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TARGETED THERAPY IN METASTATIC MELANOMA: CLINICAL EVALUATION AND BIOMARKERS FOR RESPONSE

Degree Project in Medicine

Christoffer Kvarnström

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Supervisors: Henrik Jespersen and Lars Ny

Jubileumskliniken, Department of Oncology

Table of content

List of abbreviations

AJCC	American Joint Committee on Cancer
BMI	Body Mass Index
BOR	Best Overall Response
CDKN2A	Cyclin Dependent Kinase inhibitor 2A
CI	Confidence Interval
CM	Cutaneous Melanoma
CRR	Clinical Response Rate
CR	Complete Response
CTLA4	Cytotoxic T-Lymphocyte-Associated protein 4
DOR	Duration Of Response
ECOG	Eastern Cooperative Oncology Group
HR	Hazard Ratio
LDH	Lactate Dehydrogenase
MAPK	Mitogen-Activated Protein Kinase
ORR	Overall Response Rate
OS	Overall Survival
PD	Progressive Disease
PD1	Programmed cell Death protein 1
PFS	Progression Free Survival
PR	Partial Response
PS	Performance Status
RECIST	Response Evaluation Criteria In Solid Tumours
RTK	Receptor Tyrosine Kinase
SD	Stable Disease
TNM	Tumour Node Metastases
ULN	Upper Limit Normal
UV	Ultraviolet

Abstract

Targeted therapy in metastatic melanoma: Clinical evaluation and biomarkers for response
Degree project, programme in medicine, 2018
Christoffer Kvarnström
Supervisors: Lars Ny and Henrik Jespersen
Department of Oncology, Sahlgrenska University Hospital, Gothenburg, Sweden

Background: Previous trials has demonstrated efficacy for dabrafenib + trametinib in treating metastatic melanoma. In this setting S100b is often used as biomarker in clinical routine but has not yet been validated for the purpose.

Aim:

1) To evaluate the use of the BRAF-MEK-inhibitors dabrafenib and trametinib in patients with metastatic melanoma at a single institution in a clinical setting.
2) To evaluate the use of S100b as a biomarker for treatment response and disease progression in the same population.

Method: Retrospective descriptive study of medical records from all patients starting treatment with dabrafenib and trametinib between 1st of February 2016 to 31st of January 2018. Survival analysis and description of S100b concentrations in relation to treatment response and disease progression.

Results: 59 patients received dabrafenib and trametinib for the first time. The clinical response rate (CRR) was 83.1%. The overall survival (OS) rate at 12 months was 65.0%. Median OS was 15 months. The progression free survival (PFS) rate at 12 months was 20.6%. Median PFS was 6.5 months. Patients with brain metastases had a significantly lower OS compared to other patients (median 9.3 months compared to 19.9 months). 94.6% of the patients responding to treatment had a concordant decrease in S100b concentration. 50% of the patients not responding to treatment had a decrease in S100b concentration. Approximately 2/3 of all patients that progressed in their disease had an elevated S100b concentration at date of progression (+/- 1 week), or 2-14 weeks before disease progression. In a separate cohort of 12 patients who had previously been treated with a line of BRAF-MEK-inhibitors, 83.3% responded to treatment.

Conclusion: Patient treated with dabrafenib + trametinib in this clinical evaluation had lower OS compared to previous trials. There are however important differences between the treated populations, e.g. in this evaluation, a large subpopulation of patients had brain metastases, which significantly lowered survival. Most patients respond and benefit from treatment but mostly only for a limited amount of time. This is also applicable for patients receiving treatment a second time. The role of S100b as a useful biomarker for predicting treatment response and disease progression could not be affirmed in this setting.

Key words: Metastatic melanoma, BRAF, MEK, S100b, clinical outcome

Background

Melanocytes and melanoma

Melanoma is a type of malignant neoplasm that originate from melanin producing cells, melanocytes. Melanocytes are cells derived from the neural crest that under development colonize the skin, eye and to a lesser extent other tissues, e.g. meninges and anogential tract. Melanin is a pigmenting molecule that gives rise to the colour of our skin, hair and eyes. Its main function is to protect other skin cells, e.g. keratinocytes from ultraviolet (UV) radiation induced DNA-damage. When exposed to UV-radiation the keratinocyte stimulates the melanocyte to melanin production and proliferation (1).

Melanoma most frequently occur in the skin i.e. cutaneous melanoma (CM) but can also occur at other sites such as the eye (uvea, conjunctiva) and mucosal organs (e.g. sinonasal, oral, anorectal, vulvovaginal and penile) (2).

Epidemiology

Worldwide CM is the 19th most common cancer with an estimated number of 290 000 new cases in 2018 (3). In Sweden CM is the fifth most common cancer with 4 151 new cases in 2016 and an age standardized incidence of 41.6/100 000 in men and 36.3/100 000 in women (4). The incidence rate is increasing rapidly with a yearly increase around 5% based on the latest 10-year period. However, the mortality rate has been relatively unchanged around 5% at the same period (5). The relative 5-year survival is 90.5% in men and 95% in women. It was the cause of death for 514 patients in 2016. The median age for diagnosis was 67 in 2016 (6).

