 mg once daily, plus binimetinib, 45 mg twice daily, with vemurafenib, 960 mg twice daily, or encorafenib, 300 mg once daily, (COLUMBUS part 1). Baseline patient characteristics are summarized in **Table I**. The primary endpoint of coBRIM and COLUMBUS trial was PFS. Primary endpoint of COMBI-v was OS. Key inclusion criteria were comparable across the studies including patients with unresectable stage III or stage IV BRAF-V600-mutated melanoma, adequate organ functions and ECOG PS 0 or 1. Patients with untreated brain metastases were not eligible.

In this analysis the subgroups with normal and elevated LDH have been statistically analysed using a model for making indirect comparisons of the magnitude of treatment effects without losing the power of randomization (Bucher analysis) (11).

Statistical analysis

The aim of this analysis was the indirect comparison of PFS and OS as well as ORR in the subgroups with elevated LDH levels using the Bucher method. For our analysis, data cut-off dates of 9 May 2014 (PFS), 16 January 2015 (ORR) and 28 August 2015 (OS) for coBRIM; 17 April 2014 (PFS, ORR) and 13 March 2015 (OS) for COMBI-v and 19 May 2016 (PFS) and 7 November 2017 (OS) for COLUMBUS part 1 were used. Due to data availability, the PFS analysis comparing coBRIM with COMBI-v was done using local assessment data, while the comparison with COLUMBUS part 1 used data from the independent central review. As defined by the particular study protocol, all enrolled patients were included in the analysis. The Bucher analysis was based on the assessments of benefit of the Federal Joint Committee (G-BA) for vemurafenib plus cobimetinib (module 5), dabrafenib plus trametinib (module 4) and encorafenib plus binimetinib (module 4) as well as data from Dummer et al. (3). Median OS and PFS were calculated using the Kaplan–Meier method.

RESULTS

Patients' characteristics

Baseline patients' characteristics of the 3 studies are summarized in Table I. The percentage of patients with elevated baseline levels of LDH was higher in the coBRIM cohort being treated with vemurafenib plus cobimetinib ($n = 112$, 46%) than in the combination arms of the COMBI-v ($n = 118$, 34%) and the COLUMBUS ($n = 55$, 29%) trials (χ^2, $p < 0.001$). Other prognostic factors, such as ECOG PS and number of patients with M1c disease according to the AJCC 2009 staging system, were comparable across the 3 trials (Table I) (2, 12, 13). Of note, there was a low number of patients with first-line modern immunotherapy in the COLUMBUS part 1 trial (encorafenib + binimetinib group: 7 (4%) ipilimumab, 1 (1%) anti-PD-1 or anti-PD-L1; vemurafenib group: 7 (4%) ipilimumab, 0 (0%) anti-PD-1 or anti-PD-L1).

Progression-free survival

Median PFS in the combination arms was 12.3 months for vemurafenib and cobimetinib (coBRIM, data cut-off 16 January, 2015, median follow-up 14.2 months), 11.4 months for dabrafenib and trametinib (COMBI-v, data cut-off 17 April, 2014, median follow-up 10 months) and 14.9 months for encorafenib and binimetinib (COLUMBUS part 1, data cut-off 19 May, 2016, median follow-up 14.4 months) (1–3). In all 3 studies median PFS was significantly longer in the combination arms than in those treated with vemurafenib monotherapy. Table I shows the corresponding hazard ratios (HR) for progression or death comparing vemurafenib and dual MAPKi regimes.

Table I. Patient characteristics

	coBRIM		COMBI-v		Columbus Part 1		
Therapy	V + C	V + P	D + T	V	E+B	E	V
Patients (*n*)	247	248	352	352	192	194	191
Primary endpoints	PFS		OS		PFS (E+B vs. V)		
Secondary endpoints	OS, ORR, DoR, PFS		PFS, ORR, DoR, S		PFS (E+B vs. E), BOR, DoR, S, etc.		
Median age, years	56	55	55	54	57	54	56
LDH ≥ ULN, %	46	43	34	32	29	24	27
ECOG PS, %							
0	76	67	71	70	71	72	73
1	24	33	29	30	29	28	27
M1c, %	59	62	63	59	64	62	65
Disease sites, ≥ 3, *n*	–		50	43	45	44	46
mPFS, months	12.3	7.2	11.4	7.3	14.9	9.6	7.3
HR (95% CI)	0.58 (0.46–0.72)		0.56 (0.46–0.69)		0.54 (0.41–0.71)[a]		
ORR, %	70	50	64	51	63	51	40
mOS, months	22.3	17.4	nr	17.2	–	–	–
HR (95% CI)	0.70 (0.55–0.90)		–		–		

[a]Hazard ratio (HR) for encorafenib (E)+ binimetinib (B) vs. vemurafenib (V).
C; cobimetinib; P; placebo; D; dabrafenib; T; trametinib; PFS; progression-free survival; OS; overall survival; ORR; objective response rate; DoR; duration of response; S; safety; LDH; lactate dehydrogenase; ULN; upper limit of normal; ECPG PS; Eastern Cooperative Oncology Group performance status; mPFS; median progression-free survival; CI; confidence interval; mOS; median overall survival.

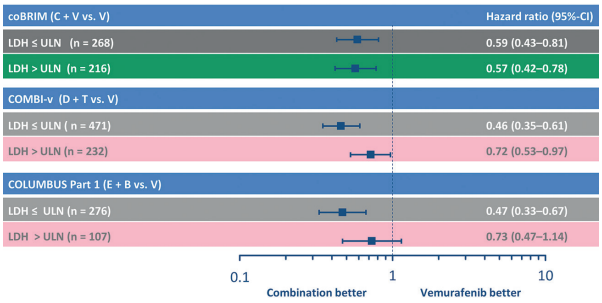

Fig. 1. Progression-free survival (PFS) subgroup analyses for lactate dehydrogenase (LDH) for coBRIM (data cut-off 16 January 2015), COMBI-v (data cut-off 17 April 2014) and COLUMBUS Part 1 (data cut-off 19 May 2016). C: cobimetinib; V: vemurafenib; D: dabrafenib; T: trametinib; E: encorafenib; B: binimetinib; CI: confidence interval; ULN: upper limit of normal (references 1–3, 14 and G-BA-Bericht: Dossier zur Nutzenbewertung von Dabrafenib. Stand 16.09.2015).

Across the 3 trials, the subgroups with normal baseline level of LDH clearly benefited from the combination therapy (**Fig. 1**), whereas only the coBRIM trial could show an equal advantage for the subgroup with elevated baseline levels of LDH (HR 0.57 (95% confidence interval (95% CI), 0.42–0.78), data cut-off 16 January 2015) (Fig. 1) (1). Kaplan–Meier estimates of PFS for the subgroups with elevated LDH derived from coBRIM and COMBI-v are shown in **Fig. 2**A.

Using the Bucher method, an indirect comparison of PFS, OS and ORR data from coBRIM, COMBI-v and COLUMBUS part 1 was performed. In this indirect comparison, a non-significant risk reduction for progression or death for patients with elevated baseline LDH receiving vemurafenib plus cobimetinib (data cut-off 9 May 2014) was found. When compared with dabrafenib plus trametinib (data cut-off 17 April 2014), a 24% risk reduction (HR 0.76 (95% CI, 0.48–1.23)) and compared with encorafenib plus binimetinib (data cut-off 19 May 2016), an 11% risk reduction (HR 0.89 (95% CI, 0.50–1.58)) was observed (**Table II**).

Overall response rate

The objective response rate was 70% (172/247 patients) in the vemurafenib plus cobimetinib group vs. 50% (124/248 patients) in the vemurafenib plus placebo group (coBRIM, data cut-off 16 January 2015); 64% (226/351 patients) in the dabrafenib plus trametinib group vs. 51% (180/350 patients) in the vemurafenib monotherapy group (COMBI-v, data cut-off 17 April 2014) and 63% (121/192 patients) in the encorafenib plus binimetinib group vs. 40% (77/191 patients) in the vemurafenib monotherapy groups (COLUMBUS part 1, data cut-off 19 May 2016) (1–3). The indirect comparison showed an advantage for vemurafenib plus cobimetinib (data cut-off 16 January

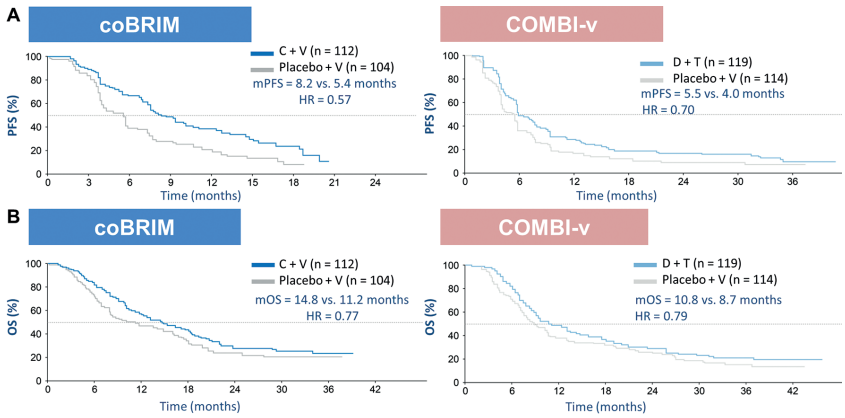

Fig. 2. Kaplan–Meier estimates for (A) median progression-free survival (mPFS) and (B) median overall survival (mOS) in the subgroups with elevated lactate dehydrogenase (LDH) for coBRIM (data cut-off 16 January 2015 for PFS and 28 August 2015 for OS) and COMBI-v (data cut-off 17 April 2014 for PFS and 13 March 2015 for OS). C: cobimetinib; V: vemurafenib; D: dabrafenib; T, trametinib; HR: hazard ratio (reference 1, and Robert C. ESMO 2016. Oral presentation).

Table II. Bucher analysis for the subgroups with elevated lactate dehydrogenase (LDH)

	coBRIM V+C vs. V HR (95% CI)	COMBI-v D+T vs. V HR (95% CI)	V+C vs. D+T HR (95% CI)	coBRIM V+C vs. V HR (95% CI)	COLUMBUS E+B vs. V HR (95% CI)	V+C vs. E+B HR (95% CI)
mPFS	0.55 (0.38; 0.79)	0.72 (0.53; 0.97)	0.76 (0.48; 1.23)	0.65 (0.45; 0.94)	0.73 (0.47; 1.14)	0.89 (0.50; 1.58)
ORR	1.81 (1.34; 2.44)	1.34 (0.99; 1.83)	1.35 (0.88; 2.07)	–	–	–
mOS	0.77 (0.56; 1.07)	0.81 (0.59; 1.10)	0.95 (0.61; 1.49)	0.77 (0.56; 1.07)	0.95 (0.63; 1.43)	0.81 (0.48; 1.37)

V: vemurafenib; C: cobimetinib; D: dabrafenib; T: trametinib; E: encorafenib; B: binimetinib; mPFS: median progression-free survival; ORR: objective response rate; mOS: median overall survival; HR: hazard ratio; CI: confidence interval; mOS: median overall survival.

2015) compared with dabrafenib plus trametinib (data cut-off 17 April 2014) in the subgroup with elevated baseline levels of LDH (HR 1.35 (95% CI, 0.88–2.07). ORR data for COLUMBUS were not available.

Overall survival

Median OS in the combination arms was 22.3 months for vemurafenib and cobimetinib (coBRIM, data cut-off 28 August 2015) and was not reached for dabrafenib and trametinib (COMBI-v, data cut-off 17 April 2014) (2) and encorafenib and binimetinib (COLUMBUS, data cut-off 19 May 2016) (3). In updated analyses, the OS was significantly longer in the combination arms compared with BRAF inhibitor monotherapy across all 3 trials (**Fig. 3**) (14, 15). Fig. 2B shows OS survival curves for coBRIM and COMBI-v for the subgroups with elevated LDH. For COLUMBUS, such OS data from patients with elevated LDH were not available.

In the subgroup of patients with elevated baseline levels of LDH, the indirect comparison showed a similar risk of death for patients receiving vemurafenib plus cobimetinib (data cut-off 28 August 2015) or dabrafenib plus trametinib (data cut-off 13 March 2015) (HR 0.95 (95% CI, 0.61–1.49)) (Table II). A slight and non-significant risk reduction for death by 19% was found when coBRIM was compared with COLUMBUS part 1 (data cut-off 7 November 2017) (HR 0.81 (95% CI, 0.48–1.37)) (Table II).

DISCUSSION

This study undertook an indirect analysis in BRAF-V600 mutated patients treated with combined BRAF and MEK inhibition with elevated baseline levels of LDH. In pooled analyses reported recently, baseline levels of LDH, ECOG PS, number of involved organ systems and baseline sum of longest diameter of target lesions (SLDs) were identified as key predictive factors for PFS and OS in BRAF-V600-mutated patients treated with combined BRAF and MEK inhibition (6–8). In particular, baseline LDH level was the strongest predictive factor across all 3 trials. Therefore, our analysis focused on the comparison of patients with elevated baseline LDH level to further investigate PFS, ORR and OS comprehensively across the 3 first-line targeted therapy (TT) trials in this particular subgroup.

Two major clinical decisions need to be addressed in patients with advanced BRAF-mutant melanoma. Firstly, patients can either receive an anti-PD-1 based immunotherapy or dual MAPKi. Since no prospective head-to-head data are available, this decision is based mainly on patient characteristics and preference as well as the physicians' preference. Looking at data from a survey conducted in melanoma experts, symptomatic disease, a high tumour burden and elevated baseline LDH are features associated with using dual MAPKi as first-line treatment (16). Recently, we were able to show this association in a retrospective study (17). When comparing consecutive patients receiving either dual MAPKi ($n=195$) or PD-1 monotherapy ($n=106$), the TT cohort was significantly enriched with patients showing non-pulmonary visceral metastases and an elevated LDH. Secondly, the specific regime needs to be chosen. If dual MAPKi is recommended, 3 different combinations are approved by FDA and EMA. In terms of overall efficacy, no regime seems to be superior to the others. However,

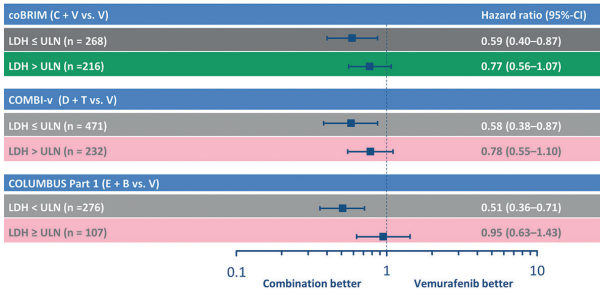

Fig. 3. Overall survival (OS) subgroup analyses for lactate dehydrogenase (LDH) for coBRIM (data cut-off 28 August 2015), COMBI-v (data cut-off 17 April 2014) and COLUMBUS Part 1 (data cut-off 17 November 2017). C: cobimetinib; V: vemurafenib; D: dabrafenib; T: trametinib; E: encorafenib; B: binimetinib; CI: confidence interval; ULN: upper limit of normal. (1–3, 14 and G-BA-Bericht: Dossier zur Nutzenbewertung von Dabrafenib. Stand 16.09.2015).

a given combination might provide greater benefit in a particular subgroup of patients.

Although indirect comparisons of pivotal trials warrant great caution, the usage of vemurafenib as comparator coBRIM, COMBI-v and COLUMBUS part 1 allows to perform a Bucher analysis (18). Looking at the vemurafenib monotherapy arms, results indicate similar response to treatment and prognosis due to resemblance across multiple endpoints (e.g. PFS and OS) in the respective vemurafenib groups (1, 3, 17). Prognostic factors, such as ECOG PS and degree of organ involvement, were comparable across the trials (Table I) (2, 12, 13). However, the coBRIM trial included the highest percentage of patients with elevated baseline levels of LDH in the combination arm (46%) compared with only 34% in the COMBI-v and 29% in the COLUMBUS trial (1–3). In real-world datasets, up to 48% of patients receiving dual MAPKi first-line showed an elevated LDH resembling the coBRIM cohort (19, 20). Although an elevated baseline LDH accounts for a worse outcome, the HR for progression or death in the total trial populations was comparable across the 3 trials (Table I). This indicated an advantage of vemurafenib plus cobimetinib in the subgroup with elevated baseline LDH. The coBRIM data confirmed this hypothesis regarding a PFS advantage of combined BRAF and MEK inhibition with vemurafenib plus cobimetinib compared with a vemurafenib monotherapy independent of the baseline LDH level (Fig. 1). Consequently, BRAF-V600 mutated patients with an elevated baseline LDH level might benefit from a combined TT with vemurafenib and cobimetinib to a similar extend as patients with normal LDH do when compared with vemurafenib monotherapy. This could not be demonstrated for dabrafenib plus trametinib or encorafenib plus binimetinib (Fig. 1). The Bucher analysis confirmed these findings showing a non-significant advantage for vemurafenib plus cobimetinib in the subgroup with elevated baseline LDH compared with dabrafenib plus trametinib and encorafenib plus binimetinib regarding PFS (Table II). Although this retrospective indirect Bucher analysis does not allow an exclusion of all selection bias, our results indicate that the LDH level should be considered when choosing a specific BRAF and MEK inhibitor to achieve disease control.

In contrast to the PFS data our analysis did not affirm a beneficial effect of vemurafenib plus cobimetinib compared with dabrafenib and trametinib, and showed only a slight advantage compared with encorafenib and binimetinib regarding OS. Likewise, the coBRIM data do not provide an OS advantage for combined TT compared with vemurafenib monotherapy. Therefore, the choice of a particular BRAF and MEK inhibitor combination seems to have no impact on OS. However, when interpreting efficacy results, such as OS, prior and subsequent treatment regimens, such as immunotherapies, as well as prognostic factors apart from LDH have to be taken into consideration, creating a potential bias.

Besides efficacy, safety and tolerability are of high clinical relevance and have an impact on treatment recommendations. Distinct patterns of treatment-related adverse events can be found in melanoma patients receiving dabrafenib + trametinib, vemurafenib + cobimetinib or encorafenib + binimetinib. Pyrexia is most frequently observed in patients receiving dabrafenib + trametinib, while vemurafenib + cobimetinib causes the highest number of cutaneous adverse events (AEs), and encorafenib + binimetinib leads to more nausea and constipation than the other combinations (1–3). In an indirect comparison similar to ours, a lower incidence of treatment-related AEs was found for dabrafenib + trametinib compared with vemurafenib + cobimetinib (18). However, when looking at any AE, serious AEs or AEs leading to treatment discontinuation, no differences were observed. Regarding OS and PFS (dabrafenib + trametinib vs. vemurafenib + cobimetinib), Daud et al. calculated a HR of 0.94 and 1.05, respectively, when applying the Bucher method. In contrast to our indirect comparison, earlier data cuts were used and most importantly, the total patient populations of the combination arms were analysed.

We cannot provide any data explaining the differences observed. Lactate accumulating in the tumour microenvironment might cause acidification, decreasing the pH (19). Since it is known that bioavailability of dabrafenib is dependent on pH, while that of vemurafenib is not (20), our hypothesis is that antineoplastic activity of dabrafenib, but not vemurafenib, is pH dependent. Experimental and pharmacokinetic data are needed to test this hypothesis.

Elevated LDH is a very important biomarker in advanced melanoma, and has been incorporated into the AJCC Melanoma Staging system since 2009 (5). Three recent pooled analyses confirmed an elevated LDH as predictive factor for shorter PFS and OS in melanoma patients receiving combined TT (6–8). In the real-world setting, melanoma patients receiving palliative MAPKi first-line have poor prognostic features, including, but not limited to, elevated LDH (21, 22). There might be other subgroups in which a particular treatment regime might tend to be superior to the others. However, taking other biomarkers, such as involvement of particular organs or the sum of lesions diameters, into account was not possible. Patient cohorts are slightly heterogeneous across the 3 trials and more importantly, the way the trials are reported limits the availability of data for comparisons.

In conclusion, there is no statistically significant difference in efficacy between the 3 TT couples using the Bucher method. However, our data indicate a trend towards a lower risk for progression or death in melanoma patients with elevated LDH when receiving vemurafenib + cobimetinib in comparison with dabrafenib + trametinib and encorafenib + binimetinib as first-line therapy. In light of the current preference to use dual MAPKi instead of immune-checkpoint blockade in pa-

tients with poor prognostic features including elevated LDH, our indirect analysis might provide a rationale to use a specific treatment regime. However, this finding needs to be validated prospectively. Although a Bucher analysis partially retains the randomization of the individual trials, data provided by an indirect comparison must be interpreted with caution.

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Conflicts of interest. VG has received honoraria from Bristol-Myers Squibb (BMS) and reports travel support from Novartis, Pierre Fabre Pharmaceuticals, BMS, Merck Sharp & Dohme (MSD) and Sanofi Genzyme; outside the submitted work. TA reports travel support from Novartis, personal fees and travel support from BMS, outside the submitted work. CG reports grants and personal fees from Novartis, personal fees from Pierre Fabre, grants and personal fees from Roche, during the conduct of the study; personal fees from Amgen, grants and personal fees from BMS, personal fees from MSD, grants and personal fees from Neracare, personal fees from Philogen, personal fees from Sanofi, outside the submitted work. K-MT reports advisory roles for or has received honoraria from Roche, Novartis, Pierre Fabre, BMS, MSD and LEO; travel support from Roche, Novartis, Pierre Fabre, BMS and LEO; outside the submitted work. PM reports personal fees, non-financial support and other from Pierre Fabre, GSK, MSD, Merk Germany, Roche, BMS, Novartis and Sanofi; outside the submitted work. AH has received clinical trial support from Amgen, BMS, Merck Serono, MSD, Novartis, Philogen, Pierre Fabre, Provectus, Regeneron and Roche and honoraria or consultancy fees from Amgen, BMS, Merck Serono, MSD, Novartis, OncoSec, Philogen, Pierre Fabre, Provectus, Regeneron and Roche. BS reports advisory roles for or has received honoraria from Pierre Fabre Pharmaceuticals, Incyte, Novartis, Roche, BMS and MSD, research funding from BMS, Pierre Fabre Pharmaceuticals and MSD, and travel support from Novartis, Roche, BMS, Pierre Fabre Pharmaceuticals and Amgen; outside the submitted work.

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4. **"Combined immune checkpoint blockade for metastatic uveal melanoma: a retrospective, multi-center study."** Heppt, M. V., **T. Amaral,** K. C. Kähler, L. Heinzerling, J. C. Hassel, M. Meissner, N. Kreuzberg, C. Loquai, L. Reinhardt, J. Utikal, E. Dabrowski, A. Gesierich, C. Pföhler, P. Terheyden, K.-M. Thoms, L. Zimmer, T. K. Eigentler, M. C. Kirchberger, H. M. Stege, F. Meier, M. Schlaak and C. Berking (2019). Journal for ImmunoTherapy of Cancer **7**(1): 299.

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RESEARCH ARTICLE Open Access

Combined immune checkpoint blockade for metastatic uveal melanoma: a retrospective, multi-center study

Markus V. Heppt[1,2], Teresa Amaral[3,4], Katharina C. Kähler[5], Lucie Heinzerling[2], Jessica C. Hassel[6], Markus Meissner[7], Nicole Kreuzberg[8], Carmen Loquai[9], Lydia Reinhardt[10], Jochen Utikal[11], Evelyn Dabrowski[12], Anja Gesierich[13], Claudia Pföhler[14], Patrick Terheyden[15], Kai-Martin Thoms[16], Lisa Zimmer[17], Thomas K. Eigentler[3], Michael C. Kirchberger[2], Henner M. Stege[9], Friedegund Meier[10], Max Schlaak[1] and Carola Berking[1,2*]

Abstract

Background: Uveal melanoma (UM) is highly refractory to treatment with dismal prognosis in advanced stages. The value of the combined checkpoint blockade with CTLA-4 and PD-1 inhibition in metastatic UM is currently unclear.

Methods: Patients with metastatic or unresectable UM treated with ipilimumab in combination with a PD-1 inhibitor were collected from 16 German skin cancer centers. Patient records of 64 cases were analyzed for response, progression-free survival (PFS), overall survival (OS), and safety. Clinical parameters and serum biomarkers associated with OS and treatment response were determined with Cox regression modelling and logistic regression.

Results: The best overall response rate to combined checkpoint blockade was 15.6% with 3.1 and 12.5% complete and partial response, respectively. The median duration of response was 25.5 months (range 9.0–65.0). Stable disease was achieved in 21.9%, resulting in a disease control rate of 37.5% with a median duration of the clinical benefit of 28.0 months (range 7.0–65.0). The median PFS was 3.0 months (95% CI 2.4–3.6). The median OS was estimated to 16.1 months (95% CI 12.9–19.3). Regarding safety, 39.1% of treated patients experienced a severe, treatment-related adverse event according to the CTCAE criteria (grade 3: 37.5%; grade 4: 1.6%). The most common toxicities were colitis (20.3%), hepatitis (20.3%), thyreoiditis (15.6%), and hypophysitis (7.8%). A poor ECOG performance status was an independent risk factor for decreased OS ($p = 0.007$).

Conclusions: The tolerability of the combined checkpoint blockade in UM may possibly be better than in trials on cutaneous melanoma. This study implies that combined checkpoint blockade represents the hitherto most effective treatment option available for metastatic UM available outside of clinical trials.

Keywords: Ipilimumab, Nivolumab, Combined immune checkpoint blockade, Uveal melanoma, Biomarker

* Correspondence: Carola.Berking@uk-erlangen.de
[1]Department of Dermatology and Allergy, Munich University Hospital (LMU), Frauenlobstr. 9-11, 80337 Munich, Germany
[2]Department of Dermatology, University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Ulmenweg 18, 91054 Erlangen, Germany
Full list of author information is available at the end of the article

Background

Uveal melanoma (UM) is a malignant tumor of the eye that originates from the pigment cells of the choroid layer or the ciliary body which is clinically and biologically distinct from cutaneous melanoma. Although the incidence is much lower than that of cutaneous melanoma, UM belongs to the most common malignant intraocular tumors in adults [1]. In approximately 50% of all cases, patients develop distant metastasis during the course of the disease, which affects predominantly the liver. Clinical risk factors for metastases are posterior localization in the eye, tumor size of more than 10 mm, and presence of vascular loops. Molecular biomarkers associated with a higher risk of metastasis are monosomy 3 or genomic alterations of BAP-1 [2]. Once distant metastases have occurred, the prognosis is dismal with an average survival time of approximately 1 year across all therapeutic regimens [3].

Patients with metastatic UM have so far benefited little or not at all from the treatment innovations achieved in cutaneous melanoma in recent years. Neither targeted therapy with MEK inhibitors nor checkpoint blockade with ipilimumab or PD-1 inhibitors as monotherapy was able to significantly improve the prognosis of patients with UM [4, 5]. The response rates were consistently in the single-digit percentage range in a panel of previous studies [6–9]. In cutaneous melanoma, combined checkpoint blockade with ipilimumab and nivolumab revealed response rates and survival outcomes superior to PD-1 inhibitor monotherapy, albeit at the cost of high immune-related toxicity [10]. However, the significance of combined checkpoint blockade in UM is unclear and has only been investigated in case reports and small case series [6, 11, 12]. In this study, we evaluate the clinical course of 64 patients with metastatic UM who received combined checkpoint blockade. We report clinical outcomes with respect to response, survival, and adverse events (AE). Furthermore, clinical and laboratory parameters were investigated which may have prognostic value in UM patients treated with checkpoint blockade.

Patients and methods

Patient population and study approval

This study was designed as a retrospective multi-center explorative analysis. Patients were included if they had a diagnosis of stage IV UM and received combined checkpoint blockade of ipilimumab with a PD-1 inhibitor in any treatment line. A follow-up period of at least 3 months was required. The clinical data of 64 patients from 16 German skin cancer centers who met the inclusion criteria were investigated. The cases were collected from June 23, 2018 to October 4, 2019. Clinical data and the treatment outcomes of interest were extracted from the original patient records and merged into a central database prior to analysis. This study was approved by the institutional review board of the medical faculty of the Munich University Hospital (approval number 413–16 UE) and was conducted in accordance with the principles of the Helsinki Declaration in its current version.

Data collection and treatment outcomes

The clinical data recorded at baseline prior to immunotherapy comprised demographics with Eastern Cooperative Oncology Group (ECOG) performance status, available information on the genotype, sites of metastasis, number of organ systems affected by metastases, and previous antineoplastic therapies. As potential serum biomarkers, lactate dehydrogenase (LDH), C-reactive protein (CRP), and the relative counts of lymphocytes (RLC), neutrophils (RNC), and eosinophils (REC) were specifically collected from patient charts and analyzed for their prognostic value [13, 14].

Combined checkpoint blockade was carried out using different treatment schedules (Table 1). Ipilimumab was given at either 3 mg/kg or 1 mg/kg body weight for up to 4 treatment cycles. Nivolumab was applied at 1 mg/kg together with ipilimumab, followed by 3 mg/kg every 2 weeks (Q2W) as maintenance therapy. Treatment with pembrolizumab was applied every 3 weeks (Q3W) at 2 mg/kg. Patients were treated until disease progression or until the development of unacceptable toxicity. AE were retrospectively graded by the site investigators based on the patient records and clinical outcomes according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 published by the National Institutes of Health in 2017. Immune-related adverse events were managed according to pertinent guidelines and algorithms that were previously published [15, 16]. Besides, fatal adverse events and events leading to permanent discontinuation of treatment were specifically recorded and evaluated. The best radiologic response to treatment was assessed by the site investigators and indicated as complete response, partial response, stable disease, or progressive disease based on the RECIST criteria version 1.1 [17]. Complete response and partial response were summarized as best overall response rate (ORR). Complete response, partial response, and stable disease were summarized as disease control rate (DCR).

Statistical analyses

Overall survival (OS) and progression-free survival (PFS) were calculated as the time from the initiation of the first cycle of combined checkpoint blockade until melanoma-specific or treatment-related death and disease progression, respectively. Time-to-event analyses were calculated where death or progression were considered as events. If neither occurred or if patients were lost to follow-up, the date of the last documented presentation was used as a

Table 1 Baseline characteristics of the patient population

	Patient population *n* = 64 (100%)
Gender	
Male	33 (51.6)
Female	31 (48.4)
Age	
< 60 years	28 (43.8)
≥ 60 years	36 (56.2)
GNAQ	
Mutated	8 (12.5)
Wildtype	8 (12.5)
Unknown	48 (75.0)
GNA11	
Mutated	10 (15.6)
Wildtype	5 (7.8)
Unknown	49 (76.6)
ECOG status	
0	49 (76.6)
1	11 (17.2)
2	1 (1.6)
3	1 (1.6)
Unknown	2 (3.1)
Serum LDH	
Normal	28 (43.8)
Elevated (>ULN)	33 (51.6)
Unknown	3 (4.7)
Previous systemic therapies	
0	50 (78.1)
1	12 (18.8)
≥ 2	2 (3.1)
Previous ipilimumab monotherapy	2 (3.1)
Previous PD-1 inhibitor monotherapy	12 (18.8)
Liver-directed therapies	
0	33 (51.6)
1	30 (46.9)
≥ 2	1 (1.6)
Metastatic sites[a]	
Liver	58 (90.6)
Lung	23 (35.9)
Bone	17 (26.6)
Lymph nodes	12 (18.8)
CNS	4 (6.3)
Treatment regimen	
Ipilimumab 3 mg/kg + nivolumab 1 mg/kg Q3W, followed by nivolumab 3 mg/kg Q2W	59 (92.2%)
Ipilimumab 1 mg/kg + pembrolizumab 2 mg/kg Q3W, followed by pembrolizumab 2 mg/kg Q3W	5 (7.8%)

[a]Multiple metastatic sites per patient were possible (values do not sum up to 100%); *abbreviations*: *CNS* Central nervous system, *Q2W* Every two weeks, *Q3W* Every three weeks

censored observation. The survival and progression probabilities were indicated with the Kaplan-Meier method for censored failure time data assuming proportional hazards. The survival curves were compared with the log-rank test [6]. The duration of the clinical response and clinical benefit was defined as time from treatment initiation to progressive disease if a response or stable disease was achieved, respectively. The time to response was defined as time from treatment start until a response was evident radiologically.

Cox proportional hazards regression modelling was applied to investigate the relationship of clinical risk factors and serum biomarkers with OS. Cox regression was performed as a univariate and multivariate analysis in a stepwise approach [6]. Imputation of missing data was not allowed and patients with missing values of a given parameter were excluded from the analysis. Hazard ratios (HR) with 95% confidence intervals (CI) were calculated to quantify the impact on survival. *P*-values were calculated based on Wald statistics [6]. The association of treatment response as a categorical variable with clinical characteristics or serum biomarkers was investigated with the Chi-square test and logistic regression, as appropriate. In all cases, two-tailed *p*-values were calculated and considered significant with values $p < 0.05$. All analyses were carried out with SPSS statistics version 23.0 (IBM) or GraphPad Prism version 5.01 (GraphPad Software).

Results

A total of 64 (100%) patients with metastatic UM were included. Fifty patients (78.1%) were naïve to systemic treatment and received combined checkpoint blockade as first-line systemic therapy. Regarding genotype, the presence of monosomy 3 as risk factor was specifically investigated in 7 patients and identified in 2 of them. BRAF, NRAS and KIT were analyzed and reportedly wildtype as expected in 30, 22, and 20 patients, respectively. Mutations and inactivations of MBD4 which were previously linked to a hypermutator profile with high sensitivity to PD-1 inhibition were not investigated in any case [18, 19].

Previous ipilimumab and PD-1 inhibitor monotherapy were applied in 2 (3.1%) and 12 (18.8%) cases, respectively. Both patients treated with ipilimumab before showed PD. Specifically, 4 patients (6.3%) had received nivolumab and 8 (12.5%) pembrolizumab before. In 4 cases, SD was achieved while 8 patients showed PD upon PD-1 inhibitor monotherapy. The median duration of the clinical benefit was 6.5 months in the 4 patients with SD. Liver-directed therapies were reported in 31 patients (48.4%). Most patients had an ECOG status of 0 ($n = 49$, 76.6%). Serum LDH was elevated in 33 cases (51.6%) at baseline. Other baseline characteristics are listed in detail in Table 1. Ipilimumab plus nivolumab was given in 59 patients (92.2%), while 5 patients (7.8%) received ipilimumab plus pembrolizumab. The median number of treatment cycles was 3 (range 1–4) for the combination of ipilimumab with a PD-1 inhibitor in the induction phase, and 0 (range 0–27) for PD-1 inhibitor maintenance therapy in the overall population. A total of 19 patients (29.7%) received a PD-1 inhibitor maintenance therapy. Among these, the median number of PD-1 inhibitor cycles was 3 (range 1–27).

The best ORR to combined checkpoint blockade was 15.6% ($n = 10$) relating to the entire population (4 patients were not evaluable for a radiologic response). Two patients achieved a complete response (3.1%) and 8 (12.5%) a partial response. The median duration of response was 25.5 months (range 9.0–65.0). Stable disease was achieved in further 14 cases (21.9%), resulting in a disease control rate of 37.5% with a median duration of the clinical benefit of 28.0 months (range 7.0–65.0) (Table 2). The median PFS was 3.0 months (95% CI 2.4–3.6). The median OS was estimated to 16.1 months (95% CI 12.9–19.3) with a median follow-up period of 9.2 months (95% CI 7.8–10.6) (Fig. 1).

The median time to response in patients with CR or PR after treatment initiation was 12 weeks (range 5–31). For the patients with SD, the median duration until the benefit was observed also amounted to 12 weeks (range 9–30). Interestingly, all 4 patients with SD after previous single PD-1 inhibitor blockade had PD to combined checkpoint blockade. Among the remaining 8 patients with PD after previous single PD-1 inhibitor blockade, one achieved a PR to combined checkpoint blockade. Thus, these data suggest that the effects of single and combined checkpoint blockade were observed independently from each other.

A total of 78 AE were reported in 39 patients. Thus, the majority of patients developed any treatment-related AE (60.9%). Of all events, 37 AE were graded as severe (grade 3 + 4). They were observed in 25 patients (39.1%; grade 3: 37.5%; grade 4: 1.6%). The treatment was discontinued in 25 cases (39.1%) due to unacceptable toxicity. However, no treatment-related deaths occurred during treatment or the observation period. The most common events were colitis (20.3%), hepatitis (20.3%),

Table 2 Best response rates to combined checkpoint blockade

	Cases (%)	Cumulative percentage (%)
Complete response	2 (3.1)	3.1
Partial response	8 (12.5)	15.6 (ORR)
Stable disease	14 (21.9)	37.5 (DCR)
Progressive disease	36 (56.3)	93.8
Unknown	4 (6.3)	100
Total	64 (100)	100

Abbreviations: *ORR* Objective response rate, *DCR* Disease control rate

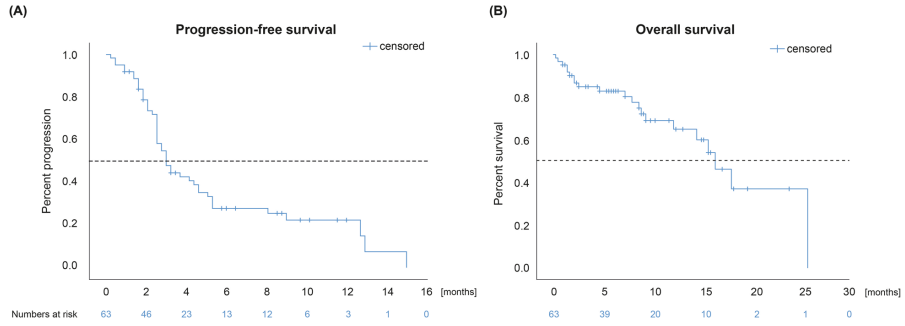

Fig. 1 Kaplan-Meier estimates of the patient population for **a** progression-free survival (PFS) and **b** overall survival (OS). The median PFS and OS was estimated to 3.0 months (95% CI 2.4–3.6) and 16.1 months (95% CI 12.9–19.3), respectively. One patient was not included in the Kaplan-Meier analysis for PFS and OS due to missing data

thyreoiditis (15.6%), hypophysitis (7.8%), fever (4.7%), and myalgia with myositis (4.7%). In all 5 cases with hypophysitis, the individual hormone axes including ACTH, cortisol, FSH, LH, TSH, and testosterone were investigated but not specifically graded. In 3 cases, the pituitary gland was enlarged in MRI examinations. All patients received systemic replacement of hydrocortisone. All AE are listed in Additional file 1.

In univariate Cox regression, ECOG status (p = 0.000096), the presence of bone metastasis (p = 0.011), and the best response to checkpoint blockade (p = 0.002) were significantly associated with OS (Additional file 2). The risk factors ECOG status, serum LDH, serum levels of CRP, and presence of bone metastasis were further integrated into a multivariate Cox regression model. Of these factors, a significant association with OS was confirmed for ECOG status (p = 0.007) only (Table 3, Fig. 2a).

We recently identified a prognostic score of the serum biomarkers LDH, CRP, and relative eosinophil count (REC) in a cohort of 94 UM patients receiving PD-1 inhibitors [6]. The score assigns one risk point for each unfavorable factor, i.e., elevated LDH, elevated CRP, and a REC < 1.5%, defining four distinct prognostic groups (low, intermediate, high, and very high risk). Each patient receiving combined checkpoint blockade was assigned to a risk group and the score was validated with Kaplan-Meier estimates. Due to a small sample size, patients with low and intermediate risk were pooled. The risk groups showed significantly different survival probabilities (p = 0.000005). The median survival times were superior for the low plus intermediate group (17.7 months, 95% CI 14.7–20.8) compared to the high (15.4 months, 95% CI 12.7–18.2) and very high risk group (7.1 months, 95% CI 0.0–16.2) (Fig. 2b). However, the score neither correlated with the response rate (p = 0.609) nor with the DCR (p = 0.446), suggesting that it was generally prognostic but not specifically predictive for the response to combined checkpoint blockade.

Subgroup analysis were performed for patients with metastasis to the central nervous system (CNS) at treatment initiation and for the treatment responders. Four patients showed an involvement of the CNS. Two of them had neurological symptoms. Two patients achieved SD, 2 showed PD. The median PFS for the CNS subgroup was 3.0 months (95% CI 0.0–6.1) while the median OS was not reached. In contrast, none of the treatment responders (CR or PR) had CNS involvement when the treatment was initiated (Table 4). The median time from detection of the primary tumor to metastatic disease was 43 months among the responders. Data on the assessment of the risk of metastasis formation of the primary tumors were sparse, as e.g. the presence of monosomy 3 or the MBD4 status was not investigated in any of the responders.

Table 3 Multivariate Cox regression analysis of clinical parameters and serum biomarkers

Parameter	Category	HR (95% CI)	*P*-value
ECOG status	n.a. (ordinal)	3.19 (1.36–7.47)	0.007*
LDH	normal	1	0.428
	elevated (>ULN)	1.83 (0.41–8.08)	
CRP	normal	1	0.534
	elevated (>ULN)	1.73 (0.31–9.74)	
Bone metastasis	no	1	0.331
	yes	2.02 (0.49–8.27)	

Four parameters were included in the multivariate Cox regression analysis. Of these factors, ECOG status was significantly associated with overall survival in this model. *Abbreviations*: *CI* Confidence interval, *n.a.* not applicable, *ULN* Institutional upper limit of normal, *LDH* Lactate dehydrogenase, *CRP* C-reactive protein; *p<0.05.

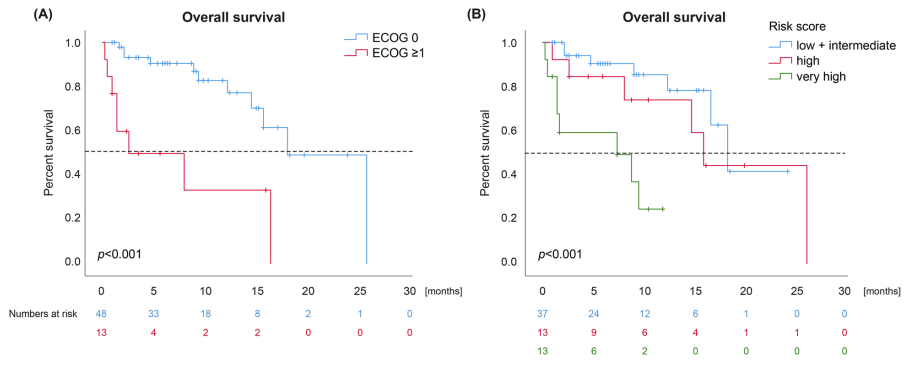

Fig. 2 a Kaplan-Meier estimates for overall survival (OS) according to ECOG performance status. The median OS was 17.7 months (95% CI 13.1–22.3) for ECOG 0 versus 2.5 months (95% CI 0.0–9.6) for ECOG ≥1. Three patients were not included due to missing data. **b** Kaplan-Meier estimates for OS according to the prognostic score based on the serum parameters LDH, CRP, and REC. The groups with low and intermediate risk were pooled due to a small number of cases. The median OS was 17.7 months (95% CI 14.7–20.8) in the low plus intermediate group versus 15.4 months (95% CI 12.7–18.2) in the high risk group versus 7.1 months (95% CI 0.0–16.2) in the very high risk group. The *p*-values indicated were calculated with the log-rank test. One patient was not included due to missing data

Discussion

Here, we present a comparatively large cohort of patients with metastatic UM who were treated with combined checkpoint blockade. We detected a 15.6% ORR, with a 3.1% complete and 12.5% partial response rate. This response rate is in line with our previous report showing 16% ORR, although only 12 patients were evaluable for their radiologic response and the follow-up time was short [6]. Another case series was recently published from a single-center experience where 2 out of 8 patients treated with nivolumab and ipilimumab had a partial response [11]. Other preliminary data on the efficacy of the combined checkpoint blockade have been proposed as conference abstracts, but appear preliminary to date. Najjar et al. reported results from a multicenter, retrospective analysis in 66 patients from 11 U.S. centers, revealing an ORR of 13% and a DCR of 31% [20]. In addition to these estimates in a real-world setting, prospective trials are currently underway. A preliminary analysis of the Spanish phase II trial GEM1402 (NCT02626962) showed an ORR of 12% and disease stabilization in 52% of cases [21]. Another phase II trial is currently ongoing in the U.S. in 30 patients with UM (NCT01585194). A recently presented interim analysis revealed an ORR of 17% and disease control in 50% [22]. Thus, we conclude that the ORR of 15.6% identified in this population is a solid estimate for the efficacy of combined checkpoint blockade in UM and a good indicator of what we can expect from the final analyses of the prospective trials. This regimen appears to be significantly superior compared to the sobering efficacy values observed with ipilimumab and PD-1 inhibitor monotherapy [6–9, 23–26]. Considering the data available so far, we conclude that the increase of ORR of the combined blockade versus PD-1 inhibition alone amounts to approximately 10%. Further evidence for a better efficacy of the combined regimen is supported by the observation of complete responders, albeit to a small extent. This is notable as UM is considered a “cold” tumor due to a low mutational burden and a unique immunosuppressive tumor microenvironment [27–29]. Further research is urgently needed to identify the radiologic, immunologic, and molecular determinants for treatment response in this small subset of patients. Regarding safety, the rate of severe AE was lower compared to the events reported in the pivotal trial in cutaneous melanoma (CheckMate-067) [30]. In particular, the occurrence of potentially life-threatening grade 4 AE was surprisingly low, suggesting that the regimen may be better tolerated in UM. However, it is also conceivable that the retrospective design and the small number of cases of this study causes an underreporting of AE.