Risk factors

The predominant established external risk factor for development of CM is exposure to UV-radiation (sun or indoor tanning devices). There is also a difference in risk comparing different forms of exposure. Intermediate high exposure gives a higher risk compared to more

continues exposure whereas a history of sunburn is associated with the highest risk (7). Other well established risk factors for development of CM are fair skin type, family history of melanoma, previous melanoma or other skin cancer, multiple or large melanocytic naevi and immunosuppression therapy (8).

Pathogenesis

CM is associated with several mutation involving different cellular pathways. Mutations can be acquired throughout life (i.e. somatic mutation) or be inherited (i.e. germline mutation) such as the case with the cyclin dependent kinase inhibitor 2A (CDKN2A) mutation observed in approximately 20-40% of the cases with familial melanoma (familial melanoma constitutes 5-10% of all cases of melanoma) (5, 9). CDKN2A is a gene coding for the two proteins p16 and p14 that acts as tumour suppressors regulating the cell cycle. Consequently loss of function in CDKN2A leads to an unregulated cell cycle which increase the probability for tumour development (10).

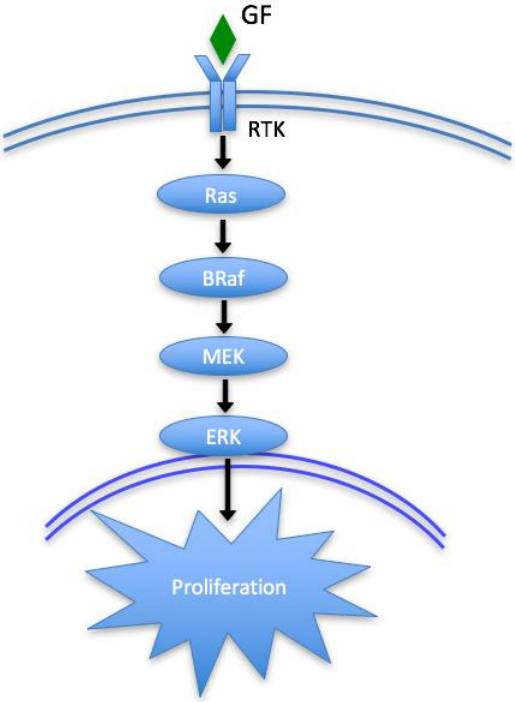


Figure 1. The mitogen-activated protein kinase (MAPK) pathway. Abbreviations: GF, growth factor; RTK, receptor tyrosine kinase. Figure by Henrik Jespersen

BRAF and NRAS (somatic mutations) are oncogenes regulating the mitogen-activated protein kinase (MAPK) pathway that govern cell proliferation. Simplified, in physiological conditions the MAPK-pathway is activated by a ligand binding in to the receptor tyrosine kinase (RTK) found on the plasma membrane of the cell. Once activated, RTK activates the intracellular NRAS enzyme which downstream activates an enzymatic cascade involving BRAF, MEK and ERK that ultimately leads to an expression of genes involved in cell proliferation and differentiation (Figure 1) (11).

Gain of function mutations in BRAF and NRAS are the most common genetic alterations in CM. A BRAF-mutation is present in approximately 40-50% of all CM with the BRAF-V600E-mutation being the predominant variant. A NRAS-mutation is present in approximately 15-20% of all CM (12, 13). Other examples of gene mutation often found in CM are NF1, TERT and tumour suppressors PTEN and TP53 (12).

Classification

The primary CM is classified according to the world health association into the major pathological subtypes superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma and acral lentiginous melanoma depending on clinical and histopathological appearance (14). There are also more unusual forms of CM such as desmoplastic melanoma, naevoid melanoma, hypo- and amelanocytic melanoma (14, 15). This classification has no prognostic value in itself. It is however associated with different epidemiological and clinical features. For example, superficial spreading melanoma is known to occur more often in younger individuals at anatomical sites exposed to intermediate UV-radiation and desmoplastic melanoma for its tendency to reoccur locally after excision. Furthermore, sentinel node (first lymph node(s) linked to the primary tumour via lymphatic drainage) metastasis is less common in desmoplastic melanoma (14).

The TNM (Tumour, Node, Metastases) -classification system does not involve the subtypes mentioned above. Tumour thickness (Breslow thickness) and the presence of ulceration which are factors associated with a higher probability of metastasis is used for staging the primary CM into different T-stages. The N-stage defines the presence of locoregional lymph node metastases, in-transit metastases, satellite metastases and/or microsatellite metastases. The M-stage defines the presence of distant metastasis depending on anatomical site and serum levels of lactate dehydrogenase (LDH). Elevated LDH and brain metastases are factors associated with the poorest prognosis. Clinically, melanoma is staged from I-IV, I-II indicates T-stage,

III indicates N-stage and IV indicates M-stage (16). For a more detailed description of TNM-system for melanoma see supplementary appendix.