Among clinical parameters and serum biomarkers, only the ECOG performance status was a consistent prognostic factor in multivariate analysis. Other parameters such as serum LDH, CRP, and the REC showed a significant association neither with OS nor with the treatment response when they were considered as single factors. However, when integrated into a prognostic score, they were useful for risk stratification and discriminated groups with distinct survival probabilities. Thus, the risk score identified previously in a distinct

Table 4 Characterization of the responders to combined checkpoint blockade ($n = 10$)

Response	Duration of response (months)	Time to response (weeks)	Treatment cycles (induction + maintenance)	Gender	Time to metastasis from primary tumor (months)	Age at treatment onset (years)	Available molecular/ genetic analysis	ECOG	LDH	CRP	Risk score	Previous systemic treatment	Previous liver-directed treatment	Sites of metastasis	AE ≥ grade 3 (CTCAE v5.0)
PR	14	5	4 + 0	female	9	46	mutated: GNAQ (Q209P)	0	normal	normal	low	nivolumab (PD)	TACE	liver	yes (colitis)
PR	9	10	1 + 0	female	92	54	mutated: GNAQ (Q209P); ALK, MET; wildtype: BRAF, NRAS, GNA11, BAP1	0	normal	normal	low	none	none	lung	yes (colitis)
PR	23	10	4 + 0	female	33	77	mutated: GNA11	0	elevated	unknown	low	none	chemo-saturation	liver, mesenteric fat tissue	yes (Guillain-Barré syndrome)
PR	55	13	3 + 0	male	408	67	mutated: GNAQ	0	normal	elevated	high	none	surgery	liver, nodal	yes (colitis, hypophysitis)
PR	65	19	4 + 0	female	168	60	mutated: GNAQ; wildtype: BRAF, NRAS, KIT, GNA11	0	elevated	normal	high	none	TACE	liver, lung, ovarial, cervix, omentum	no
CR	53	12	4 + 5	male	unknown	59	unknown	0	unknown	unknown	low	none	surgery	lung	no
CR	50	12	4 + 16	male	unknown	45	unknown	0	elevated	elevated	very high	none	none	liver, bone, pelvic	no
PR	26	13	3 + 0	female	14	67	unknown	0	elevated	normal	intermediate	none	SIRT	liver, lung	yes (uveitis)
PR	25	14	4 + 1	female	30	56	wildtype: BRAF, NRAS, KIT; expression PD-L1 20%; polysomia of chromosome 12	0	elevated	elevated	high	none	none	liver, lung	no
PR	11	31	4 + 27	female	53	73	wildtype: BRAF, KIT, KRAS, NRAS, NF1, CDKN2A, CDK4	0	elevated	unknown	intermediate	none	chemo-saturation	liver, bone, nodal, renal	no

Abbreviations: *CR* Complete response, *PR* Partial response, *ECOG* Eastern Cooperative Oncology Group, *LDH* Lactate dehydrogenase, *CRP* C-reactive protein, *TACE* Transarterial chemoembolization, *SIRT* Selective internal radiation therapy, *AE* Adverse event(s), *CTCAE* Common Terminology Criteria for Adverse Events

cohort was successfully validated in this population [6]. As there was a significant association neither with the ORR nor the DCR, we conclude that the score is generally prognostic but not specifically predictive for the response to checkpoint blockade.

The major limitations of this study are its retrospective design and the lack of a control group. When compared to historical controls, the median OS of 16.1 months is superior to survival estimates from other studies. Recently, the median OS benchmark for metastatic UM was identified as 10.2 months in a meta-analysis on individual data from 912 patients pooled from 29 trials [31]. Another analysis on individual-level data from 2494 patients proposed a median OS of 1.07 years across all treatment modalities. In this context, the OS observed in our cohort treated with combined checkpoint blockade appears more favorable, although external cohorts should be interpreted with caution and the comparison may be subject to significant confounding. A further limitation comes from the paucity of molecular and genetic analysis on the primary and metastatic tumors which are urgently needed to better characterize and understand the pattern of treatment response in UM.

Conclusions

Altogether, our study implies that combined checkpoint blockade represents the hitherto most effective treatment option available for metastatic UM available in routine care outside of clinical trials. Based on our analysis and preliminary data from others, we hypothesize that the ORR achieved with combined checkpoint blockade will be 15–17%. Future trials are warranted to identify specific biomarkers for treatment response.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s40425-019-0800-0.

Additional file 1. Adverse events of combined checkpoint blockade according to frequency.

Additional file 2. Univariate Cox regression analysis of clinical and laboratory parameters.

Abbreviations

AE: Adverse event; CI: Confidence interval; CRP: C-reactive protein; CTCAE: Common Terminology Criteria for Adverse Events; DCR: Disease control rate; ECOG: Eastern Cooperative Oncology Group; HR: Hazard ratio; LDH: Lactate dehydrogenase; ORR: Overall response rate; OS: Overall survival; PFS: Progression-free survival; Q2W: Every two weeks; Q3W: Every three weeks; REC: Relative eosinophil count; RLC: Relative lymphocyte count; RNC: Relative neutrophil count; UM: Uveal melanoma

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Authors' contributions

All authors made substantial contributions to the manuscript. MVH, MS and CB designed the study. MVH, TA, KCK, LH, JCH, MM, NK, CL, LR, JU, ED, AG, CP, PT, K-MT, LZ, TKE, MCK, HMS and FM were involved in the acquisition of the dataset and interpreted the data. MVH and CB drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the institutional review board of the medical faculty of the Munich University Hospital (approval number 413–16 UE) and was conducted in accordance with the principles of the Helsinki Declaration in its current version.

Consent for publication

Not applicable.

Competing interests

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Author details

[1]Department of Dermatology and Allergy, Munich University Hospital (LMU), Frauenlobstr. 9-11, 80337 Munich, Germany. [2]Department of Dermatology, University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Ulmenweg 18, 91054 Erlangen, Germany. [3]Department of Dermatology, Center for Dermatooncology, University Hospital Tübingen, Liebermeisterstr. 25, 72076 Tübingen, Germany. [4]Portuguese Air Force Health Care Direction, Lisbon, Portugal. [5]Department of Dermatology, University Hospital Schleswig-Holstein, Campus Kiel, Rosalind-Franklin-Str. 7, 24105 Kiel, Germany. [6]Skin Cancer Center, Department of Dermatology and National Center for Tumor Diseases (NCT), University Hospital Heidelberg, Im Neuenheimer Feld 460, 69120 Heidelberg, Germany. [7]Department of Dermatology, Venereology and Allergology, Goethe University, Theodor-Stern Kai 7, 60590 Frankfurt am Main, Germany. [8]Department of Dermatology and Venereology, Skin Cancer Center at the Center of Integrated Oncology (CIO) Köln Bonn, University Hospital of Cologne, Kerpenerstr. 62, 50937 Cologne, Germany. [9]Department of

Dermatology, University Medical Center Mainz, Langenbeckstr. 1, 55131 Mainz, Germany. [10]Department of Dermatology, Skin Cancer Center, Medical Faculty and University Hospital Carl Gustav Carus, TU Dresden, Fetscherstr. 74, 01307 Dresden, Germany. [11]Skin Cancer Unit, German Cancer Research Center (DKFZ) and Department of Dermatology, Venereology and Allergology, University Medical Center Mannheim, Ruprecht-Karl University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany. [12]Department of Dermatology, Klinikum Ludwigshafen, Bremserstr. 79, 67063 Ludwigshafen, Germany. [13]Department of Dermatology, University Hospital Würzburg, Josef-Schneider Straße 2, 97080 Würzburg, Germany. [14]Department of Dermatology, Saarland University Medical School, Kirrbergerstr, 66421 Homburg/Saar, Germany. [15]Department of Dermatology, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. [16]Department of Dermatology, University Medical Center Göttingen, Robert-Koch-Str. 40, 37075 Göttingen, Germany. [17]Department of Dermatology, University Hospital, University Duisburg-Essen, Hufelandstr. 55, 45147 Essen, Germany.

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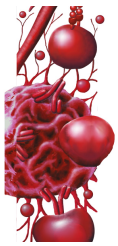

Immunotherapy plus surgery/radiosurgery is associated with favorable survival in patients with melanoma brain metastasis

Teresa Amaral*[,1,2], Ioanna Tampouri[1], Thomas Eigentler[1], Ulrike Keim[1], Bernhard Klumpp[3], Vanessa Heinrich[4], Daniel Zips[4,7], Frank Paulsen[4,7], Irina Gepfner-Tuma[5,7], Marco Skardelly[5,6,7], Marcos Tatagiba[5,6,7], Ghazaleh Tabatabai[5,7], Claus Garbe[1] & Andrea Forschner[1]

[1]Center for Dermatooncology, Department of Dermatology, University Hospital Tuebingen, Tuebingen, Germany
[2]Portuguese Air Force Health Direction, Paço do Lumiar, 1649-020, Lisbon, Portugal
[3]Department of Radiology, University Hospital Tuebingen, Tuebingen, Germany
[4]Department of Radiation Oncology, University Hospital Tuebingen, Tuebingen, Germany
[5]Interdisciplinary Division of Neuro-Oncology, Hertie Institute for Clinical Brain Research, University Hospital Tuebingen, Tuebingen, Germany
[6]Department of Neurosurgery, University Hospital Tuebingen, Tuebingen, Germany
[7]Centre for CNS Tumors at the Comprehensive Cancer Centre Tuebingen-Stuttgart, University Hospital Tuebingen, Tuebingen, Germany
*Author for correspondence: Tel.: +49 0 7071 29 84553; Fax: +49 0 7071 29 5187; teresa.amaral@med.uni-tuebingen.de

Aim: Melanoma brain metastases (MBM) are associated with a dismal prognosis. Few clinical trials evaluated the impact of immunotherapy (IT) and targeted therapy (TT) alone or in combination with surgery and radiotherapy in this population. **Patients & methods:** Retrospective analysis of data from 163 patients diagnosed with MBM between January 2014 and December 2016. Prognostic factors of overall survival were analyzed using Kaplan–Meier survival curves, classification and regression tree and multivariate Cox regression analysis. **Results:** The median follow-up was 25 months; median overall survival (mOS) for all patients was 7 months. For patients receiving IT, the mOS was 13 months and 7 months for patients receiving TT or chemotherapy (CT). The mOS for patients treated with surgery/radiosurgery in combination with IT, TT and CT was 25, 14 and 11 months, respectively. **Conclusion:** New systemic therapies, especially IT, improve mOS in patients with MBM, particularly when combined with surgery/radiosurgery upfront.

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Keywords: chemotherapy • dominant systemic therapy • immunotherapy • melanoma brain metastases • overall survival • radiosurgery • stereotactic radiation • surgery • targeted therapy • whole brain irradiation

Melanoma brain metastases (MBM) are associated with the most unfavorable prognosis of all metastatic melanoma patients. In the eighth AJCC classification, an independent substage was defined in stage IV of melanoma for patients with MBM, as these patients usually have the shortest survival time [1]. Prior to the introduction of new systemic therapies, median overall survival (mOS) for patients with MBM and normal lactate dehydrogenase (LDH) was 6 months, and for patients with elevated LDH was 3 months [2].

Few clinical studies evaluated the influence of new systemic therapies in patients with MBM [3,4] and, in the large Phase III trials the presence of MBM was an exclusion criteria [5–8].

Our retrospective study assesses the influence of new therapies using a collective of subsequent patients diagnosed with MBM, and treated with a multimodal approach.

We analyzed prognostic factors and survival considering the type of systemic therapy received and the combination of systemic therapy with surgery and/or radiotherapy. We also describe the multiple subsequent treatments using swimmer plots outlining the dominant therapy for each patient.

The primary end point of this analysis is overall survival; intracranial disease control and intracranial response are outside the scope of this manuscript, and will be analyzed and reported separately.

Future Medicine

Patients & methods

Patients' collective

We included in our analysis 163 consecutive patients diagnosed with MBM between January 2014 and December 2016. The cut-off data analysis was January 2018. The patients' collective was identified using data available in the German Central Malignant Melanoma Registry (CMMR) from the University Department of Dermatology in Tuebingen, Germany. Information from CMMR was compiled in a Statistical Package for Social Sciences (SPSS) table including – gender; date of birth; date of first diagnosis, date of entry stage IV disease and date of MBM diagnosis; stage at first diagnosis; localization of distant metastases; and histopathological characteristics from the primary tumor. The ethics committee of the Medical Faculty of the University of Tuebingen approved this study (Reference 136/2017 BO2).

Documentation of prognostic & predictive factors

Besides the data from the CMMR previously described, further information was retrieved from the electronic patient files. These additional data included BRAF status, previous therapies (if any) for stage IV disease, LDH, Eastern Cooperative Oncology Group performance status (ECOG PS), presence or absence of neurological symptoms, number of MBM, type of systemic therapy received after MBM diagnosis, type of local therapy (surgery and/or radiotherapy), best response to each treatment and time of last follow-up (FU) or death from any cause. Melanoma-specific graded prognostic assessment (GPA) that combines Karnofsky performance status and number of MBM [9] was calculated for each patient. The cut-off criterion of three MBM is used not only in the GPA score but also in other analyses similar to ours. We are aware that this cutoff is currently being discussed and others that include a higher number of MBM might be used in the future. However, since this analysis included patients diagnosed with MBM between 2014 and 2016, and this was the cutoff used for ablative therapy at that time, we understand that it should be applied in our analysis as well.

The data previously mentioned was included in the SPSS table using corresponding variables.

Analysis of dominant therapy

Currently, multiple systemic therapies are available for patients with MBM and it is difficult to determine the prognostic influence of the individual therapies. Conventional analyses followed the concept of investigating first-line therapies and second-line therapies separately. With the current expanded and complex use of immunotherapy (IT), targeted therapy (TT) and chemotherapy (CT) for stage IV melanoma, such evaluation does not seem to be reasonable, as the decisive therapeutic influence can be attributed to the first-line therapy as well as to the therapies received in the second or third line. Considering this background, we propose to identify and evaluate the therapy that was 'dominant' for the course of the disease in each patient. The dominant systemic therapy was defined as the therapy that has been administered for the longest period of time or has achieved the best disease control or response. For each patient, two authors (T Amaral, C Garbe) defined independently which therapy should be regarded as the dominant therapy.

In order to be able to evaluate this reliably, we developed multiple systemic therapies swimmer plots and divided the patients in three groups – patients with more than 12 months OS that were still alive, patients with more than 12 months OS that were already deceased and patients with less than 12 months OS. Each therapy episode and the best objective response (intracerebral + extracerebral) were displayed on a time axis. This enabled us to define the dominant therapy for each patient and the combination of dominant systemic therapy with surgery and/or radiotherapy. By applying the concept of dominant therapies, we obtained four different 'systemic therapy groups': IT (CTLA-4 ± PD-1 or PD-1 inhibitors), TT (BRAF ± MEK inhibitors), CT (carboplatin/paclitaxel, dacarbazine or temozolomide) and no systemic therapy. All systemic therapies mentioned throughout the article refer to this concept. The colored circle before each patient's swimmer plot identifies the dominant therapy for this particular patient, and the number refers to the OS in days.

Combination of dominant therapy & surgery/radiosurgery

Since the nature of intracranial intervention plays a role in the outcome of patients with MBM, survival analysis for the combination of dominant therapy and surgery and/or radiotherapy was also performed. For all patients who underwent surgery/radiosurgery (S/RS), an MRI examination is performed for the postoperative control and response evaluation. For this analysis, the following possible combinations were used: S/RS + IT, S/RS + TT,

S/RS + CT, S/RS with no systemic treatment, whole brain irradiation (WBRT) with or without systemic treatment and no radiotherapy (with or without systemic treatment).

Statistics

FU time was defined as the date of last FU or death minus the date of MBM diagnosis. Survival probabilities were calculated using the date of diagnosis of MBM. In the OS analyses, all causes of death were considered. Survival curves and median survival with 95% confidence intervals (95% CIs) were obtained according to the Kaplan–Meier (KM) estimators and compared using the log-rank test. The following factors were included in the classification and regression tree analysis and in the multivariate Cox regression analysis: gender, *BRAF* status, number of MBM, ECOG PS, LDH, presence or absence of extracerebral disease, previous systemic therapy, dominant therapy and combination of dominant therapy and surgery and/or radiotherapy (6 groups as described above). Survival analyses were performed with SPSS v.24 (SPSS Inc., IL, USA). STATA® program v15 was used to generate the final version of KM survival curves and R version 3.4.3 by the R Foundation of Statistical Computing (Vienna, Austria) was used to generate the swimmer plots, classification and regression tree and multivariate forest plot analysis [10].

Results

Patients' characteristics

Table 1 summarizes the patients' characteristics. The median age at the time of MBM diagnosis was 63 years (54.0–74.0). The majority of patients (92%) had cerebral and extracerebral disease and half of the patients had already received at least one systemic therapy for stage IV melanoma before being diagnosed with MBM. When analyzing the prognostic factors, 56% had ≤3 MBM, 52% harbored a BRAF mutation, 43% had elevated LDH and 70.6% had ECOG 0. Finally, 28.2% were GPA class 3.5–4.0. The median FU was 25 months (95% CI: 21.4–28.6) and at the time of data cut-off analysis, 19% of the patients were still alive.

Systemic therapies swimmer plots

As mentioned before, we divided the patients in three subgroups according to OS – more than 12 months OS and still alive at time of cut-off analysis; more than 12 months OS and already deceased and less than 12 months OS. We used the 12 months' limit due to the fact that previous reports and analyses for melanoma brain metastases show median overall survivals less than 12 months and to produce an easily understandable graphic representation and swimmer plots of the three groups.

In the first subgroup (Figure 1A; n = 31), IT was the dominant therapy in the majority of the patients (23 patients), followed by TT (five patients). Three patients did not receive systemic therapy. 13 patients (42%) obtained a complete intracranial and extracranial response (CR), based on the RECIST criteria [11].

In the second subgroup (Figure 1B; n = 23), IT was the dominant therapy in 12 patients; TT in eight patients, CT in two patients and one patient received no systemic therapy. In this subgroup, no CRs were observed.

Finally, in the third subgroup (Figure 1C; n = 109), a third of the patients did not receive systemic therapy (33 patients). TT was the dominant therapy in 31 patients, IT in 29 patients and CT in 16 patients.

Overall survival

Figure 2A–D shows the KM curves for the prognostic factors identified (number of MBM, LDH, previous systemic therapy and GPA class). In the subgroup of patients with ≤3 MBM the mOS was 10 months, significantly superior to the 4 months mOS observed in the group with >3 MBM (95% CI: 7.1–12.9 and 2.7–5.3). There was no significant difference in the mOS between patients with normal and elevated LDH at the time of MBM diagnosis: 7 and 6 months, respectively (95% CI: 3.3–10.7 and 3.8–8.2). In the subgroup of patients that have not received previous systemic therapy, the mOS was significantly higher compared with those already treated for stage IV disease – 10 versus 6 months (95% CI: 7.0–12.9 and 5.0–6.9). Finally, for patients in GPA class 3.5–4.0, the mOS was 11 months, in GPA class 2.5–3.0, it was 10 months, in GPA class 1.5–2.0, it was 6 months and in GPA class 0.0–1.0 the mOS was 4 months (95% CI: 5.9–16.1; 7.1–12.9; 4.6–7.4 and 2.9–5.1, respectively).

Figure 2E represents the KM OS curves considering the dominant therapy classification. Patients treated with IT had an mOS of 13 months, significantly higher compared with the other subgroups – 7 months mOS for TT; 7 months mOS for CT and 3 months mOS for the subgroup not receiving systemic therapy. The 1 year (1 y) OS was 53.1% for patients receiving IT and 29.5% for those treated with TT. The 2 y OS was 37.5 and 8.4%, respectively.

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Table 1. Patients characteristics.

	All patients	Overall survival			p-value
		>12 m alive	>12 m deceased	≤12 m	
N	163	31	23	109	
Age					
<55 y	44	19.4%	34.8%	27.5%	0.670
≥55 y < 70 y	58	45.2%	30.4%	33.9%	
≥70 y	61	35.5%	34.8%	38.5%	
Gender					
Males	93	45%	57%	61%	0.311
Females	70	55%	43%	39%	
Extracerebral disease					
Yes	150	90%	87%	94%	0.526
No	13	10%	13%	6%	
Previous therapy					
Yes	81	32%	35%	58%	**0.013**
No	82	68%	65%	42%	
LDH					
Normal	66	55%	44%	36%	0.183†
Elevated	69	39%	26%	47%	
Not available	28	6%	30%	17%	
Number of BM					
≤3	92	77%	70%	48%	**0.005**
>3	71	23%	30%	52%	
***BRAF* status**					
Mutated	84	45%	70%	49%	0.233†
Wild-type	74	45%	30%	49%	
Not available	5	10%	0	2%	
ECOG PS					
0	115	84%	74%	66%	0.147
≥ 1	48	16%	26%	34%	
GPA					
0.0–1.0	33	7%	17%	25%	**0.054**
1.5–2.0	59	29%	26%	40%	
2.5–3.0	25	19%	17%	14%	
3.5–4.0	46	45%	39%	21%	

Clinical features and disease characteristics from all patients are presented, as well as their distribution in three subgroups used to perform the graphic representation in Figure 1 (patients with OS >12 months, still alive at the time of analysis; patients with OS <12 months and already dead at the time of analysis; patients with OS ≤12 months). To determine the relationship between these characteristics in the three different subgroups, we used crosstabs and results are presented in the last column.
Bold p-values represent those that are significant.
†Analysis performed only for patients with available data.
BM: Brain metastasis; ECOG PS: Eastern Cooperative Oncology Group performance status; GPA: Melanoma-specific graded prognostic assessment; LDH: Lactate dehydrogenase; m: Month; Y: Year.

Figure 2F shows OS analysis for the combination between the dominant therapy and local therapies. For patients treated with S/RS + IT, the mOS was 25 months compared with 7 months for patients receiving the combination S/RS + TT. For each of the previous subgroups, the 1 y OS was 69.4 and 62.5% and the 2 y OS was 50.6 and 19.4%, respectively. The mOS for patients treated with WBRT ± systemic therapy was 5 months, and the 1 y OS and 2 y OS were 12.7 and 6.8%, respectively. Table 2 provides more information on OS analysis of systemic therapy and combination with local therapy.

Figure 3 provides OS data stratified by the presence of symptoms and treatment with corticosteroids at the time of MBM diagnosis. The mOS for asymptomatic and symptomatic patients was 7 and 4 months (95% CI: 4.6–9.4 and 0.9–4.1; p = 0.359) and for patients not treated and treated with corticotherapy was 8 and 4 months (96% CI: 5.4–10.6 and 1.7–6.3; p = 0.053).

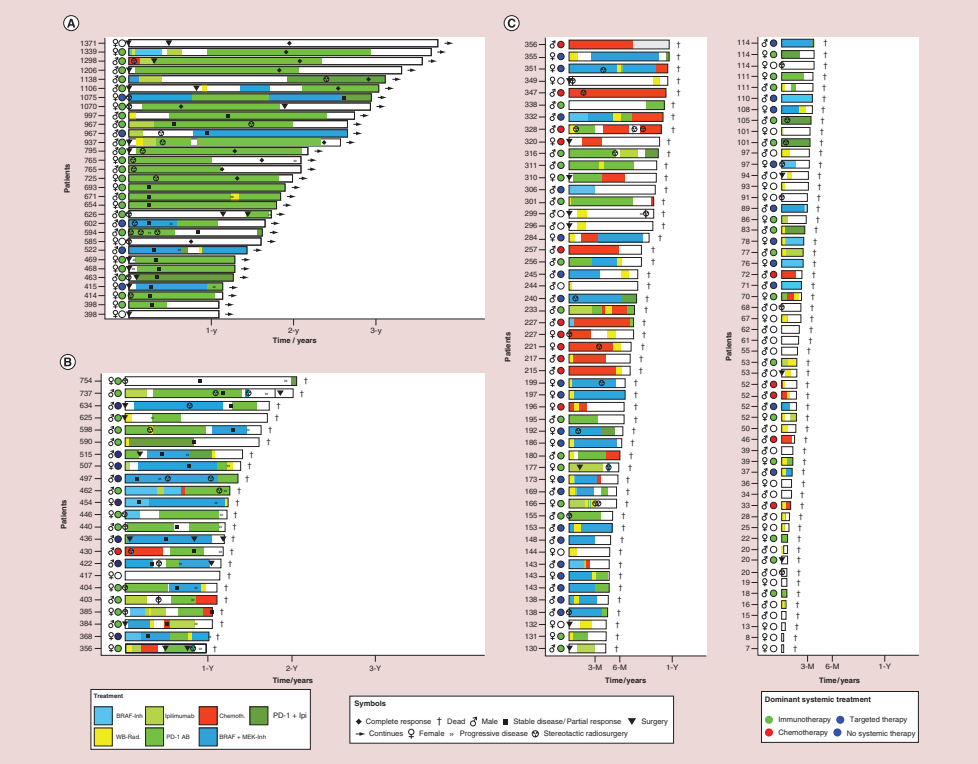

Figure 1. Multiple therapies swimmer´s plots. (A) Patients with OS >12 months, still alive. **(B)** Patients with OS >12 months deceased. **(C)** Patients with OS ≤12 months. The number displayed before each patient's individual swimmers plot represents the OS in days.
OS: Overall survival.

Finally, the mOS for all patients was 7 months (95% CI: 5.4–8.6) and the 1 year and 2 years OS was 32.5 and 18.7%, respectively. Table 2 provides more information on the mOS for all therapy groups and on the 1 and 2 years OS.

Classification & regression tree

The classification and regression tree is shown in Figure 4. Node 1 represents the most significant prognostic factor for this population, which is S/RS. Two groups were then generated: Node 2 (patients treated with S/RS) and Node 5 (patients not treated with S/RS, that includes patients treated with WBRT and patients who did not receive radiotherapy). In both groups, the effect of the combination with dominant therapy is subsequently evaluated. The best mOS (17.4 months) was observed in the group of patients treated with S/RS and IT or TT (Node 3). For patients treated with S/RS + CT or no systemic therapy (Node 4) the mOS was 9.9 months. In the group of patients not treated with S/RS but receiving systemic therapy (Node 6), the mOS was 6.6 months for those treated with IT (Node 7) and 6.2 months for those treated with TT or CT (Node 8). The shortest mOS (2 months) was observed in the subgroup of patients that did not receive S/RS or systemic therapy (Node 9).

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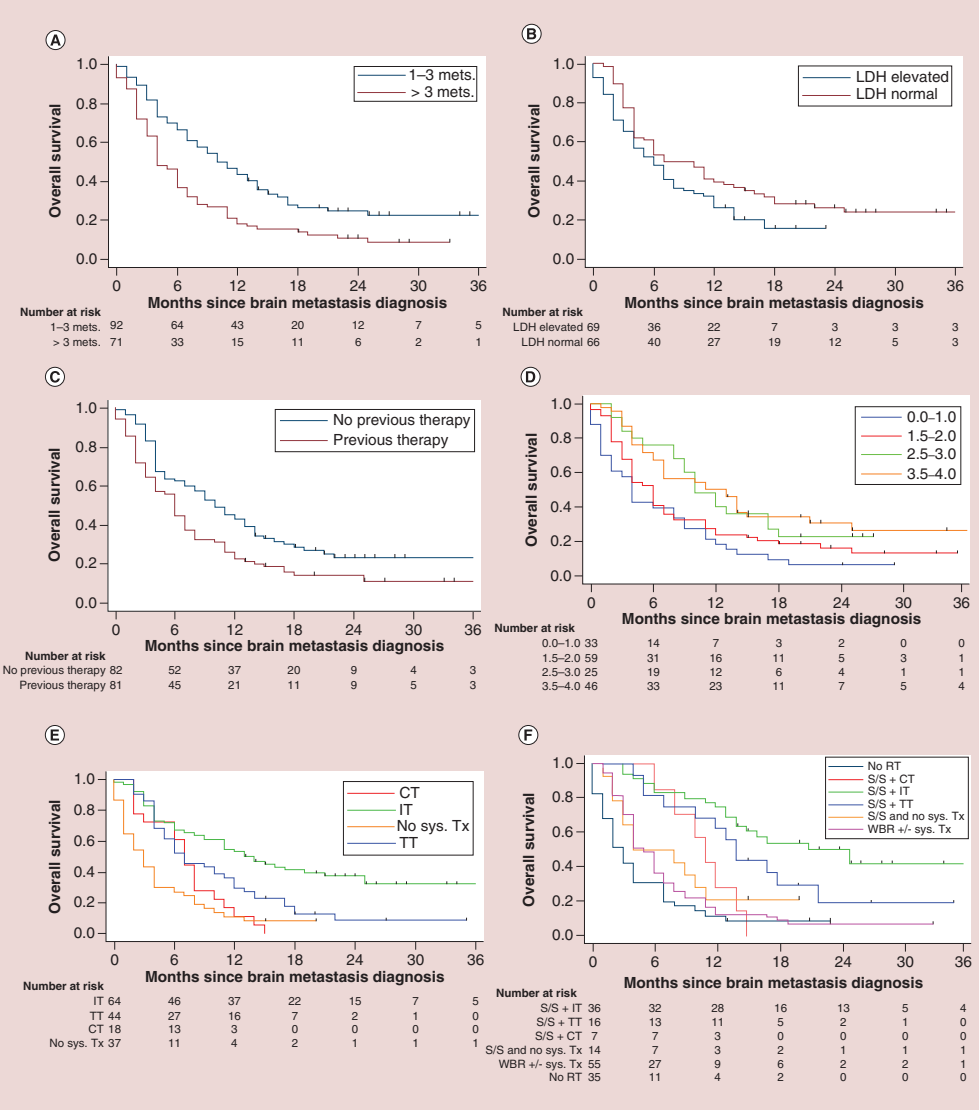

Figure 2. Overall survival. Stratified by **(A)** number of brain metastases (1–3 vs >3), p smaller than 0.0001; **(B)** LDH (elevated vs normal), p = 0.053; **(C)** prior systemic therapy (yes vs no), p = 0.005; **(D)** melanoma-specific GPA, p = 0.002; **(E)** dominant systemic therapy (IT vs TT vs CT vs No sys. Tx), p smaller than 0.0001; **(F)** combination dominant systemic therapy and local therapy (S/S + IT vs S/S + TT vs S/S + CT vs S/S and no sys. Tx vs WBR ± sys. Tx vs no RT), p smaller than 0.0001.
CT: Chemotherapy; GPA: Graded prognostic assessment; LDH: Lactate dehydrogenase; IT: Immunotherapy; No sys. Tx: No systemic therapy; RT: Radiotherapy; S/S: Surgery/radiosurgery; TT: Targeted therapy; WBR: Whole brain irradiation.

Table 2. Median overall survival and 1 year and 2 years overall survival for dominant systemic therapies and combination with local therapies.

	Median OS (Months; 95% CI)	OS (%; 95% CI)	
		1 year OS	2 years OS
Systemic therapy ($p < 0.0001$)			
Immunotherapy	13 (8.1–17.8)	53.1 (40.9–62.2)	37.5 (25.3–49.6)
Targeted therapy	7 (3.8–10.2)	29.5 (16–43)	8.4 (0–18)
Chemotherapy	7 (5.6–8.4)	11.1 (0–25.6)	–
No systemic therapy	3 (1.5–4.8)	10.8 (0.8–20.8)	–
Radiotherapy + systemic therapy ($p < 0.0001$)			
S/SRS + immunotherapy	25 (14.6–35.4)	69.4 (54.3–84.4)	50.6 (33.5–67.5)
S/SRS + targeted therapy	14 (12.1–15.9)	62.5 (38.8–86.2)	19.4 (0–41.3)
S/SRS + chemotherapy	11 (8.4–13.6)	28.6 (0–62.1)	–
S/SRS without systemic therapy	4 (0–2.1)	21.4 (0–43)	–
Whole brain irradiation ± systemic therapy	5 (3.9–6.1)	12.7 (3.9–21.5)	6.8 (0–13.7)
No radiotherapy	3 (1.7–4.2)	11.4 (0.8–22)	–

CI: Confidence interval; OS: Overall survival; S/SRS: Surgery/radiosurgery.

Multivariate analysis

In the multivariate analysis (Figure 5), the type of therapy (combination of systemic therapy with surgery and/or radiotherapy) remained a significant prognostic factor (hazard ratio [HR] for S/RS + IT = 0.25) along with ECOG PS (HR: 1.67). The number of MBM was borderline significant (HR: 1.52). The other factors evaluated – gender, BRAF status, presence of extracerebral disease, LDH at the time of diagnosis and previous systemic therapy for stage IV disease were not significant in the multivariate analysis.

Discussion

The main finding of our study is the considerable impact of IT on OS of patients with MBM, particularly in combination with S/RS.

The mOS of patients receiving predominantly IT was significantly longer (mOS = 13 months) than for those treated with other systemic therapies, namely TT and CT. Surprisingly, the mOS for patients predominantly treated with TT and CT was the same (mOS = 7 months) in our population. The combination of systemic immunotherapy and S/RS showed an impressive mOS of 25 months. The 1 year and 2 years OS rates were 69.4 and 50.6%, higher than previously reported [12–16]. However, it should be taken into consideration that these studies, contrary to ours, analyzed the outcomes of monotherapies with CTLA-4 and PD1 inhibitors only.

The inclusion of S/RS and WBRT in the analysis already implies a selection bias. S/RS is mainly used in patients with ≤3 MBM while WBRT is used exclusively in patients with >3 MBM. By using this approach in the clinical practice, we are selecting patients with the best prognostic factors to receive predominantly S/RS upfront, which might partially explain the best outcomes with this approach. However, the subgroup of patients treated with S/RS and systemic therapies (Figure 2F, groups 1–4) also includes patients with >3 MBM. In this case, S/RS was not performed upfront but later on at the time of progression of individual brain metastases. In a retrospective analysis, this selection bias cannot be completely avoided, and prospective studies are required to answer the questions that remain unanswered here.

As for the patients treated with S/RS + TT, the mOS was 14 months and the 1 year OS rate was 62.5% (95% CI: 38.8–86.2), which is better than compared with previously reported data [4,17].

55 patients treated with WBRT were included (Figure 2F). In this subgroup, the mOS was only 5 months. In the two subgroups of patients that either did not receive systemic therapy or radiotherapy, the mOS was 3 months. In view of the fact that IT and TT are available in clinical practice, and also achieve good results in MBM, treatment with WBRT should be judged critically.

For patients who did not receive S/RS but were treated with IT (Figure 4; Node 7), the mOS was 6.6 months, which does not exceed the mOS for patients receiving S/RS and CT or no systemic therapy (Node 4). However,

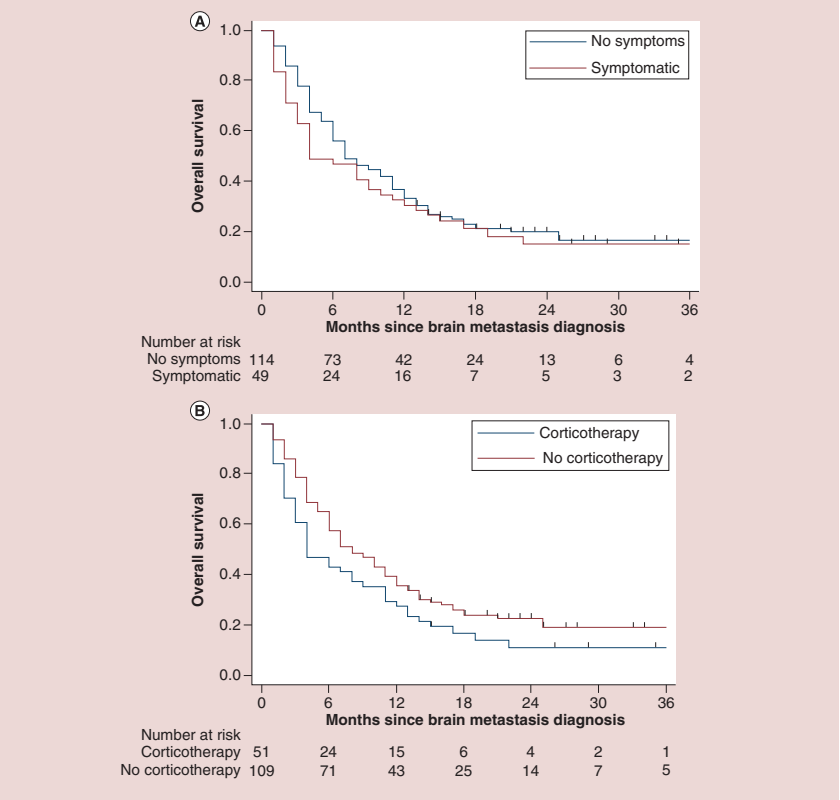

Figure 3. Overall survival. Stratified by **(A)** presence of symptoms (no symptoms vs symptomatic), p = 0.359; and **(B)** therapy with corticosteroids (no corticotherapy vs corticotherapy) at the time of MBM diagnosis, p = 0.053.
MBM: Melanoma brain metastasis.

in the first group a higher percentage of the patients (>20%) were long-term survivors. We understand that this favors IT as first-line systemic therapy when S/RS is not possible.

Our analysis shows that the previous therapies for stage IV disease have a prognostic significance in this population, as well as the number of MBM and performance status, already described by other authors [2,4,18,19]. In our population, LDH, presence of symptoms and corticotherapy at the time of MBM diagnosis were not significant.

A considerable large subgroup (23% of the patients) did not receive any systemic treatment for MBM. The majority of these patients were either heavily pretreated for metastatic disease before developing MBM or were clinically unfit, resulting in an OS <12 months.

Retrospective analysis including real-world patients' data remain important since trials addressing specific questions associated with the treatment of patients with MBM are scarce. Results available from the ABC [3] and CheckMate 204 [20] trials showed that the combination of CTLA-4/PD-1 inhibitors is safe and active in patients with asymptomatic MBM, with similar intra- and extra-cranial responses, and with better outcomes when given upfront. In the COMBI-MB [4] trial, the first interim analysis showed that, for BRAF mutated patients with

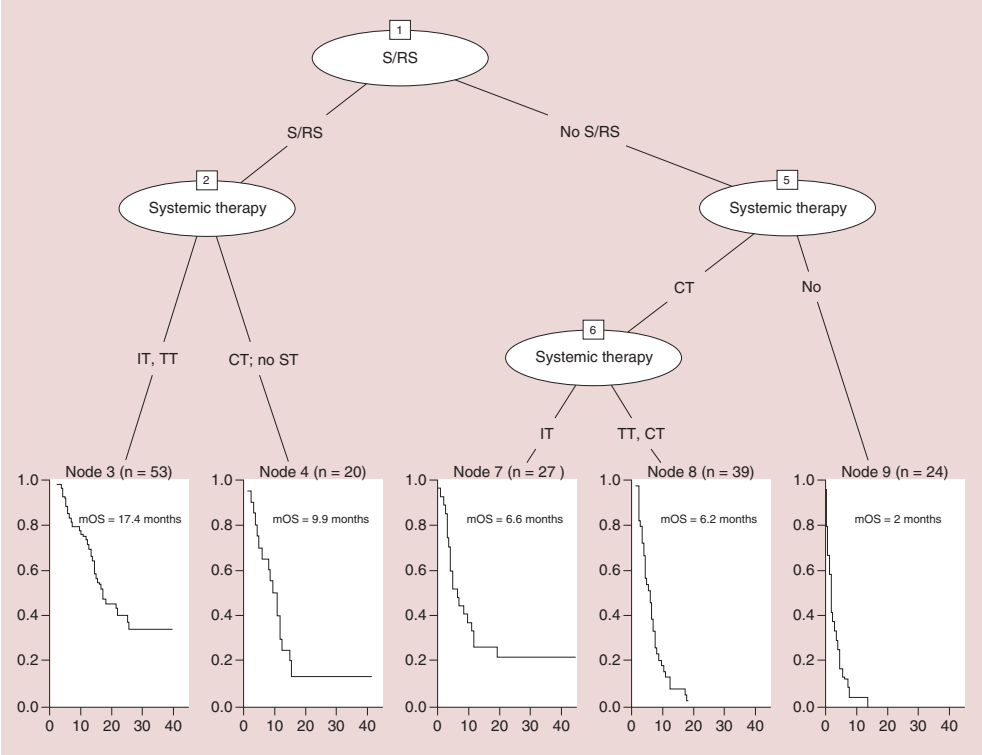

Figure 4. Classification and regression tree analysis. Y-axis represents the OS probability and X-axis represents time in months. In Node 5 are included the patients who received WBRT and those who did not receive any radiotherapy.
CT: Chemotherapy; IT: Immunotherapy; mOS: Median overall survival; n: Number of patient; no ST: No systemic therapy; OS: Overall survival; S/RS: Surgery/radiosurgery; TT: Targeted therapy; WBRT: Whole brain irradiation.

asymptomatic MBM, ECOG PS 0/1 and no previous intracranial local therapy, the mOS was 10.8 months, with a 1 year OS of 46%, supporting the use of the combination dabrafenib/trametinib in this subgroup.

The following limitations need to be considered when examining the results. This is a retrospective analysis from a single center. However, we present data from a rather large number of patients obtained from a very detailed dataset. We included patients diagnosed in 2014, for whom the currently first-line IT and TT were not yet available. The therapeutic approach changed considerably since then. A selection bias has to be considered. The patients with better prognostic factors (≤3 MBM, not progressing under previous therapy and with higher GPA score) mostly received S/RS and IT, which could explain the better outcomes observed. Hence, the results obtained by comparing groups 1–4 (which include patients treated with S/RS and therefore with better prognostic factors upfront) with groups 5 and 6 need to be interpreted with caution (Figure 2F).

Our study also presents strengths. In this study, we present OS data from patients with MBM, for which very few data are available. Most of the information available is focused on intracranial response and intracranial disease control. The analysis based on multiple therapies swimmer plots, enabled us to identify the dominant therapy for the individual patient outcome. The results of our study show that this analytic approach can successfully identify effective combinations of systemic treatments with surgery and/or radiotherapy. Although a selection

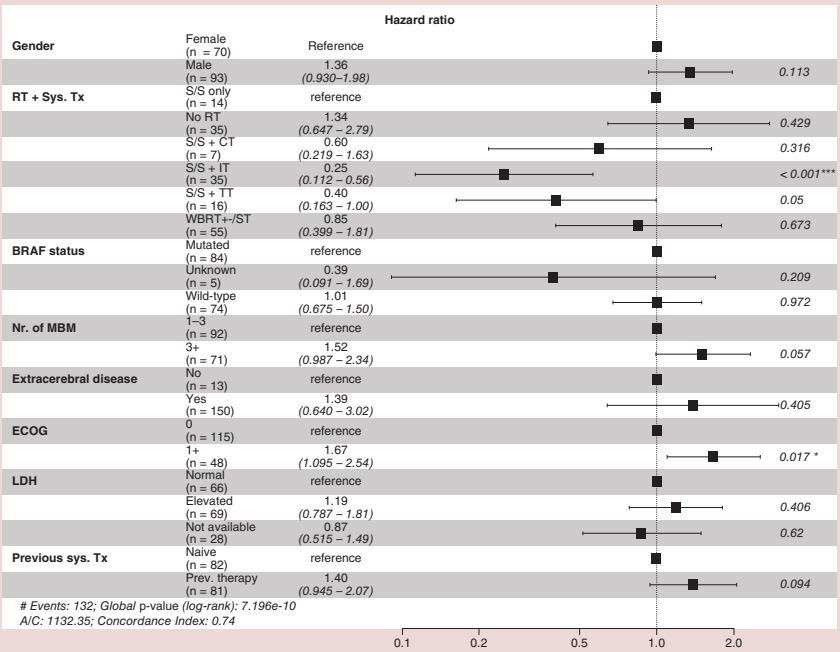

Figure 5. COX multivariate analysis.
ECOG: Eastern Cooperative Oncology Group performance status; LDH: Lactate dehydrogenase; RT + Sys. Tx: Radiotherapy plus systemic therapy; S/S: Surgery/radiosurgery; S/S + CT: Surgery/radiosurgery plus chemotherapy; S/S + IT: Surgery/radiosurgery plus immunotherapy; S/S + TT: Surgery/radiosurgery plus targeted therapy; No RT: No radiotherapy; Nr. of MBM: Number of melanoma brain metastases; Previous Sys. Tx: Previous systemic therapy; WBRT ± ST: Whole brain irradiation with or without systemic therapy.

bias was present regarding the prognostic factors previously mentioned, the multivariate analysis showed that the combination of S/RS + IT seems to be the best approach, with a clear survival advantage. The median FU after MBM diagnosed was 25 months, which is highly uncommon, supporting our long-term outcomes. Our results are based on data from the CMMR, which is a very detailed database, continuously updated, with well-documented therapy and FU information.

Conclusion

This retrospective study included 163 patients with MBM treated with a multidisciplinary approach in a tertiary center. This analysis confirmed the prognostic significance of the number of cerebral metastases, the ECOG performance status and GPA score that combines these two aspects. The LDH value was only marginally significant.

The new systemic therapies, especially IT, improve the OS of patients with MBM, especially when combined with ablative therapies (S/RS). Finally, our results suggest that in MBM, local therapy should be considered as the first approach whenever possible. IT or TT should be the systemic therapies of choice. If upfront S/RS is not possible, first-line IT should be considered, which is also in line with the results from the ABC and CheckMate 204 clinical trials.

Future perspective

The therapeutic approach for patients with MBM changed significantly in the last years. Previously, OS for these patients did not exceed 4–6 months, depending on the publications considered. However, IT and TT changed the spectrum of therapeutic options for these patients, which resulted in improved disease control and survival.

Based on the results from clinical trials, combined IT (CTLA-4 + PD-1 inhibitors) seems to be the systemic therapy with the best outcomes in patients with MBM. The combination of dabrafenib and trametinib also showed an improvement in OS in patients with MBM and BRAF mutation. To be noted, intracranial and extracranial response do not seem to differ both for IT and TT.

Nonetheless, some questions remain open. The best combination of systemic and local therapy is yet to be determined. Should they be given concurrently or sequential? Data show that concurrent therapy does not seem to increase intracranial toxicity, contrary to what might be expected.

Ongoing clinical trials will show the role of the triple combination therapy (PD-1/PD-L1 inhibitors + BRAF/MEK inhibitors) in stage IV melanoma. The triple combination in MBM should also be investigated, particularly if no limiting toxicity is observed in the current ongoing trials.

Currently, the cutoff of the MBM for receiving ablative therapy is also the subject of an intense discussion. However, the exact number is not consensual and depends on several factors including the experience of the treating multidisciplinary team. New concepts that are not limited by the number of MBM are being investigated. For patients with more than three MBM, should we treat locally the lesions that will most probably cause symptoms or are progressing, and initiate/continue systemic therapy or should we skip the local therapy and focus predominantly in systemic approaches?

Finally, we need to mention the group of patients with symptomatic MBM that are normally excluded from clinical trials. Moreover, immunotherapy might be delayed in these patients since the therapy for symptomatic MBM includes corticosteroids. This aspect is particularly important for patients with BRAF wild-type melanoma who have no other valid systemic therapeutic options and other strategies that help mitigate this problem need to be addressed.

Summary points

- Treatment of patients with melanoma brain metastases (MBM) has changed significantly in the last years, but remains challenging.
- Immunotherapy (particularly the combination of CTLA-4 and PD-1 inhibitors), and targeted therapy (BRAF + MEK inhibitors) have shown to improve disease control and survival in patients with asymptomatic MBM.
- A total of 163 consecutive patients diagnosed with MBM between 2014 and 2016 were included in this analysis.
- The dominant therapy for each patient was defined based on the therapy duration and outcome of each systemic therapy.
- Prognostic significance factors were number of cerebral metastases, Eastern Cooperative Oncology Group performance status, melanoma-specific graded prognostic assessment score and previous systemic therapies received for stage IV disease. In our population, lactate dehydrogenase, presence of symptoms and corticotherapy at the time of MBM diagnosis were not significant.
- The median overall survival (mOS) of patients receiving IT was significantly longer (mOS = 13 months) than for those receiving targeted therapy (TT) and chemotherapy (mOS = 7 months for both TT and CT).
- The combination of IT and surgery/radiosurgery (S/RS) showed an mOS of 25 months. The 1 year and 2 years OS rates were 69.4 and 50.6%.
- In our analysis, IT and TT improve mOS in patients with MBM, particularly when combined with S/RS.
- If upfront S/RS is not possible, first-line IT should be considered.

Financial & competing interests disclosure

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Ethical conduct of research

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

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6. **"Combined immunotherapy with nivolumab and ipilimumab with and without local therapy in patients with melanoma brain metastasis: a DeCOG* study in 380 patients." Amaral, T.,** F. Kiecker, S. Schaefer, H. Stege, K. Kaehler, P. Terheyden, A. Gesierich, R. Gutzmer, S. Haferkamp, J. Uttikal, C. Berking, D. Rafei-Shamsabadi, L. Reinhardt, F. Meier, A. Karoglan, C. Posch, T. Gambichler, C. Pfoehler, K. Thoms, J. Tietze, D. Debus, R. Herbst, S. Emmert, C. Loquai, J. C. Hassel, F. Meiss, T. Tueting, V. Heinrich, T. Eigentler, C. Garbe and L. Zimmer (2020). Journal for ImmunoTherapy of Cancer 8(1): e000333.

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Combined immunotherapy with nivolumab and ipilimumab with and without local therapy in patients with melanoma brain metastasis: a DeCOG* study in 380 patients

Teresa Amaral ,[1] Felix Kiecker,[2] Sarah Schaefer,[3] Henner Stege,[4] Katharina Kaehler,[5] Patrick Terheyden,[6] Anja Gesierich,[7] Ralf Gutzmer,[8] Sebastian Haferkamp,[9] Jochen Uttikal,[10,11] Carola Berking,[12,13] David Rafei-Shamsabadi ,[14] Lydia Reinhardt,[15] Friedegund Meier,[15] Ante Karoglan,[16] Christian Posch,[17,18] Thilo Gambichler,[19] Claudia Pfoehler,[20] Kai Thoms,[21] Julia Tietze,[22] Dirk Debus,[23] Rudolf Herbst,[24] Steffen Emmert,[25] Carmen Loquai,[4] Jessica C Hassel,[3] Frank Meiss,[14] Thomas Tueting,[16] Vanessa Heinrich,[26] Thomas Eigentler,[1] Claus Garbe,[1] Lisa Zimmer,[27] *German Dermatological Cooperative Oncology Group

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For numbered affiliations see end of article.

Correspondence to
Dr Teresa Amaral;
teresa.amaral@med.uni-tuebingen.de

ABSTRACT

Background Nivolumab combined with ipilimumab have shown activity in melanoma brain metastasis (MBM). However, in most of the clinical trials investigating immunotherapy in this subgroup, patients with symptomatic MBM and/or prior local brain radiotherapy were excluded. We studied the efficacy of nivolumab plus ipilimumab alone or in combination with local therapies regardless of treatment line in patients with asymptomatic and symptomatic MBM.

Methods Patients with MBM treated with nivolumab plus ipilimumab in 23 German Skin Cancer Centers between April 2015 and October 2018 were investigated. Overall survival (OS) was evaluated by Kaplan-Meier estimator and univariate and multivariate Cox proportional hazard analyses were performed to determine prognostic factors associated with OS.

Results Three hundred and eighty patients were included in this study and 31% had symptomatic MBM (60/193 with data available) at the time of start nivolumab plus ipilimumab. The median follow-up was 18 months and the 2 years and 3 years OS rates were 41% and 30%, respectively. We identified the following independently significant prognostic factors for OS: elevated serum lactate dehydrogenase and protein S100B levels, number of MBM and Eastern Cooperative Oncology Group performance status. In these patients treated with checkpoint inhibition first-line or later, in the subgroup of patients with BRAFV600-mutated melanoma we found no differences in terms of OS when receiving first-line either BRAF and MEK inhibitors or nivolumab plus ipilimumab (p=0.085). In BRAF wild-type patients treated with nivolumab plus ipilimumab in first-line or later there was also no difference in OS (p=0.996). Local therapy with stereotactic radiosurgery or surgery led to an improvement in OS compared with not receiving local therapy (p=0.009), regardless of the timepoint of the local therapy. Receiving combined immunotherapy for MBM in first-line or at a later time point made no difference in terms of OS in this study population (p=0.119).

Conclusion Immunotherapy with nivolumab plus ipilimumab, particularly in combination with stereotactic radiosurgery or surgery improves OS in asymptomatic and symptomatic MBM.

INTRODUCTION

Melanoma brain metastasis (MBM) is a known characteristic for poor prognosis. The median overall survival (mOS) in the era of chemotherapy was 4 months and decreased to 2 months in patients with elevated lactate dehydrogenase (LDH).[1 2] The response of MBM to chemotherapy was approximately 5%. This applies to both, drugs that cross the blood-brain barrier, such as temozolomide and fotemustine, and to drugs that do not cross the blood-brain barrier, such as dacarbazine.[3 4] The American Joint Committee on Cancer has acknowledged the negative impact of brain metastasis on the prognosis of patients with melanoma in its latest eighth edition staging system by defining this subgroup as M1d.[5]

With the introduction of targeted treatment (BRAF/MEK inhibitors) and immune checkpoint inhibitors, the prognosis of metastatic melanoma has drastically improved.[6–8] In contrast to ample data on the efficacy of

novel therapies in stage IV melanoma without MBM, there are only a few small studies on the efficacy of these drugs in patients with cerebral disease. This lack of information is mainly due to the fact that large phase II/III multicenter studies systematically excluded patients with MBM, particularly if symptomatic or previously treated with local therapy, such as stereotactic radiosurgery and surgery (STR/surgery). The first studies investigating targeted therapy and immune checkpoint inhibitors in patients with MBM showed that these therapies were also very effective intracranially.[9–12] Currently available data suggest that PD-1-based immunotherapy and particularly combined immunotherapy with nivolumab and ipilimumab (NIVO+IPI) might be more effective than BRAF/MEK inhibitors.[8 13] In two retrospective studies with patients with MBM, the authors reported that patients receiving immunotherapy had a mOS between 13 and 14.8 months (95% CI: 8.1 to 17.8 and 9.9 to 19.7, respectively), while in those receiving targeted therapy the mOS was only 7 and 10 months (95% CI: 3.8 to 10.2 and 7.8 to 11.7, respectively).[14 15] This difference was also present when these systemic therapies were given in combination with stereotactic radiosurgery, favoring the combination with immunotherapy, which resulted in a mOS between 21–25 months (95% CI: 12.9 to 29.1 and 14.6 to 35.4, respectively) and 12.9–14 months with targeted therapy (95% CI: 12.9 to 29.1 and 9.1 to 16.7, respectively).

Our study provides real-world outcome data from 23 German skin cancer centers, retrospectively assessing the activity of NIVO+IPI alone or in combination with local therapies regardless of treatment line in patients with asymptomatic and symptomatic MBM.

We addressed the following questions: (a) Which prognostic factors for OS can be identified in patients with MBM treated with combined immunotherapy? (b) Does local therapy (STR/surgery) improves survival in patients with MBM treated with NIVO+IPI? (c) Are STR/surgery more effective when given before or after combined immunotherapy? (d) Is there a difference in terms of survival when combined immunotherapy is given as a first-line treatment for MBM or later in the course of the disease? (e) In patients with BRAF V600-mutated melanoma, which first-line systemic therapy for MBM translates into better OS: first-line immunotherapy or first-line targeted therapy? (f) Is there a difference in terms of OS when patients with symptomatic and asymptomatic MBM receive NIVO+IPI? Since a total of 380 patients were included, we were able to perform subgroup analyses with reasonable statistical power.

METHODS

Patients' characteristics and treatments

We used pseudo-anonymized forms to document retrospective data from patients with MBM treated with NIVO+IPI between April 2015 and October 2018. All participating centers received the mentioned pseudo-anonymized forms including the prespecified information to be collected. Data were extracted from patients' medical records in 23 German skin cancer centers either by medical doctors or by clinical research documentation professionals, depending on the site. Patients were included regardless of previous local or systemic therapies, provided that they received combined immunotherapy for treating MBM.

Multiple MBM were irradiated by whole brain irradiation with opposite lateral field in mask technique. Stereotactic radiosurgery was used to irradiate small brain metastasis. Neuroimaging consisted of a stereotactic three-dimensional T1-weighted postcontrast Magnetic Ressonance Imaging (MRI) acquisition und an planning CT scan. Selection of dosimetry parameters (maximum dose, marginal isodose and number of isocenters) was made according to size, shape, localization and relationship for brain metastasis to critical structures. Target localization was referenced to a coordination system and target position was tracked during treatment. The data cut-off date was October 31, 2018.

Statistical Analysis

We performed univariate and multivariate Cox regression analysis to evaluate the impact of baseline patient and disease characteristics on OS. Cox multivariate analysis included the following factors: sex, BRAF mutation status, number of MBM, Eastern Cooperative Oncology Group performance status (ECOG-PS) as categorical variables and age, LDH level and protein S100B level as both categorical and numerical variables. The use of corticosteroids at the start of combined immunotherapy was also documented. As these data are rather complex regarding dosage and duration of each individual treatment, they will be analyzed in a separate investigation.

OS and follow-up (FU) time were calculated considering the date of MBM diagnosis and last patient contact or death. Kaplan-Meier estimates were used for the calculation of OS. Differences between groups were assessed using the log-rank test. Patients were grouped considering the timing of combined immunotherapy (first-line or not first-line) for treatment of MBM and according to BRAF mutation status (BRAFV600 mutant or BRAF wild type). Pretreatment protein S100B and LDH values were assigned categorical variables (normal, elevated and 2-fold or 10-fold elevated), according to the institutional upper limit of normal. Patients with missing values were excluded from the respective analysis. Further subgroups considering the number of MBM, presence of neurological symptoms and ECOG-PS were defined. To investigate the effect of local therapies on OS, data from patients receiving STR/surgery were compared with data from patients not receiving local therapies. Timing of local therapy and its effect on OS were analyzed by defining two groups: STR/surgery before start of NIVO+IPI treatment or STR/surgery at a later time point. Patients treated with whole brain radiotherapy (WBRT) were evaluated

Table 1 Patients characteristics of the whole collective (n=380) considering combined immunotherapy at first line or not at first line

Baseline characteristics	Total N (%)	CombiIT first line	CombiIT not first line	P value*
Sex				
Male	240 (63.2)	165 (66)	75 (57.7)	0.111
Female	140 (36.8)	85 (44)	55 (42.3)	
Age (years) at the time of CombiIT				
<54	153 (40.3)	90 (36)	63 (48.5)	**0.024**
54–64	105 (27.6)	69 (27.6)	36 (27.7)	
>64	122 (32.1)	91 (36.4)	31 (23.8)	
BRAF status				
BRAF wild type	138 (36.3)	112 (44.8)	26 (20)	**<0.0001**
BRAF mutant	242 (63.7)	138 (55.2)	104 (80)	
LDH level†				
Normal	189 (51.4)	131 (54.1)	58 (46.0)	0.223
Elevated	133 (36.1)	85 (35.1)	48 (38.1)	
2×>ULN	46 (12.5)	26 (10.8)	20 (15.9)	
S100B level†				
Normal	109 (32.8)	69 (31.2)	40 (36.4)	0.597
Elevated	156 (47)	106 (47.7)	50 (45.4)	
10×>ULN	67 (20.2)	47 (21.1)	20 (18.2)	
Number of MBM at the time of CombiIT†				
1–3	167 (46.8)	127 (53.6)	40 (33.3)	**<0.0001**
>3	190 (53.2)	110 (46.4)	80 (66.7)	
ECOG-PS†				
0	249 (66.4)	168 (67.7)	81 (63.8)	0.741
1	87 (23.2)	55 (22.2)	32 (25.2)	
>1	39 (10.4)	25 (10.1)	14 (11)	
Presence of symptoms†				
Yes	60 (31)	44 (32.1)	16 (28.6)	0.629
No	133 (69)	93 (67.9)	40 (71.4)	
Local therapy				
STR/surgery‡	220 (57.9)	135 (54)	85 (65.4)	**0.011**
No local therapy	90 (23.7)	71 (28.4)	19 (14.6)	
WBRT	70 (18.4)	44 (17.6)	26 (20)	

Bold values indicate statistically significant results.
*Pearson's χ^2 test.
†Denotes variables for which the missing/unknown values were excluded from the analysis.
‡Ten patients (4.5%) received only surgery. Four patients receiving STR/surgery before combined immunotherapy and two patients receiving STR/surgery after combined immunotherapy were treated with the two techniques within an interval of 2 weeks.
CombiIT, nivolumab plus ipilimumab; ECOG-PS, Eastern Cooperative Oncology Group performance status; MBM, melanoma brain metastases; n, number of patients in each subgroup; STR, stereotactic radiosurgery; ULN, upper level normal; WBRT, whole brain radiotherapy.

separately. Results are reported as two-sided p values with 95% CIs. Statistical significance was set at p<0.05.

RESULTS

Patients characteristics

A total of 380 patients with MBM and NIVO+IPI treatment were included in the analysis (table 1, online supplementary figure S1). Thirty-seven per cent of the patients were females and median age at the time of MBM diagnosis was 58 years (IQR 49–68). The majority of melanomas (63.7%) carried a BRAFV600 mutation.

In the univariate Cox regression analysis (table 2), we found the following significant prognostic factors for OS: LDH level, favoring patients with normal LDH (p<0.0001), number of MBM (p=0.001) favoring patients with 1–3 MBM and ECOG-PS (p=0.001) favoring patients with ECOG-PS=0. No significant OS difference was observed for baseline S100B level (p=0.099), BRAFV600

Table 2 Impact of baseline patient and disease characteristics on overall survival: univariate and multivariate Cox regression analysis

		Univariate analysis		Multivariate analysis	
	Total N (%)	HR (death) (95% CI)	P value	HR (death) (95% CI	P value
Gender					
Male	240 (63.2)	1		1	0.855
Female	140 (36.8)	0.94 (0.69 to 1.27)	0.682	1.35 (0.70 to 1.50)	
Age (years) at the time of CombiIT					
<54	153 (40.3)	1	0.616	1	0.689
54–64	105 (27.6)	1.13 (0.78 to 1.62)		1.17 (0.75 to 1.81)	0.491
>64	122 (32.1)	1.19 (0.83 to 1.70)		1.20 (0.76 to 1.90)	0.428
BRAF status					
BRAF wild type	138 (36.3)	1	0.962	1	
BRAF mutant	242 (63.7)	0.99 (0.72 to 1.37)		1.13 (0.76 to 1.67)	0.548
LDH level*					
Normal	189 (51.4)	1	**<0.0001**	1	0.069
Elevated	133 (36.1)	1.24 (0.88 to 1.74)		1.05 (0.69 to 1.59)	0.831
2×>ULN	46 (12.5)	2.53 (1.67 to 3.83)		1.80 (1.05 to 3.09)	**0.031**
S100B level*					
Normal	109 (32.8)	1	0.099	1	0.325
Elevated	156 (47)	1.35 (0.92 to 2.00)		1.39 (0.90 to 2.11)	0.135
10×>ULN	67 (20.2)	1.61 (1.02 to 2.54)		1.30 (0.76 to 2.24)	0.341
Number of MBM at the time of CombiIT*					
1–3	167 (46.8)	1	**0.001**	1	**0.008**
>3	190 (53.2)	1.74 (1.26 to 2.40)		1.67 (1.14 to 2.44)	
ECOG-PS*					
0	249 (66.4)	1	**0.001**	1	**0.006**
1	87 (23.2)	1.3 (0.91 to 1.87)		1.31 (0.87 to 1.99)	0.188
>1	39 (10.4)	2.58 (1.66 to 4.00)		2.42 (1.39 to 4.20)	**0.002**
Presence of symptomatic MBM*					
No	133 (69)	1			
Yes	60 (31)	1.46 (0.96 to 2.23)	0.078	N/A	

Bold values indicate statistically significant results (p<0.05).
*Denotes variables for which the missing/unknown values were excluded from the analysis.
CombiIT, nivolumab plus ipilimumab; ECOG-PS, Eastern Cooperative Oncology Group performance status; MBM, melanoma brain metastases; N
, number of patients in each subgroup; N/A, not performed for this factor, since information was available to only 50% of the patients; ULN, upper level normal.

mutation status (p=0.962), age groups (p=0.616), sex (p=0.682) and presence of symptomatic MBM (p=0.078). Multivariate Cox regression analysis using categorical variables (table 2) showed that the number of MBM (p=0.008) and ECOG-PS (p=0.006) were independent prognostic factors for OS. In the multivariate Cox regression analysis using numerical variables for age, serum LDH and protein S100B (online supplementary table S4), the following prognostic factors were found to be an independently associated with OS: LDH (p=0.001), protein S100B (p=0.001), number of MBM (p=0.017) and ECOG-PS (p=0.041).

Overall survival analysis considering systemic and local therapy

The mOS for the whole cohort was 19 months (95% CI: 15.9 to 22.0) and the median FU time was 18 months (IQR 9–28 months). The 1-year, 2-year and 3-year OS rates were 69%, 41.1% and 30.1%, respectively (table 3; figure 1A; 95% CI: 63.5 to 74.5; 34.9 to 47.9 and 22.2 to 37.9, respectively).

Figure 1B–E show the Kaplan-Meier OS curves considering BRAF mutation status, serum LDH level, number of MBM, protein S100B level, and online supplementary figure S2A–D show the Kaplan-Meier OS curves according to age groups, sex, ECOG-PS and presence of symptomatic MBM. The results shown are in line with what has been previously described in the univariate Cox regression analysis (table 2), that is, there is a statistically significant difference between the groups analyzed regarding serum LDH level (p<0.0001), number of MBM (p=0.001) and ECOG-PS (p<0.0001).

Stratifying for the best intracranial response (figure 1F), best OS was observed in patients with complete response (CR) and the difference between the subgroups with CR, partial response (PR), stable disease (SD) and progressive disease (PD) was statistically significant (p<0.0001). The mOS for patients with an intracranial CR or SD was not reached and for patients with PR and PD was 42 and 10 months, respectively (table 3; 95% CI: 22.6 to 61.4; 16.7 to 23.3, respectively). Patients achieving an intracranial CR had an improved 1-year OS rate of 92.7% compared with those with PD with a 1-year OS rate of only 39% (95% CI: 82.9 to 100; 31.4 to 46.6, respectively). Patients with SD showed favorable OS that was better than those with PR at 2 years and similar to PR at 3 years. The subgroups of patients with PR and SD did not differ significantly regarding serum LDH level, protein S100B, number of MBM, ECOG-PS or presence of extracerebral metastases.

Local therapy (STR/surgery) also improved OS (table 3, figure 2A): patients who received local therapy (at any time point of the course of the disease) reached a mOS of 24 months compared with patients without local therapy with only 16 months (p=0.009; 95% CI: 19.6 to 28.4 and 7.6 to 24.4, respectively). There was no significant difference in terms of patients' characteristics in these two groups, except for S100B level and presence of symptomatic MBM (online supplementary table S1). However, we need to acknowledge that information regarding the presence of symptomatic MBM was missing in approximately 50% of the patients.

When analyzing the time point of local therapy (ie, before or after NIVO+IPI), we found no significant difference in terms of patients' characteristics (online supplementary table S2) and the mOS was similar in the two subgroups (figure 2B; p=0.110). However, there seems to be a trend for a benefit of STR/surgery upfront (mOS=26 months vs 16 months; 95% CI: 21.1 to 30.9; 10.8 to 21.2, respectively). Patients who received WBRT had a mOS of 8 months (table 3, figure 2C; 95% CI: 4.9 to 11.0) and were analyzed separately.

No OS difference was observed for patients receiving first-line NIVO+IPI compared with those that received combined immunotherapy later (figure 2D; p=0.119). When looking at the patients' characteristics from these two groups, there was a significant difference between them regarding age, BRAFV600 mutation status, number of MBM and treatment with local therapy (STR/surgery) at the time of starting NIVO+IPI (table 1). These differences might contribute for similar OS outcomes regardless of therapy line.

In the subgroup of patients with BRAFV600 mutation (242 patients), 83 received first-line treatment with BRAF/MEK inhibitors, 138 received first-line NIVO+IPI and all received combined immunotherapy for MBM in the course of the disease. There was no OS difference when comparing first-line targeted therapy with first-line combined immunotherapy (figure 2E; p=0.085). The line of treatment for combined immunotherapy (first-line or not first-line) had no effect on survival outcome in patients with BRAF wild-type melanoma (figure 2F; p=0.996).

Regarding presence of symptomatic MBM, information was available for only 193 patients (online supplementary figure S2D), but there is a trend benefiting patients with asymptomatic MBM (p=0.065). However, if we consider only the patients that received first-line NIVO+IPI for MBM (n=137), the difference in OS between symptomatic and asymptomatic MBM is not significant (p=0.084; data not shown).

Safety

In the present cohort, a total of 236 (62%) patients were reported to have at least one immune-related adverse events (irAEs). In 142 (37%) patients, no irAEs were documented and there was no available information in 2 (1%) patients.

We found no difference (Pearson's χ^2 test) in terms of onset of irAEs in patients with 1–3 MBM compared with patients with >3 MBM (p=0.069). Regarding the onset of irAEs in patients who received STR/surgery versus those who did not receive STR/surgery, there was also no significant difference between the two groups (p=0.657). Finally, when analyzing the relation between receiving STR/surgery or not, and the interruption of therapy

Table 3 Median OS and 1-year, 2-year and 3-year OS rates

	mOS (months) (95% CI)	1-year OS (%; 95% CI)	2-year OS (%; 95% CI)	3-year OS (%; 95% CI)
All patients	19 (15.9 to 22.0)	69 (63.5 to 74.5)	41.1 (34.9 to 47.9)	30.1 (22.2 to 37.9)
Number of MBM				
1–3	29 (16.9 to 41.4)	71.2 (63.6 to 78.8)	57.0 (46.8 to 67.2)	42.3 (28.6 to 56.0)
>3	14 (10.2 to 17.9)	52.1 (44.3 to 59.9)	32.2 (23.9 to 40.4)	22.7 (13.5 to 31.9)
BRAF status				
BRAF wild type	19 (14.9 to 23.0)	61.3 (51.9 to 70.7)	40.1 (28.3 to 51.9)	N/A
BRAF mutant	18 (14.1 to 21.9)	60.7 (53.8 to 67.6)	42.0 (34.2 to 49.8)	27.3 (18.1 to 36.5)
LDH level				
Normal	21 (15.1 to 26.9)	69.3 (61.6 to 76.7)	45.9 (36.3 to 55.5)	32.6 (20.4 to 44.6)
Elevated	19 (12.8 to 25.1)	58.4 (48.8 to 68.0)	40.1 (29.1 to 51.1)	32.9 (19.9 to 45.8)
2×>ULN	7 (6.1 to 7.9)	32.1 (17.6 to 46.6)	22.9 (8.0 to 37.8)	8.6 (5.5 to 22.7)
S100B level				
Normal	22 (18.2 to 25.8)	78.4 (68.6 to 88.2)	44.7 (30.8 to 58.6)	36.1 (20.6 to 51.6)
Elevated	17 (9.6 to 24.4)	57.0 (48.2 to 65.8)	42.8 (32.8 to 52.8)	32.3 (20.5 to 44.6)
10×>ULN	17 (8.2 to 25.8)	53.5 (40.4 to 66.6)	30.9 (15.8 to 45.9)	20.6 (1.2 to 40.0)
Best intracerebral response				
CR	Not reached	92.7 (82.9 to 100)	85.6 (69.3 to 100)	N/A
PR	42 (22.6 to 61.4)	86.9 (76.9 to 96.9)	62.9 (46.0 to 79.8)	55.1 (34.5 to 75.7)
SD	Not reached	93.6 (86.5 to 100)	83.6 (71.1 to 96.1)	50.2 (19.0 to 81.4)
PD	10 (16.7 to 23.3)	39.0 (31.4 to 46.6)	20.0 (13.1 to 26.9)	12.8 (6.0 to 19.3)
CombiIT				
First line	17 (10.7 to 23.9)	56.4 (48.9 to 63.8)	44.7 (35.9 to 53.5)	27.9 (11.2 to 44.6)
Not first line	21 (17.8 to 24.2)	67.9 (59.9 to 75.9)	41.9 (32.7 to 51.1)	31.6 (21.8 to 41.4)
BRAF mutant patients				
First-line targeted therapy	22 (17.2 to 26.77)	65.6 (55.2 to 76)	44.3 (34.5 to 57.7)	32.0 (20 to 44)
First-line CombiIT	16 (7 to 25)	53.6 (43.2 to 64)	42.9 (30.7 to 55.1)	N/A
BRAF wild-type patients				
First-line CombiIT	21 (10.2 to 31.8)	59.6 (52.6 to 73.4)	47 (33.8 to 60.1)	47 (33.8 to 60.1)
First-line not CombiIT	19 (16.3 to 21.7)	68.3 (50.1 to 74.2)	31.9 (11.5 to 52.3)	31.9 (11.5 to 52.3)
STR/surgery (at any time point)				
Yes	24 (19.6 to 28.4)	70.6 (63.7 to 77.5)	49.5 (40.9 to 58.1)	36.5 (26.3 to 46.7)
No	16 (7.6 to 24.4)	53.2 (41.0 to 65.4)	40.9 (26.6 to 55.2)	N/A
WBRT	8 (4.9 to 11.0)	40.7 (28.4 to 53.0)	20.8 (9.4 to 32.2)	10.4 (1.4 to 22.2)
STR/surgery				
Upfront	26 (21.1 to 30.9)	72.5 (65.1 to 79.9)	50.9 (41.3 to 60.5)	39.5 (28.3 to 50.7)
Later	16 (10.8 to 21.2)	63.7 (47.6 to 79.8)	44.3 (24.9 to 63.7)	22.2 (1.5 to 45.9)
ECOG-PS				
0	22 (16.4 to 27.6)	65.7 (59.0 to 72.4)	47.1 (39.1 to 55.1)	36.4 (26.4 to 46.4)
1	18 (7.3 to 28.7)	52.3 (40.1 to 64.5)	38.0 (34.1 to 519)	22.2 (6.1 to 38.3)
>1	8 (7.3 to 17.1)	49.3 (31.8 to 66.7)	23.5 (5.5 to 41.5)	5.9 (5.1 to 16.9)
Presence of symptomatic MBM				
No	19 (10.7 to 27.2)	62.5 (53.4 to 71.8)	45.4 (34.6 to 56.2)	35.1 (21.8 to 48.4)

Continued

Table 3 Continued

	mOS (months) (95% CI)	1-year OS (%; 95% CI)	2-year OS (%; 95% CI)	3-year OS (%; 95% CI)
Yes	12 (7.0 to 17.0)	46 (32.1 to 59.9)	28.1 (13.8 to 42.4)	15.0 (0 to 30.7)

CombiIT, nivolumab plus ipilimumab; CR, complete response; ECOG-PS, Eastern Cooperative Oncology Group performance status; MBM, melanoma brain metastases; mOS, median overall survival; PD, progressive disease; PR, partial response; SD, stable disease; STR, stereotactic radiosurgery; ULN, upper level normal; WBRT, whole brain radiotherapy.

due to irAEs, we again found no significant difference between the two groups (p=0.913).

DISCUSSION

The present study shows that combined immunotherapy with NIVO+IPI can result in improved survival of patients with MBM, comparable to results in other stage IV patients. This is particularly true if intracranial CR, PR or SD has been achieved. The type of intracranial response is a strong predictor for OS. In our cohort, the 2-year OS rates of patients with SD, PR and CR ranged from 63% to 86%, whereas patients with PD had a 2-year OS rate of only 20% (table 3). Similar favorable results have been reported in the ABC trial, a randomized phase II study of nivolumab or NIVO+IPI in patients with MBM.[16] The 3-year intracranial PFS was above 90% for patients with asymptomatic, treatment-naïve MBM achieving an intracranial CR, and above 50% for patients with PR. We have no explanation why in our cohort patients with SD did better than patients with PR.

In our study, the 1-year and 2-year OS rate were 69% and 41%, respectively, in line with previous reports.[9 10] In the already mentioned ABC trial, patients who received

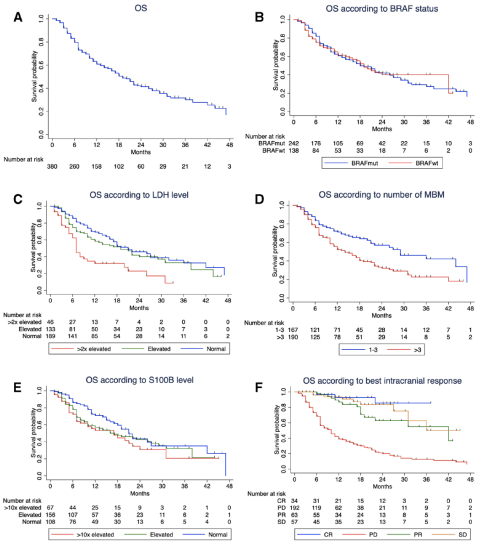

Figure 1 Kaplan-Meier curves for overall survival (A) and considering the different factors: (B) BRAF status; (C) LDH level; (D) number of melanoma brain metastases (MBM) at the time of therapy with nivolumab+ipilimumab; (E) protein S100B level; (F) best intracranial response. CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

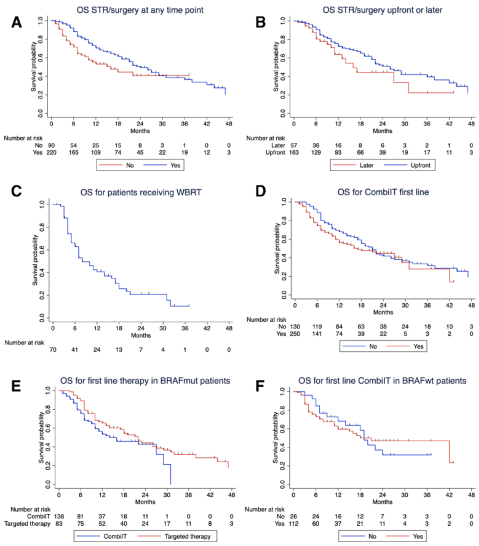

Figure 2 Kaplan-Meier curves for overall survival (OS) according to the following factors: (A) local therapy (STR/surgery, stereotactic radiosurgery or surgery); (B) time of local therapy (before or after combined immunotherapy with nivolumab+ipilimumab); (C) for patients receiving whole brain radiotherapy (WBRT); (D) combined immunotherapy for melanoma brain metastasis (MBM) in first line or later; (E) first-line therapy in patients harboring a BRAF mutation and (F) combined immunotherapy first line or later in BRAF wild-type patients. Patients treated with WBRT were excluded from the analysis in figure 2A. Ten patients (4.5%) from the STR/surgery group (n=220) received only surgery. In the Kaplan-Meier analysis in figure 2B, four patients receiving STR/surgery before combined immunotherapy and two patients receiving STR/surgery after combined immunotherapy were treated with the two techniques in an interval of 2 weeks.

combined immunotherapy had a 1-year and 2-year OS rate of 63%[16] and in the Checkmate-204 trial the reported 1-year OS rate was even higher (81.5%).[10] The survival rates in these trials are higher than those reported in our cohort. Compared with the ABC trial and the Checkmate-204 trial, which included patients with asymptomatic MBM and treatment-naïve BRAF wild-type patients, 31% (60/193) of the patients in our trial had symptomatic MBM and 20% of the BRAF wild-type patients were pretreated. In the Checkmate-204 trial, 17% of the patients had received previous systemic therapy for MBM and 52% had only one MBM compared with 34% pretreated patients and 53% patients with more than three MBM in our cohort.

Two studies evaluating pembrolizumab in patients with MBM also reported similar outcomes.[17 18] The first study evaluated treatment with pembrolizumab monotherapy in 23 patients with one or more asymptomatic and untreated MBM. With a longer follow-up of 38 months, the mOS time was 17 months (95% CI: 10 months to not reached) and the 2-year OS was 48%. These are in line with our results for patients who did not receive STR/surgery for whom the mOS was 16 months (95% CI: 7.6 to 24.4) and the 2-year OS rate was 41%. However, in this trial, only asymptomatic patients were included and 87% had <3 MBM, a population with potentially better outcome that the one included in our report. In the second study, Anderson *et al* reported the results of the combination from pembrolizumab and radiation therapy in 21 patients with MBM. Despite the low number of patients included, the percentage of lesions that had a CR (>30%), was higher than previously reported with systemic therapy or STR alone.

The combination of immunotherapy and local therapy with stereotactic irradiation or surgery improved patients' survival compared with patients who only received NIVO+IPI. This benefit might be related to a synergic effect between radiotherapy and immunotherapy that has been demonstrated both in preclinical and clinical studies.[19–23] The combination of radiation and immune checkpoint inhibitors seems to be effective both in the irradiated and non-irradiated lesions, and this effect might be associated with the activation of cytotoxic T-cells and reduction of myeloid-derived suppressor cells.[18 24 25]

The benefit of combining local and systemic therapy in MBM has been previously shown by our group and others, with mOS that range from 14 to 25 months and 1-year OS rates between 58% and 78% in the groups that received local and systemic therapy, clearly superior to the outcomes of patients receiving only systemic therapy (mOS between 6 and 13 months and 1-year OS rates ranging from 34% to 53%).[14 15 26–33]

In our study, the time point at which the patients received local therapy did not seem to play a significant role in OS: local therapy performed upfront or after initiation of NIVO+IPI resulted in similar OS rates, with a trend benefiting local therapy upfront (mOS 26 months vs 16 months). Different retrospective studies have also addressed this question, and, similar to our cohort, upfront local therapy seems to have better outcomes (mOS of 11–23 months in the group receiving local therapy upfront and 3–9 months in patients receiving local therapy after systemic therapy).[34 35]

There is still an ongoing debate whether some patients might be better served with systemic therapy alone, as we see very positive outcomes.[9–11 36] Not applying local therapy reduces local complications, potential cognitive impairment and might be particularly adequate for patients with a low number of asymptomatic MBM. This question along with the best sequence regarding local therapy is being addressed in ongoing clinical trials, and in the future, we might be better equipped to decide which patients to treat with the different modalities.[37 38]

In this study, there was a high proportion of patients with BRAFV600-mutated melanoma (63%), but similar to other publications where this subgroup represents between 52% and 65% of the patients.[14 15 26 28] Previously, it has been postulated that even in patients with BRAFV600-mutated MBM, first-line systemic treatment should consist of combined immunotherapy. Our analysis showed that there was no difference in OS of patients receiving first-line NIVO+IPI or first-line targeted therapy followed by combined immunotherapy (p=0.085). The two subgroups did not differ significantly (online supplementary table S4), except for the number of MBM, where a higher proportion of patients with >3 MBM received first-line targeted therapy (p=0.002). Our results in this subgroup need to be interpreted with caution since we have not included patients with BRAFV600 mutation who only received targeted therapy.

In the multivariate Cox regression analysis, we identified LDH, S-100B, ECOG-PS and number of MBM as independent prognostic factors. These prognostic factors have already been described in previous analyses,[8 14 39–41] but to the best of our knowledge, S100B has only been described as independent prognostic factor for checkpoint inhibitor immunotherapy in one monocentric study.[42] It is interesting, however, that both tumor markers, LDH and S100B, remained independent prognostic factors in the multivariate analysis, suggesting that these non-invasive and easy to determine blood parameters can and should be used early in the course of the disease to inform about patients' prognosis.

Regarding the presence of symptomatic MBM, there was no OS differences between patients with and without symptoms (p=0.065), but a trend can be seem showing that patients with symptomatic MBM have worse prognosis that those who are asymptomatic (1-year OS rate 46% and 63%, respectively). In other prospective studies investigating similar cohorts, the OS rate ranged from 66% at 6 months[43] to 31% at 12 months.[16] Unfortunately, information regarding the presence of symptomatic MBM is missing in approximately 50% of the patients in our study, and therefore, definitive conclusions cannot be drawn from our data.

Strengths of this investigation are that data from 23 German-certified skin cancer centers with high standards

for data quality were included. Three-hundred and eighty patients were analyzed which is thus far the largest published cohort of patients with MBM managed in a routine clinical setting. This high number of patients allowed us to perform subgroup analyses, with results of reasonable sensitivity. Furthermore, this study provides long-term follow-up data of patients with MBM covering a period of up to 18 months.

The study limitations are related to its retrospective design. Patients were included regardless of previous systemic and local therapies prior to the combined immunotherapy and thus some heterogeneity of the study population might have contributed to differences in survival outcomes observed in our cohort. The decision to offer local therapy or not was probably influenced by the number and size of MBM. Additionally, the maximum number of MBM considered to be treated individually by STR/surgery might vary between different centers. We have not evaluated intracranial toxicities. However, this aspect might have been considered when planning local therapy and targeted therapy in patients with BRAFV600-mutated melanoma, influencing the systemic therapy offered as well as the therapy sequence in this subgroup.

In conclusion, our study shows that treatment with NIVO+IPI, particularly in combination with STR/surgery improves survival of patients with MBM. Results presented herein also suggest that local therapy with STR/surgery either before or after starting combined immunotherapy might be advantageous to prolonging OS.

Author affiliations
[1]Center for Dermatooncology, Department of Dermatology, Eberhard Karls University of Tuebingen, Eberhard Karls University of Tuebingen, Tubingen, Germany
[2]Skin Cancer Center, Department of Dermatology, Charité Universitätsmedizin Berlin, Berlin, Germany
[3]Skin Cancer Center, Department of Dermatology and National Center for Tumor Diseases (NCT), University Hospital Heidelberg, Heidelberg, Germany
[4]Department of Dermatology, University Medical Center Mainz, Mainz, Germany
[5]Skin Cancer Center, Department of Dermatology, University Hospital Kiel, Kiel, Germany
[6]Skin Cancer Center, Department of Dermatology, University of Lübeck, Lübeck, Germany
[7]Department of Dermatology, University Hospital Wuerzburg, Wuerzburg, Germany
[8]Skin Cancer Center Hannover, Department of Dermatology, Hannover Medical School, Hannover, Germany
[9]Department of Dermatology, University of Regensburg, Regensburg, Germany
[10]Skin Cancer Unit, German Cancer Research Center (DKFZ), Heidelberg, Germany
[11]Department of Dermatology, Venereology and Allergology, University Medical Center Mannheim, Ruprecht-Karl University of Heidelberg, Mannheim, Germany
[12]Department of Dermatology, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität (FAU), Erlangen-Nürnberg, Germany
[13]Department of Dermatology and Allergy, University Hospital Munich, Ludwig Maximilian University, Munich, Germany
[14]Skin Cancer Center, Department of Dermatology and Venerology, Medical Centre University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany
[15]Skin Cancer Center at the University Cancer Center and National Center for Tumor Diseases Dresden, Department of Dermatology, University Hospital Carl Gustav Carus at the TU Dresden, Dresden, Germany
[16]Department of Dermatology, University Hospital Magdeburg, Magdeburg, Germany
[17]Department of Dermatology and Allergy, Technical University of Munich, School of Medicine, Munich, Germany
[18]Sigmund Freud Universität Wien, Faculty of Medicine, Wien, Austria
[19]Skin Cancer Center, Department of Dermatology, Ruhr-University Bochum, Bochum, Germany
[20]Department of Dermatology, Saarland University Medical School, Homburg/Saar, Germany
[21]Department of Dermatology, Venereology and Allergology, University Medical Center Göttingen, Göttingen, Germany
[22]Department of Dermatology and Allergology, Augsburg Medical Center, Augsburg, Germany
[23]Skin Cancer Center, Department of Dermatology, Paracelsus Medical University, General Hospital Nuremberg, Nuremberg, Germany
[24]HELIOS Klinikum Erfurt, Erfurt, Germany
[25]Clinic for Dermatology and Venereology, University Medical Center, Rostock, Germany
[26]Clinic of Radiation Oncology, Eberhard Karls University of Tuebingen, Tuebingen, Germany
[27]Department of Dermatology, University Hospital Essen, Essen, Germany

Twitter Teresa Amaral @TeresaSAmaral

Contributors Study concept: TA, TE, CG, LZ. Data collection: all authors. Data analysis: TA, TE, CG, LZ. Data interpretation: TA, RG, CB, ChP, TG, JH, FM, TE, CG, LZ. Writing: all authors. Final approval: all authors. Agreement to be accountable for all aspects of the work: all authors.

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ORCID iDs
Teresa Amaral http://orcid.org/0000-0002-2516-5181
David Rafei-Shamsabadi http://orcid.org/0000-0002-7850-3199

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7. Discussion

7.1. Systemic treatment of stage IV cutaneous melanoma

Treatment of advanced melanoma has changed amazingly in the last decade, and the results presented in this thesis confirm that. Ten years ago, only chemotherapy with dacarbazine, was approved for treating stage IV melanoma. [49] Non-approved chemotherapies, namely temozolomide, and different combinations of cytotoxic therapies were also used, with marginal benefit. Objective responses to dacarbazine were reported in approximately 25% of the patients in phase II trials. However, later investigations showed that the real percentage was about 5%-12%, and the percentage of patients that derived a long-term benefit was extremely disappointing between 1%-2%. [133-135] Intense investigation was already on the way for other therapeutic options as mentioned by Garbe et al [49], namely CTLA-4 inhibitors and BRAF/MEK inhibitors, but the data available was not enough to grasp the future impact of these therapies.

The first manuscript included in this thesis shows that, in our population of patients treated between 2011 and 2014, there was still an important percentage of patients treated with chemotherapy (51.5%; n=132), particularly dacarbazine and carboplatin plus paclitaxel. [136] Immunotherapy with checkpoint inhibitors was mostly represented by ipilimumab monotherapy. In our analysis, 42 patients treated with ipilimumab were included, representing 80.8% of all the patients treated with immunotherapy and 20% of all patients treated with first-line systemic therapy. Finally, 49 patients were treated with BRAF inhibitors, namely vemurafenib or dabrafenib, which represented 72% of the patients treated with targeted therapy, and 23% of all patients treated with first-line systemic therapy.

We showed that the OS for stage IV melanoma patients has steadily improved over the years: the 1-year (1-y) OS rate for M1a patients increased from 62% to 85%, for M1b patients improved from 53 to 74%, and for M1c patients from 33 to 52%, when compared

with data from Balch et al from 2009. [137] As the indication, for starting systemic therapy, and performing surgery in stage IV melanoma didn't change in the period analyzed, the improvement of OS in each M sub-stage was due to the availability of more effective systemic therapies.

When looking into the type of therapy received, we also saw a significant improvement compared with the historical data referred by Garbe et al. [49] The median OS (mOS) was 33 months, 16 months, and 11 months for immunotherapy, targeted therapy, and chemotherapy (95% CI: 21.7-44.3; 10.6-21.4 and 7.6-14.4, respectively) and the difference between the three groups was statistically significant (p= 0.003). Our OS results for patients treated with immunotherapy and targeted therapy were in line with the previously reported in clinical trials for treatment naïve patients receiving ipilimumab, and patients treated with dabrafenib monotherapy, i.e., the mOS was 30 months and 19 months, respectively (95% CI: 16.6-not reached and 15.2–23.7). [138,139] The 3-y OS rate for the patients treated with immunotherapy, in our cohort mostly ipilimumab, was 37.4% (95%CI: 16.6-58.2). These OS rates were slightly better than the ones from a pooled analysis reporting the 10-y OS rates of patients treated with ipilimumab monotherapy in different clinical trials.[64] However, in this pooled analysis the patients included were both treatment naïve patients and patients that have already received other systemic therapies, which might explain the better outcomes n our cohort, which included only treatment naïve patients. Schadendorf et al. showed that an OS plateau forms around the three years, with a 3-y OS rate of 21% (95% CI: 17%-24%). These results confirmed that approximately one third to one fourth of the patients receiving ipilimumab alone can derive long survival benefit, and led for the first time to a discussion whether stage IV melanoma patients could be cured. This was an historical in the melanoma field.

7.2. Primary resistance to PD-1 based immunotherapy

The immune-checkpoint inhibitors pembrolizumab, nivolumab, ipilimumab, and the combination of nivolumab plus ipilimumab are approved in Europe for the treatment of stage IV melanoma. [65,86,87] The EMA's approval was based on the results from phase II and phase III trials, showing sustained benefits in both PFS and OS. [78] [60] [79-82,84,138] The 5-y OS rate for nivolumab plus ipilimumab, nivolumab monotherapy and pembrolizumab is 52%, 44% and 34-41%, and the 5-y PFS rate is 36%, 26% and 21-29%, respectively.

Despite these very positive outcomes, primary resistance to immunotherapy, defined as the absence of benefit from immunotherapy, still is observed in a rather high percentage of patients. It is estimated to be between 40% and 65%, depending on whether patients receive first-line immunotherapy or immunotherapy after progression under other systemic therapies. [76] [90,140] Higher percentages were observed when patients were treated with ipilimumab monotherapy. [141] [142] For patients with primary resistance to immune checkpoint therapy, there are not many therapeutic options available, particularly if they have BRAF wild-type tumors.

In manuscript number 2, we analyzed the survival outcomes of stage IV melanoma patients treated with first-line PD-1 based immunotherapy between 2015 and 2018.[143] By then, pembrolizumab, nivolumab, the combination of nivolumab plus ipilimumab, and two combinations of BRAF/MEK inhibitors were already available in the clinical practice. We addressed the following questions: (1) Which factors are associated with the development of primary resistance? (2) How does survival of patients with primary resistance compare to those with disease control (CR, PR, and SD)? (3) Did the patients with primary resistance to PD-1-based immunotherapy receive further therapies, and if so, which therapies were offered and what was the outcome? We defined primary resistance as the presence of PD using the RECIST 1.1 criteria [144] at the time of the first radiological evaluation, after starting first-line PD-1 based immunotherapy.

In our cohort, we found that 40% of the patients (127/319) were primary resistant according to our definition. Our results confirm the percentages previously reported by Robert et al., and Larkin et al. [76,90,140] We further showed that patients with primary resistance have a highly significantly unfavorable PFS and OS as compared to those who achieve CR, PR or SD ($p < 0.0001$). The mOS in patients with primary resistance was only 11 months (95% CI: 9.0–13.0), which is similar to what was observed in patients receiving first-line chemotherapy in our series from 2011-2014. [136] These unfavorable outcomes stress the need for further investigation on the mechanisms associated to primary resistance to immunotherapy.

The percentage of patients achieving a CR or PR in our study, approximately 40%, was very similar to the one reported in the Checkmate 067 trial, [78] and achieving an objective response (CR or PR) was decisive for favorable OS. This seems to be true not only for the metastatic setting, as in our cohort, but also in the neo-adjuvant setting, as we saw in the OpACIN-neo trial. [145] Here, patients with an objective response, in this case a pathologic objective response, had a numerically higher long-term benefit compared to those with no pathologic objective response. The estimated 24-months relapse free survival was 97% and 36% for patients with and without a pathological objective response, respectively (95% CI: 93-100% and 17-74%).

We saw no differences in terms of OS for patients receiving PD-1 monotherapy or combination of nivolumab plus ipilimumab, but the two groups were not homogenous, and therefore no definitive conclusions can be drawn. Furthermore, the follow-up of 22 months is shorter than the one from the Checkmate 067 trial, and in this study the clinically significant OS differences were seen only after 48 months of follow-up. [78]

Finally, and similar to other series, only half of the patients with primary resistance received a second-line therapy. [146] The median PFS and the mOS were 3 months and 10 months, respectively (95%CI: 2.4-3.6 and 6.9-13.1). The response rate was extremely

low, only 11%, in patients with BRAF wild-type tumors. In patients with BRAFV600 mutated melanoma, the response rate was 66%, and slightly superior to others previously published. [147] This high response rate is explained by the fact that the majority of the patients received second-line targeted therapy with BRAF/MEK inhibitors. Also, in the metastatic setting, immunotherapy followed by targeted therapy seems to derive greater benefits than the inverse sequence. [148,149]

7.3. Combined BRAF/MEK inhibitors in Stage IV BRAFV600 mutated cutaneous melanoma

Currently, there are three different combinations of BRAF/MEK inhibitors available for treatment of stage IV BRAFV600 mutated melanoma patients: dabrafenib plus trametinib, vemurafenib plus cobimetinib and encorafenib plus binimetinib. [150-155] Monotherapy with BRAF inhibitors was abandoned due to the superior outcomes of the combination, and the only MEK inhibitor approved to be used as monotherapy is cobimetinib. [100,103,131,139]

The three BRAF/MEK combinations differ in terms of safety profile, and this is the aspect that is most frequently considered when choosing one of the three. [156,157] Contrary to the toxicity profile, the efficacy results are very similar in the metastatic setting. The response rates reported in clinical trials were 64%–67% for dabrafenib plus trametinib, 68% for vemurafenib plus cobimetinib, and 75% for encorafenib plus binimetinib. [131,139] [158] [103] The landmark survival analyses showed 2-y OS rates of 53%, 48% and 58%, respectively. [159] [100] [132]

Two major aspects need to be considered when defining the therapeutic plan in patients with advanced BRAF mutated melanoma. Firstly, patients can either receive PD-1 based immunotherapy or BRAF/MEK inhibitors. Since no prospective head-to-head data are available, this decision is based mainly on patient characteristics and preference as well

as the physicians' preference. Treating physicians tend to use first-line BRAF/MEK inhibitors in BRAFV600 mutated patients with worse prognosis, for whom a rapid response is necessary. [160] This subgroup of patients has been extensively characterized, and includes patients with symptomatic disease, high tumor volume, and elevated baseline LDH, which was the strongest predictive factor. [104,161] Secondly, the specific regime needs to be chosen. If BRAF/MEK inhibition is recommended, one the approved combinations can be chosen. However, since no head-to-head comparison is or will be available in the future, only indirect comparisons are possible. In manuscript number 3, [105] we performed such an indirect comparison, and evaluated whether patients with elevated baseline LDH could derive greater benefit from a particular BRAF/MEK combination.

ECOG PS and degree of organ involvement, were similar across the three trials evaluated. [131] [100] [103] However, the coBRIM trial included the highest percentage of patients with elevated baseline LDH in the combination arm, 46%, compared with only 34% and 29% in the COMBI-v and the COLUMBUS trials, respectively. In publications reporting data from real-world setting, up to 48% of patients receiving first-line BRAF/MEK inhibitors showed an elevated baseline LDH resembling the coBRIM cohort.[162,163] Our results show a non-significant risk reduction for progression and death in the subgroup with elevated baseline LDH receiving first-line therapy vemurafenib plus cobimetinib, compared with dabrafenib plus trametinib, and encorafenib plus binimetinib. However, we saw no OS benefit of vemurafenib plus cobimetinib compared with dabrafenib and trametinib, and only a slight advantage when comparing with encorafenib and binimetinib. Although deriving from an indirect comparison, these data may provide guidance for treatment recommendations in stage IV melanoma patients with elevated baseline LDH.

7.4. Systemic treatment of stage IV uveal melanoma

Uveal melanoma has always been associated with a poor prognosis. [164] Despite the high rates of local tumor control, most patients will eventually die of metastasis. The particular pattern of metastatic spreading is associated with the presence of liver metastasis in the vast majority of patients, and this is the leading cause of death. [165] Because of that, local therapies namely surgery, SIRT, chemosaturation and TACE have been used to approach treatment of stage IV disease. [32,35,36] [166,167] The expertise needed to execute these technics, along with a learning curve that is associated with performing liver-directed therapies, should speak for the need of referring uveal melanoma patients to a specialized center. Clinical trials specifically evaluating systemic therapy in stage IV uveal melanoma patients are scarce. Older protocols evaluating chemotherapy alone or in combination with interferon showed no survival benefit. [166,168-170] Despite the presence of driver mutations involving *GNAQ* or *GNA11* genes, and the downstream convergence on common signaling pathways such as MAPK and PI3K/AKT, targeted therapies have not been able to show clinically meaningful benefit in uveal melanoma. [171-176]

Monotherapy with immune checkpoint inhibitors, namely ipilimumab, pembrolizumab, nivolumab and atezolizumab showed modest benefits, with low response rates of 3.6% to 4.7%, median PFS between 2.6 and 3.1 months, and mOS between 7.6 and 14 months [40,41,177] Different authors have tried to identify predictive biomarkers of response to systemic therapy, but until now, none is used in the clinical practice. [178,179]

In manuscript number 4, we reported the survival outcomes of uveal melanoma patients treated with PD-1 plus CTLA-4 in a multicenter study. [39] Our results showed an overall response rate to of 15.6%, which is superior to checkpoint inhibitors monotherapy, [40,41,177] and in line with other reports on combined immunotherapy outcomes in this population. [180-182] The median duration of response was 25.5 months (range 9.0–65.0). Stable disease was achieved in 21.9%, resulting in a disease control rate of 37.5% with a median

duration of the clinical benefit of 28.0 months (range 7.0–65.0). The median PFS was 3.0 months (95% CI 2.4–3.6) and the median OS was estimated to be 16.1 months (95% CI 12.9–19.3), also superior to previous reports with monotherapy. Altogether, our study implies that combined checkpoint blockade represents the hitherto most effective treatment option available for metastatic uveal melanoma available outside of clinical trials, with an expected overall response rate of 15–17%. Considering the data available so far, we conclude that the increased overall response rate of combined immunotherapy versus PD-1 inhibition alone amounts to approximately 10%.

The results of this multicentric study have been used by the UM Cure 2020 group, which includes Portuguese investigators, to inform therapeutic options in countries where combined immunotherapy is not available or is not reimbursed. [183] Clinical trials investigating immune checkpoint inhibitors in combination with other systemic or local therapies in the metastatic setting, and targeted therapies is the adjuvant setting are ongoing or have recently been reported. [184-188] The results are not better than the ones we reported, and, therefore, there is no SOC for stage IV uveal melanoma. The European effort for boosting the investigation in this area is definitely to be commended, as only a multicentric and multinational approach can lead to a leap forward in the investigation on this area of particular need.

7.5. Systemic and local treatment of melanoma brain metastases

Patients with MBM have a particular dismal prognosis acknowledged by a new subgroup – M1d - in the 8th AJCC classification. [44] Approximately half of the patients with advanced melanoma develop brain metastases and more than 90% will also have extracerebral disease. [189] These percentages highlight the necessity of performing a complete staging at the time of stage IV diagnosis, including a brain CT scan. [190] Waiting for symptoms of

brain metastasis leads to a later diagnosis, and potentially to a worse outcome, as the tumor burden might be higher. [191] The follow up of the response to local and systemic therapy should be performed using MRI, as the brain CT scans have lower acuity in this setting. [189] In the ESMO Guidelines there are, however, no specific recommendations for follow-up in patients with MBM. [191]

Prognostic factors in MBM include the number, extent and localization of brain metastases, the presence and extent of extracerebral disease, baseline LDH, age, tumor burden, and ECOG PS.[192-194]

Four clinical trials have specifically evaluated systemic therapy in MBM – the ABC trial, the Checkmate 204 trial, the COMBI-MB trial and the BREAK-MB trial. [106-109] The two first ones evaluated PD-1 based immunotherapy in treatment naïve patients. The COMBI-MB study evaluated targeted therapy with dabrafenib and trametinib in three different cohorts of patients that included patients treated or not treated with local therapy. The BREAK-MB study evaluated dabrafenib monotherapy in patients with melanoma brain metastases with or without previous local therapy.

Manuscripts 5 and 6 evaluated the outcomes of these same therapies in two different cohorts. [112,123] In total, these two publications accessed the survival outcomes of 543 patients. Our results showed that PD-1 based immunotherapy and BRAF/MEK inhibitors are effective in patients with MBM treated in a real-world setting, similar to what has been shown in clinical trials.

The main finding in manuscript number 5 was the considerable impact of immunotherapy in OS, particularly in combination with local therapy (stereotactic radiosurgery or surgery). Combination of local therapy with immunotherapy, targeted therapy or chemotherapy resulted in a mOS of 25 months, 14 months and 11 months, respectively (95% CI: 14.6–35.4 and 12.1-15.9 and 8.4-13.6). In our collective the 1-y OS rate in the group receiving immunotherapy was higher than previously reported by other authors. [118,195-198]

This is probably explained by the fact that in our collective a significant percentage of patients received PD-1 plus CTLA-4 and local therapy, while the other studies evaluated CTLA-4 or PD-1 monotherapy in combination with local therapy. In patients treated only with systemic therapy, the 1-y OS rate of the group treated with targeted therapy was particularly lower when compared to results the COMBI-MB study - 29.5% versus 46% (95% CI: 16-43 and 33-58, respectively), [108] highlighting the need for further investigations involving this subgroup of patients.

We extensively debated the fact that no survival benefit was seen in patients treated with whole brain irradiation. The mOS was only 5 months (95% CI: 3.9-6.1), not so different from what our group reported in 2011. [199] Since effective targeted and immunotherapies are available in clinical practice, and achieve good results also when used alone, treatment with whole brain radiation should be judged critically, as the toxicity is also higher compared to stereotactic radiosurgery. [200] In our center and others, this therapy is reserved for symptomatic patients without local or systemic therapeutic options, in line with the recommendations from the European guidelines. [201] However, the definition of multiple brain metastases is foggy, and depends, in the majority of the cases, on the expertise of the treating center. A clearer definition of multiple brain metastases is needed, as we see that in some centers radiosurgery of single brain metastases is offered to patients with up to twelve brain metastases, while in others, the patient is classified as having multiple brain metastases when 4 or more metastases are present. [202] This inevitably leads to a potential bias and might explain the difference in terms of outcomes between retrospective series.

In manuscript number 6, we focused on the survival outcomes of patients with MBM treated with nivolumab plus ipilimumab in 23 centers. We confirmed that nivolumab plus ipilimumab improves survival in this subgroup, comparable to other stage IV patients. This is even more evident, in patients achieving disease control (CR, PR or SD).

Moreover, the reported 1-y and 2- y OS rates (69% and 41%) are comparable to those of the ABC trial (1-y and 2-y OS rate of 63%) and Checkmate 204 trial (1-y OS rate 81.5%). The minor differences can be explained by the different collectives evaluated in each study. Compared with the ABC and the Checkmate 204 trials, which included patients with asymptomatic MBM and treatment-naïve BRAF wild-type patients, 31% (60/193) of the patients in our study had symptomatic MBM, and 20% of the BRAF wild-type patients were pretreated. In the Checkmate 204 trial, 17% of the patients had received previous systemic therapy for MBM and 52% had only one MBM, compared with 34% pretreated patients and 53% patients with more than three MBM in our cohort.

The survival outcomes from combining local therapy and systemic therapy were in line with what we have shown in manuscript number 5, and favored the combination of local plus systemic therapy (p=0.009). Receiving local therapy upfront or later had no statistically significant impact in survival, but a trend was seen favoring local therapy upfront, confirming data previously reported by us and other groups. [121,122] Currently, several centers use the “treatment on demand” approach, i. e., patients receive local therapy only for the progressing brain metastasis, while the same systemic therapy is continued, if the extracerebral disease is stable.

In our cohort of patients treated with checkpoint inhibition first-line or later, in the sub-group of patients with BRAFV60 mutated melanoma we found no differences in terms of OS when receiving first-line either BRAF and MEK inhibitors or nivolumab plus ipilimumab (p=0.085). Based on our results we cannot produce a definitive conclusion whether BRAFV600 mutated patients should be treated upfront with targeted or immunotherapy, as we did not include the patients that only received targeted therapy. However, results from trials evaluating systemic therapy in stage IV melanoma, showed a higher survival benefit of nivolumab plus ipilimumab in the sub-group of BRAFV600

mutated melanoma.[78] In BRAF wild-type patients, we saw no difference in OS when receiving nivolumab plus ipilimumab in first-line or later (p=0.996).

7.6. Safety profile of immune checkpoint inhibitors and BRAF/MEK inhibitors

Targeted and immunotherapies have different safety profiles. This aspect has not been extensively addressed in any of the publications generated by this thesis. Yet, we have presented data on the toxicity induced by PD-1 based immunotherapy in patients treated in our center and others, and a publication is in preparation addressing toxicity associated with BRAF/MEK inhibitors, particularly cardiovascular toxicity. [203]

Table 2 and **Table 3** refer to the different toxicity in the most important clinical trials. **Figure 4** and **Figure 5** illustrate the potential mechanism associated with immune-related adverse events (irAE) and propose an algorithm to managing irAE. [204] In clinical trials, we see no significant differences between the toxicity from nivolumab and pembrolizumab, and choosing one or the other has more to do with the patients' and treating physician preferences, and the frequency of the cycles – every 2 or 4 weeks for nivolumab, and every 3 or 6 weeks for pembrolizumab.

For the combination of nivolumab plus ipilimumab versus nivolumab and ipilimumab alone, the differences are related not only to the frequency of the AE but also to their severity. The combination induces a higher percentage and more severe AE than both monotherapies isolated. [78] For ipilimumab, the onset of AE is normally between week 2 and 8, but later onset of AE has been documented. The most frequent AE reported for PD-1 monotherapy was fatigue, and grade 3 and 4 AE are less common with PD-1 monotherapy than with ipilimumab. With the combination of nivolumab plus ipilimumab, almost 95% of patients reported treatment related AE, and more than half of these AEs

were CTCAE grade 3 or higher. [90] These toxicities may develop earlier, and be apparent over a longer period of time. [205]

Regarding targeted therapy, the frequency of AE between the three combinations available is similar. [157] However, the type of AE is different, and this aspect is the one that frequently leads to choosing one or the other combination. Dabrafenib induces almost no photosensitivity compared to vemurafenib (41%). It also induces fewer keratoacanthomas and squamous cell carcinomas (7% versus 20-30%). Arthralgia (56%), fatigue (46%) and rash (41%) were commonly reported with vemurafenib treatment. [95] On the other hand, pyrexia is the most common problem associated with dabrafenib treatment, with almost 50% of the patients reporting pyrexia that leads to treatment interruption. [157] As for encorafenib plus binimetinib, the most frequent AE were gastrointestinal (28% to 40%). The cutaneous AE were manageable, and in a percentage between 3% to 13%, similar to dabrafenib plus trametinib and lower than for vemurafenib plus cobimetinib. [103] Finally, MEK inhibitors are associated with ophthalmological toxicity, which is a class effect and normally requires treatment delay. Patients with previous history of ophthalmological issues should be evaluated before treatment start. [206]

As always, patients' collaboration is the backbone for a successful management of toxicity. A structured first appointment, experienced nurses and medical doctors, as well as a dedicated ambulatory support, is one of the reasons why CTACE grade 3 and 4 AE are not so frequently seen in our population of patients.

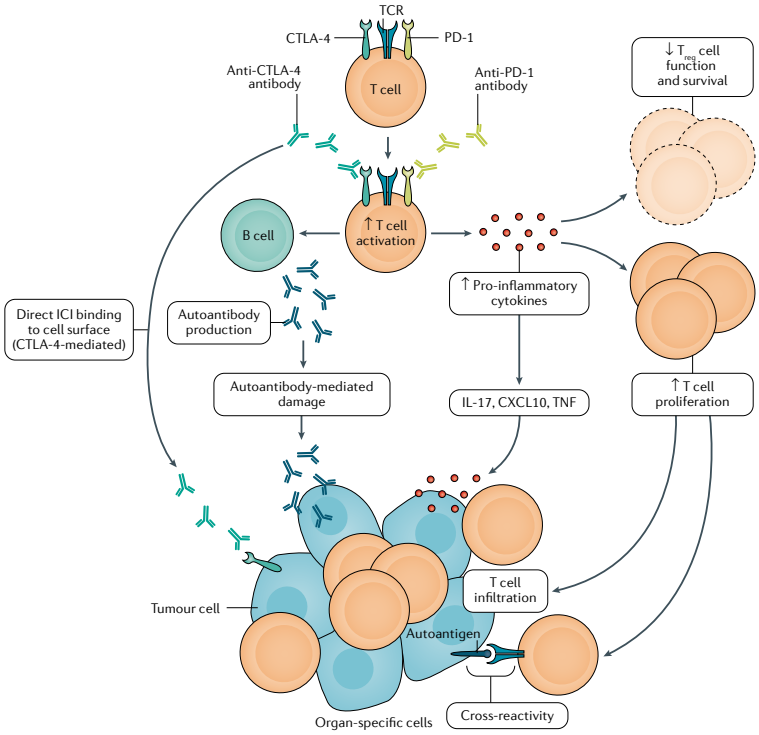

Figure 4: Mechanism of immune-related adverse events associated with immune checkpoint inhibition

The mechanisms of irAE associated with ICI depend on the type of ICI, i.e., anti-PD-1/PD-L1 or anti- CTLA-4. CTLA-4 inhibitors are able to induce as T cell activation and proliferation, impaired CD4+CD25+ regulatory T cell (Treg cell) survival, and increased counts of type 17 T helper cells. CTLAS-4 inhibitors are also able to induce cross-reactivity between anti-tumor T cells and antigens on normal cells, as well as autoantibody production. PD-1 and PD-L1 inhibitors are able to reduce Treg cell survival and Treg cell inhibitory function. They also increase cytokine production. TCR, T cell receptor; TNF tumor necrosis factor. From *Ramos-Casals et al.* [204]

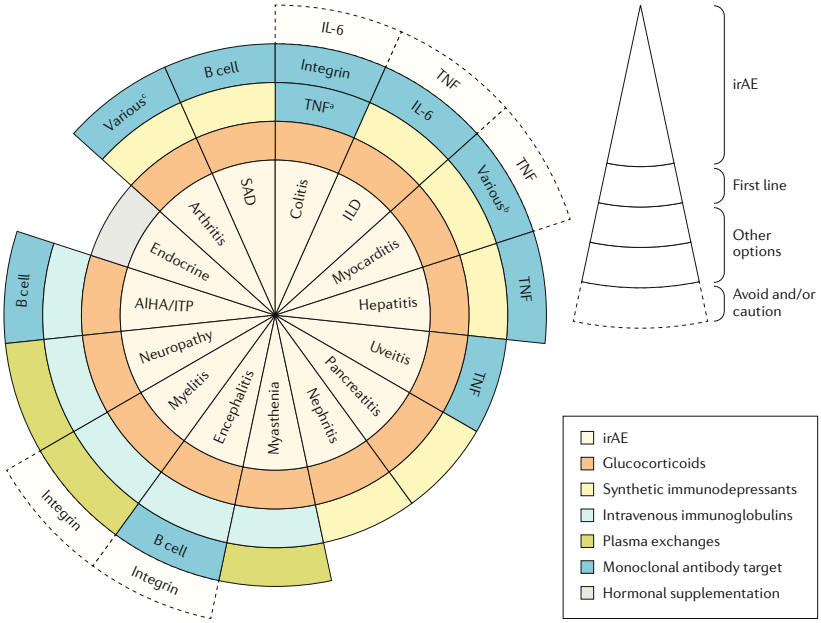

Figure 5: Proposed therapeutic algorithm for the management of immune related adverse events

The first-line therapy for patients who develop irAE while receiving treatment with ICI is glucocorticoids, except for adverse events affecting the endocrine system. Other therapies can be considered for severe or refractory cases, including other immunosuppressive therapies, intravenous immunoglobulin, plasma exchange and monoclonal antibodies. These therapeutic suggestions are based on official guidelines, retrospective analysis, published case reports, and the authors personal experience.
[a)] Avoid etanercept owing to the risk of autoimmune inflammatory colitis. [b)] Consider abatacept or alemtuzumab. [c)] Consider infliximab or tocilizumab. AIHA/ITP, autoimmune hemolytic anemia/immune thrombocytopenic purpura; ILD, interstitial lung disease; SAD, systemic autoimmune diseases; TNF, tumor necrosis factor. From *Ramos-Casals et al.* [204]

8. Limitations and conclusions

The limitations of this study were extensively described and discussed in the respective publications. They include the observational nature of the data, the absence of a population-based sample, the potential referral bias to the centers from which patients were included, the monocentric analysis in three publications, and the potentially limited follow-up time.

Nonetheless, we provided insights on patterns of care in distinctive centers in Germany, that might be different from those in other European countries, namely Portugal. This can be explained by the earlier availability of the new systemic therapies, compared to other countries, and the elevated number of German patients included in clinical trials.

By using observational data, we were able to inform on the reproducibility of clinical trials' survival data in a real-world setting. Observational data can further advise on the best strategy to be used in the design of future clinical trials.

Finally, we highlighted the need of having a well-designed and continuously updated population-based and therapy-based registry. Data derived from such registries enable clinicians and investigators to examine regional and international differences with an educated opinion on the potential causes of these disparities, and facilitate discussions on how to reduce the obstacles to optimal care.

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10. Curriculum vitae

Name	Teresa Amaral
Address	Universitäts-Hautklink Tübingen, Liebermeisterstr. 25, 72076 Tübingen, Germany
Telephone	+ 49 – (0)7071-298-4553
Fax	+ 49 – (0)7071-29-4599
Email	Teresa.amaral@med.uni-tuebingen.de; teresamaral@gmail.com

Birth date	24.11.1980
Nationality	Portuguese
Family status	Single, no children

Medical Education

2000 - 2006	Medical degree – Medical Faculty Lisbon University
2013	European Society of Medical Oncology (ESMO) Medical Oncology Examination Amsterdam
06.2014	Specialist in Medical Oncology

Medical Experience

2007-2008	General Internship - General Training in Internal Medicine, General Surgery, Gynecology, Pediatrics and Primary Care Medicine – *Centro Hospitalar Lisboa Norte (CHLN) – Hospital Pulido Valente* - Lisbon
2008 - 2014	Medical Oncology Internship – *CHLN –Hospital de Santa Maria* – Lisbon
2008 - 2014	Collaboration as co-investigator in trials in skin and ovarian cancers
2011 - 2016	Medical Oncology Unit - *Lusíadas Hospital* – Lisbon
2012	3 months fellowship (October – December) in the Skin Cancer Center in Tuebingen - Prof. Dr. med Claus Garbe working group (WG)
Since 01.2016	Skin Cancer Center - Tuebingen University, Prof. Claus Garbe WG

Other Activities

2002	Invited teacher/monitor for normal anatomy in the Medical Faculty of Lisbon University *Faculdade de Medicina Universidade de Lisboa*
2003	Invited teacher/monitor for neuroanatomy
2008 - 2009	Collaboration with *Centro de Estudos de Medicina Baseados na Evidência* (CEMBE) - Evidence Based Medicine Study Center - Lisbon
2012 - 2013	Invited speaker at the Coimbra University Medicine PhD program
2013	Invited speaker at the Lisbon University Medicine master's program
June 2018	German Approbation Medical Association of Baden-Württemberg (Ärztekammer Baden-Württemberg)
Since 2018	**Elected** - Member of the Portuguese Young Oncologists Group (*Núcleo de Internos e Jovens Oncologistas da Sociedade Portuguesa de Oncologia* - NIJE)
Since 2019	**By competitive selection -** Allumna of the European Society of Medical Oncology (ESMO) Leaders Generation Program (LGP)
Since 2019	**By invitation -** Member of the ESMO magnitude of clinical benefit scale (ESMO-MCBS) WG, ESMO clinical research observatory (ECRO) task force
Since 2019	**By invitation -** Member of theESMO clinical research observatory (ECRO) task force
Since 2019	**By invitation** - ESMO faculty member for the Melanoma faculty 2019-2023
Since 2020	**By invitation** - Member of the ESMO Social Media WG
Since 01.2020	**Elected and nominated -** Chair of the ESMO Young Oncologists Committee (YOC)

Memberships

Since 2016	ESMO member
Since 2019	Member of the *Sociedade Portuguesa de Oncologia* (SPO)
Since 2020	Member of American Society Clinical Oncology (ASCO)

Military Experience

2000 - 2006	Portuguese Air Force Academy – Aeronautic Medical Sciences
2006	Aeronautic Medicine Level 1
2006 - 2008	Portuguese Air Force Health Care Direction
2008 - 2009	Azores Air Base 4 / Aeromedical Evacuation Team
2009 - 2014	Military Hospital Lisbon
2014 - 2016	Monte Real Air Base 5
10-12.2014	NATO - Baltic Air Policing 14 Lithuania
06-07.2015	NATO – Falcon Defense 15 Romania
2016 - 2017	Portuguese Air Force Health Care Direction

11. Full list of publications

	Publication	IF
1.	Sarac E, Wilhelmi J, Thomas I, Leiter U, Keim U, Eigentler TK, Garbe C, **Amaral T**. Late recurrence of melanoma after 10 years - Is the course of the disease different from early recurrences? Journal of the European Academy of Dermatology and Venereology : JEADV. 2020; 34: 977-83.	5,248
2.	Sarac E, **Amaral T**, Keim U, Leiter U, Forschner A, Eigentler TK, Garbe C. Prognostic factors in 161 patients with mucosal melanoma: a study of German Central Malignant Melanoma Registry. Journal of the European Academy of Dermatology and Venereology : JEADV. 2020	5,248
3.	Leiter U, Heppt MV, Steeb T, **Amaral T**, Bauer A, Becker JC, Breitbart E, Breuninger H, Diepgen T, Dirschka T, Eigentler T, Flaig M, Follmann M, Fritz K, Greinert R, Gutzmer R, Hillen U, Ihrler S, John SM, Kölbl O, Kraywinkel K, Löser C, Nashan D, Noor S, Nothacker M, Pfannenberg C, Salavastru C, Schmitz L, Stockfleth E, Szeimies RM, Ulrich C, Welzel J, Wermker K, Garbe C, Berking C. S3 guideline for actinic keratosis and cutaneous squamous cell carcinoma (cSCC) - short version, part 2: epidemiology, surgical and systemic treatment of cSCC, follow-up, prevention and occupational disease. Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG. 2020; 18: 400-13.	3,664
4.	Heppt MV, Leiter U, Steeb T, **Amaral T**, Bauer A, Becker JC, Breitbart E, Breuninger H, Diepgen T, Dirschka T, Eigentler T, Flaig M, Follmann M, Fritz K, Greinert R, Gutzmer R, Hillen U, Ihrler S, John SM, Kölbl O, Kraywinkel K, Löser C, Nashan D, Noor S, Nothacker M, Pfannenberg C, Salavastru C, Schmitz L, Stockfleth E, Szeimies RM, Ulrich C, Welzel J, Wermker K, Berking C, Garbe C. S3 guideline for actinic keratosis and cutaneous squamous cell carcinoma - short version, part 1: diagnosis, interventions for actinic keratoses, care structures and quality-of-care indicators. Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG. 2020; 18: 275-94.	3,664
5.	Hilke FJ, Sinnberg T, Gschwind A, Niessner H, Demidov G, **Amaral T**, Ossowski S, Bonzheim I, Röcken M, Riess O, Garbe C, Schroeder C, Forschner A. Distinct Mutation Patterns Reveal Melanoma Subtypes and Influence Immunotherapy Response in Advanced Melanoma Patients. Cancers. 2020; 12.	6,126
6.	Glutsch V, **Amaral T**, Garbe C, Thoms KM, Mohr P, Hauschild A, Schilling B. Indirect Comparison of Combined BRAF and MEK Inhibition in Melanoma Patients with Elevated Baseline Lactate Dehydrogenase. Acta dermato-venereologica. 2020; 100: adv00174.	4,016
7.	Garbe C, Keim U, Suciu S, **Amaral T**, Eigentler TK, Gesierich A, Hauschild A, Heinzerling L, Kiecker F, Schadendorf D, Stadler R, Sunderkötter C, Tüting T, Utikal J, Wollina U, Zouboulis CC, Keilholz U, Testori A, Martus P, Leiter U, Eggermont AMM. Prognosis of Patients With Stage III Melanoma According to American Joint Committee on	32,956

Cancer Version 8: A Reassessment on the Basis of 3 Independent Stage III Melanoma Cohorts. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2020; 38: 2543-51.

8. Garbe C, **Amaral T**, Peris K, Hauschild A, Arenberger P, Bastholt L, Bataille V, Del Marmol V, Dréno B, Fargnoli MC, Grob JJ, Höller C, Kaufmann R, Lallas A, Lebbé C, Malvehy J, Middleton M, Moreno-Ramirez D, Pellacani G, Saiag P, Stratigos AJ, Vieira R, Zalaudek I, Eggermont AMM. European consensus-based interdisciplinary guideline for melanoma. Part 1: Diagnostics - Update 2019. European journal of cancer (Oxford, England : 1990). 2020; 126: 141-58. 7,275

9. Garbe C, **Amaral T**, Peris K, Hauschild A, Arenberger P, Bastholt L, Bataille V, Del Marmol V, Dréno B, Fargnoli MC, Grob JJ, Höller C, Kaufmann R, Lallas A, Lebbé C, Malvehy J, Middleton M, Moreno-Ramirez D, Pellacani G, Saiag P, Stratigos AJ, Vieira R, Zalaudek I, Eggermont AMM. European consensus-based interdisciplinary guideline for melanoma. Part 2: Treatment - Update 2019. European journal of cancer (Oxford, England : 1990). 2020; 126: 159-77 7,275

10. Forschner A, Hilke FJ, Bonzheim I, Gschwind A, Demidov G, **Amaral T**, Ossowski S, Riess O, Schroeder C, Martus P, Klumpp B, Gonzalez-Menendez I, Garbe C, Niessner H, Sinnberg T. MDM2, MDM4 and EGFR Amplifications and Hyperprogression in Metastatic Acral and Mucosal Melanoma. Cancers. 2020; 12. 6,126

11. **Amaral T**MS, Hoffmann MC, Sinnberg T, Niessner H, Sülberg H, Eigentler TK, Garbe C. Clinical validation of a prognostic 11-gene expression profiling score in prospectively collected FFPE tissue of patients with AJCC v8 stage II cutaneous melanoma. European journal of cancer (Oxford, England : 1990). 2020; 125: 38-45. 7,275

12. **Amaral T**, Seeber O, Mersi E, Sanchez S, Thomas I, Meiwes A, Forschner A, Leiter U, Eigentler T, Keim U, Garbe C. Primary Resistance to PD-1-Based Immunotherapy-A Study in 319 Patients with Stage IV Melanoma. Cancers. 2020; 12. 6,126

13. **Amaral T**, Schulze M, Sinnberg T, Nieser M, Martus P, Battke F, Garbe C, Biskup S, Forschner A. Are Pathogenic Germline Variants in Metastatic Melanoma Associated with Resistance to Combined Immunotherapy? Cancers. 2020; 12. 6,126

14. **Amaral T**, Kiecker F, Schaefer S, Stege H, Kaehler K, Terheyden P, Gesierich A, Gutzmer R, Haferkamp S, Uttikal J, Berking C, Rafei-Shamsabadi D, Reinhardt L, Meier F, Karoglan A, Posch C, Gambichler T, Pfoehler C, Thoms K, Tietze J, Debus D, Herbst R, Emmert S, Loquai C, Hassel JC, Meiss F, Tueting T, Heinrich V, Eigentler T, Garbe C, Zimmer L. Combined immunotherapy with nivolumab and ipilimumab with and without local therapy in patients with melanoma brain metastasis: a DeCOG* study in 380 patients. Journal for immunotherapy of cancer. 2020; 8. 9,913

15. Perez-Gracia JL, Awada A, Calvo E, **Amaral T**, Arkenau H-T, Gruenwald V, Bodoky G, Lolkema MP, Di Nicola M, Penel N, Vera R, Sanmamed 5,329

MF, Douillard J-Y. ESMO Clinical Research Observatory (ECRO): improving the efficiency of clinical research through rationalisation of bureaucracy. ESMO Open. 2020; 5: e000662.

16. Kaesler S, Wölbing F, Kempf WE, Skabytska Y, Köberle M, Volz T, Sinnberg T, **Amaral T**, Möckel S, Yazdi A, Metzler G, Schaller M, Hartmann K, Weide B, Garbe C, Rammensee HG, Röcken M, Biedermann T. Targeting tumor-resident mast cells for effective anti-melanoma immune responses. JCI insight. 2019; 4. 6,014

17. Heppt MV, **Amaral T**, Kähler KC, Heinzerling L, Hassel JC, Meissner M, Kreuzberg N, Loquai C, Reinhardt L, Utikal J, Dabrowski E, Gesierich A, Pföhler C, Terheyden P, Thoms K-M, Zimmer L, Eigentler TK, Kirchberger MC, Stege HM, Meier F, Schlaak M, Berking C. Combined immune checkpoint blockade for metastatic uveal melanoma: a retrospective, multi-center study. Journal for immunotherapy of cancer. 2019; 7: 299. 9,913

18. Garbe C, Keim U, Eigentler TK, **Amaral T**, Katalinic A, Holleczek B, Martus P, Leiter U. Time trends in incidence and mortality of cutaneous melanoma in Germany. Journal of the European Academy of Dermatology and Venereology : JEADV. 2019; 33: 1272-80. 5,248

19. Forschner A, Battke F, Hadaschik D, Schulze M, Weißgraeber S, Han CT, Kopp M, Frick M, Klumpp B, Tietze N, **Amaral T**, Martus P, Sinnberg T, Eigentler T, Keim U, Garbe C, Döcker D, Biskup S. Tumor mutation burden and circulating tumor DNA in combined CTLA-4 and PD-1 antibody therapy in metastatic melanoma - results of a prospective biomarker study. Journal for immunotherapy of cancer. 2019; 7: 180 9,913

20. Bochem J, Zelba H, **Amaral T**, Spreuer J, Soffel D, Eigentler T, Wagner NB, Uslu U, Terheyden P, Meier F, Garbe C, Pawelec G, Weide B, Wistuba-Hamprecht K. Peripheral PD-1+CD56+ T-cell frequencies correlate with outcome in stage IV melanoma under PD-1 blockade. PloS one. 2019; 14: e0221301. 2,870

21. **Amaral T**, Tampouri I, Eigentler T, Keim U, Klumpp B, Heinrich V, Zips D, Paulsen F, Gepfner-Tuma I, Skardelly M, Tatagiba M, Tabatabai G, Garbe C, Forschner A. Immunotherapy plus surgery/radiosurgery is associated with favorable survival in patients with melanoma brain metastasis. Immunotherapy. 2019; 11: 297-309. 2,964

22. **Amaral T**, Osewold M, Presser D, Meiwes A, Garbe C, Leiter U. Advanced cutaneous squamous cell carcinoma: real world data of patient profiles and treatment patterns. Journal of the European Academy of Dermatology and Venereology : JEADV. 2019; 33 Suppl 8: 44-51**.** 5,248

23. **Amaral T**, Tampouri I, Garbe C. How to use neoadjuvant medical treatment to maximize surgery in melanoma. Expert review of anticancer therapy. 2018; 18: 121-30. 3,573

24. Spänkuch I, Gassenmaier M, Tampouri I, Noor S, Forschner A, Garbe C, **Amaral T**. Severe hepatitis under combined immunotherapy: 7,275

	Resolution under corticosteroids plus anti-thymocyte immunoglobulins. European journal of cancer (Oxford, England : 1990). 2017; 81: 203-05.	
25.	Forschner A, Eichner F, **Amaral T**, Keim U, Garbe C, Eigentler TK. Improvement of overall survival in stage IV melanoma patients during 2011-2014: analysis of real-world data in 441 patients of the German Central Malignant Melanoma Registry (CMMR). Journal of cancer research and clinical oncology. 2017; 143: 533-40.	3,295
26.	**Amaral T**, Sinnberg T, Meier F, Krepler C, Levesque M, Niessner H, Garbe C. The mitogen-activated protein kinase pathway in melanoma part I - Activation and primary resistance mechanisms to BRAF inhibition. European journal of cancer (Oxford, England : 1990). 2017; 73: 85-92.	7,275
27.	**Amaral T**, Sinnberg T, Meier F, Krepler C, Levesque M, Niessner H, Garbe C. MAPK pathway in melanoma part II-secondary and adaptive resistance mechanisms to BRAF inhibition. European journal of cancer (Oxford, England : 1990). 2017; 73: 93-101.	7,275
28.	**Amaral T**, Meraz-Torres F, Garbe C. Immunotherapy in managing metastatic melanoma: which treatment when? Expert opinion on biological therapy. 2017; 17: 1523-38.	3,540
29.	**Amaral T**, Leiter U, Garbe C. Merkel cell carcinoma: Epidemiology, pathogenesis, diagnosis and therapy. Reviews in endocrine & metabolic disorders. 2017; 18: 517-32.	5,910
30.	**Amaral T**, Garbe C. Reply to 'Recent advances in systemic targeted therapy for cutaneous T-cell lymphoma'. Expert opinion on pharmacotherapy. 2017; 18: 1537.	2,878
31.	**Amaral T**, Garbe C. Non-melanoma skin cancer: new and future synthetic drug treatments. Expert opinion on pharmacotherapy. 2017; 18: 689-99.	2,878
32.	**Amaral T**, Nouri N, Garbe C. The safety and efficacy of cobimetinib for the treatment of BRAF V600E or V600K melanoma. Expert review of anticancer therapy. 2016; 16: 705-15.	3,573
33.	**Amaral T**, Garbe C. Acquired resistance mechanisms to immunotherapy. Annals of translational medicine. 2016; 4: 547.	3,270
34.	Macedo D, **Amaral T**, Fernandes I, Sousa AR, Costa AL, Távora I, Quintela A, Cortes P, Costa L. The Treatment of Liver Metastases in Patients with Neuroendocrine Tumors in 2012. ISRN hepatology. 2013; 2013: 702167.	0
35.	**Amaral T**M, Macedo D, Fernandes I, Costa L. Castration-resistant prostate cancer: mechanisms, targets, and treatment. Prostate cancer. 2012; 2012: 327253.	0
		Total
		209,279

12. Appendix

Ethics committee approvals

Manuscript 1

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

UNIVERSITÄTS
KLINIKUM
TÜBINGEN

Medizinische Fakultät

Ethik-Kommission

Prof. Dr. med. D. Luft
Vorsitzender

Telefon: +49 7071 29-77661
Telefax: +49 7071 29-5965
E-Mail:
ethik.kommission@med.uni-tuebingen.de

Ethik-Kommission an der Medizinischen Fakultät der Eberhard-Karls-Universität und am Universitätsklinikum Tübingen, Gartenstraße 47, 72074 Tübingen

Frau
Dr. med. Andrea Forschner
Universitäts Hautklinik
Liebermeisterstraße 25
72076 Tübingen

nachrichtlich:
Herrn Prof. Dr. med. Martin Röcken

676/2016BO2	05.10.2016	12.10.2016
unsere Projekt-Nummer	eingegangen am	Datum

Überlebensanalyse von metastasierten Melanompatienten im Stadium IV.
Prüfplan
Anschreiben vom 29.9.2016

Sehr geehrte Frau Kollegin,

die Unterlagen zu der von Ihnen geplanten Studie haben der Ethik-Kommission zur Beratung vorgelegen.

Danach bestehen gegen die Durchführung dieser Studie seitens der Kommission keine Bedenken.

Für die Durchführung Ihres Studienvorhabens wünschen wir viel Erfolg.

Mit freundlichen Grüßen

Prof. Dr. med. Dieter Luft
Vorsitzender der Ethik-Kommission

Seite 2: Allgemeine Hinweise zum Schreiben der Ethik-Kommission

Manuscript 2

Manuscript 3 – Not applicable

Manuscript 4

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

ETHIKKOMMISSION BEI DER LMU MÜNCHEN

Ethikkommission · Pettenkoferstr. 8 · 80336 München

Prof. Dr. Carola Berking
Klinik und Poliklinik für Dermatologie und Allergologie
Frauenlobstr. 9 - 11
80337 München

Vorsitzender:
Prof. Dr. W. Eisenmenger
Telefon+49 (0)89 440055191
Telefax+49 (0)89 440055192
Ethikkommission@
med.uni-muenchen.de
www.ethikkommission.med.uni-muenchen.de

Anschrift:
Pettenkoferstr. 8a
D-80336 München

08.07.2016 Hb /sc

Projekt Nr: **413-16 UE** (bitte bei Schriftwechsel angeben)

Unbedenklichkeitserklärung

Projekt: Untersuchung der Wirksamkeit von Immuncheckpunktblockade im Aderhautmelanom
Antragsteller: Prof. Dr. Carola Berking, Klinik und Poliklinik für Dermatologie und Allergologie, Frauenlobstr. 9 - 11, 80337 München

Sehr geehrte Frau Prof. Berking ,

haben Sie besten Dank für Ihr Schreiben (e-mail) vom 04.07.2016, mit dem Sie um eine Unbedenklichkeitserklärung für das o. g. Projekt bitten.

Sofern Sie Ihre Untersuchungen nur retrospektiv anhand von Daten aus den Patientenakten durchführen, die irreversibel anonymisiert sind, d. h. dass auch die Bearbeiter keinen Rückschluss auf die Daten der entsprechenden Personen erhalten, bestehen keine ethisch-rechtlichen Bedenken gegen dieses Projekt.

Vorsorglich möchte ich darauf hinweisen, dass auch bei einer positiven Beurteilung Ihres Vorhabens die Verantwortung für die Durchführung des Projektes uneingeschränkt bei Ihnen und Ihren Mitarbeitern verbleibt.

Für Ihre Untersuchungen wünsche ich Ihnen viel Erfolg.

Mit freundlichen Grüßen

Prof. Dr. W. Eisenmenger
Vorsitzender der Ethikkommission

Manuscript 5

Manuscript 6

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

UNIVERSITÄTS
KLINIKUM
TÜBINGEN

Medizinische Fakultät

Ethik-Kommission

Prof. Dr. med. Karl Jaschonek
Vorsitzender

Telefon: +49 7071 29-77661
Telefax: +49 7071 29-5965
E-Mail:
ethik.kommission@med.uni-tuebingen.de

Ethik-Kommission an der Medizinischen Fakultät der Eberhard-Karls-Universität und am Universitätsklinikum Tübingen, Gartenstraße 47, 72074 Tübingen

Frau
Dr. Teresa Amaral
Universitäts-Hautklinik
Sektion für Dermatologische Onkologie
Liebermeisterstraße 25
72076 Tübingen

nachrichtlich:
Herrn Prof. Dr. med. Martin Röcken

766/2018BO2	24.09.2018	25.09.2018
unsere Projekt-Nummer	eingegangen am	Datum

Kombinierte Immuntherapie mit Nivolumab und Ipilimumab bei Hirnmetastasierung des Melanoms: Eine multizentrische retrospektive Beobachtungsstudie.
Anschreiben vom 20.09.2018, Prüfplan vom 20.09.2018

Sehr geehrte Frau Kollegin,

die Unterlagen zu der von Ihnen geplanten Studie haben der Ethik-Kommission zur Beratung vorgelegen.
Danach bestehen gegen die Durchführung dieser Studie seitens der Kommission keine Bedenken, da die Voraussetzungen von § 13(1) LDSG-Anpassungsgesetz (Landesdatenschutz-Anpassungsgesetz) in Verbindung mit Art. 5, 6, 9, 89 der Verordnung (EU) 2016/ 679 - Datenschutz-Grundverordnung erfüllt sind.
Die Ethik-Kommission möchte darauf hinweisen, dass sich der Begriff „retrospektiv" auf bereits vorhandene Daten der Abteilung bezieht (historische Daten). Daher können von Ihrer retrospektiven Datenanalyse Daten bis einschließlich August 2018 erfasst werden. Das Studienprotokoll sollte entsprechend korrigiert werden.

Für die Durchführung Ihres Studienvorhabens wünschen wir viel Erfolg.

Mit freundlichen Grüßen

Prof. Dr. med. Karl Jaschonek
Vorsitzender der Ethik-Kommission

ALLGEMEINE HINWEISE:
Mitglieder der Ethik-Kommission: Prof. Dr. med. Henner Giedke - Psychiatrie, Prof. Dr. med. Jürgen Honegger - Neurochirurgie, Prof. Dr. med. Karl Jaschonek - Innere Medizin, Prof. Dr. med. dent. Bernd Koos – Zahnheilkunde, Prof. Dr. med. Holger Lerche - Neurologie, Prof. Dr. med. Dieter Luft - Innere Medizin, Prof. Dr. med. Klaus Mörike - Klinische Pharmakologie, Prof. Dr. med. Christian F. Poets - Kinderheilkunde, Ulrike Röllecke - Laie, Prof. Dr. iur. Dr. h.c. Georg Sandberger - Rechtswissenschaft, Prof. Dr. med. Dr. phil. Urban Wiesing - Medizinische Ethik

Universitätsklinikum Tübingen
Anstalt des öffentlichen Rechts
Sitz Tübingen
Geissweg 3 • 72076 Tübingen
Tel: 07071/29-0
www.medizin.uni-tuebingen.de
Steuer-Nr. 86156/09402
USt.-ID: DE 146 869 674

Aufsichtsrat
Ulrich Steinbach (Vorsitzender)
Vorstand
Prof. Dr. Michael Bamberg (Vorsitzender)
Gabriele Sonntag (Stellv. Vorsitzende)
Prof. Dr. Karl Ulrich Bartz-Schmidt
Prof. Dr. Ingo B. Autenrieth
Klaus Tischler

Baden-Württembergische Bank Stuttgart
BLZ 600 501 01 Konto-Nr. 7477 5037 93
IBAN: DE 41 6005 0101 7477 5037 93
BIC (SWIFT-Code): SOLADEST600
Kreissparkasse Tübingen
BLZ 641 500 20 Konto-Nr. 14 144
IBAN: DE 79 6415 0020 0000 0141 44
BIC (SWIFT-Code): SOLADES1TUB