Diagnosis and treatment

CM often starts in a new developed neavus that grows rapidly, has an asymmetric shape with irregular borders and multiple colours. These changes can also occur in an already pre-existing neavus (15). The suspected CM is then excised for a definite histopathological diagnosis. After histopathological diagnosis an extended excision is made with margins depending on Breslow depth and the presence of ulceration. A sentinel node biopsy is recommended for patient with Breslow depth (measured from granular epidermis to the deepest invasive cell at the base of the tumour) >1mm or ulceration, clinical monitoring is recommended for patients with Breslow depth <1mm without ulceration (5). A sentinel node biopsy gives extra prognostic information on melanoma specific survival in addition to Breslow depth and ulceration status (17).

For patients with no metastases in sentinel node clinical monitoring is recommended. Patients with positive clinical occult metastases in sentinel node are recommended monitoring with e.g. ultrasound (5). A lymph node dissection is no longer recommended for these patients since a randomized trial showed no benefit in survival for patients who underwent lymph node dissection compared to patients monitored with ultrasound (5, 18). Instead only patients with clinical detectable lymph node metastases are recommended for surgery. This after ruling out general (M-stage) disease via radiological examination. After surgery adjuvant treatment with immunotherapy or targeted therapies is recommended for most patients (5).

Inoperable stage III melanoma and stage IV melanoma is treated systemically with immunotherapy, targeted therapy or chemotherapy. Other treatments available are

intratumoural injections with oncolytic virus, electrochemotherapy, isolated limb perfusion, isolated limb infusion and radiotherapy (5).

Chemotherapy

Historically metastatic melanoma has been associated with limited treatment options and a very poor prognosis. Treatment has mainly been limited to chemotherapy with the alkylating agents dacarbazine and temozolomide. Temozolomide is a prodrug to dacarbazine with the benefit of oral administration (compared to dacarbazines intravenous) and in contrast to dacarbazine has the ability to penetrate the blood brain barrier. The overall response rate (ORR) for dacarbazine/temodal is approximately 10-15% and the treatment does not have an impact on overall survival (OS) (19).

Combination therapies such as CDBT (cisplatin, dacarbazine, carmustine and tamoxifen), CVD (cisplatin, vinblastine, dacarbazine) and PC (paclitaxel, carboplatin) has been tested. These combinations has a higher ORR compared to dacarbazine/temodal but is associated with a greater toxicity and has not demonstrated any impact on OS (19).

In recent years several new drugs have been made available for treating metastatic melanoma. These drugs have revolutionized the field and includes immunotherapy with cytotoxic T-lymphocyte-associated protein 4 (CTLA4) inhibitors and programmed cell death protein 1 (PD1) inhibitors together with drugs targeting the MAPK-pathway. These treatments have more patients responding to treatment compared to chemotherapy and a far better efficacy prolonging survival (20-23). Chemotherapy is therefore today only considered as a late palliative option when treatment is failing on these novel drugs or when patients are not suitable for them (5).

Immunotherapy

Nivolumab and pembrolizumab are monoclonal antibodies that inhibits the PD1-receptor

present on T-cells from activation (24). PD1-ligands are expressed on the surface of antigen presenting cells and by some malignant cells including melanoma cells (25). Upon activation the PD1-receptor inhibits activation of the T-cell, thus inhibiting this receptor promotes an immune stimulating effect that helps eradicate cancer cells (26). Clinical studies has shown a benefit in OS and progression free survival (PFS) in patients with metastatic melanoma treated with nivolumab compared to dacarbazine with an ORR around 40% (21). PD1 inhibitors were introduced as clinical routine treatment in Sweden in September 2015 and is now considered first choice for treatment of metastatic melanoma independent of BRAF-mutation status for most patients (5).

Ipilimumab is a CTLA4-inhibitor that acts in a similar way as PD-1 inhibitors (i.e. by blocking inhibitory signals in T-cells) (24). Ipilimumab has been proven to be less effective compared to nivolumab regarding OS and PFS and is associated with a greater toxicity (27). Ipilimumab is therefore not recommended in monotherapy for treating metastatic melanoma in Sweden (5). However, the combination of nivolumab plus ipilimumab is more effective in regards of ORR compared to nivolumab or ipilimumab in monotherapy but comes with the cost of a greater toxicity (28, 29). Nivolumab plus ipilimumab is currently used only for selected patients with metastatic melanoma in Sweden (5).

Targeted therapies

Targeted therapies for metastatic melanoma include the BRAF-inhibitors vemurafenib (Zelboraf®) and dabrafenib (Tafinlar®) together with the MEK-inhibitors trametinib (Mekinist®) and cobimetinib (Cotellic®). In Sweden vemurafenib was first to be introduced as monotherapy in clinical routine 2013 followed by dabrafenib 2014. In 2016 combination therapy with dabrafenib plus trametinib replaced monotherapy (30) and is frequently used in patients with metastatic melanoma harbouring a BRAF-V600-mutation (5).

Vemurafenib and dabrafenib are molecules acting on the MAPK-pathway by inhibiting the BRAF-enzyme which ultimately leads to reduced proliferation in BRAF-mutated cells (31, 32). Adverse events include photosensitivity, increased frequency of squamous cell carcinoma and keratoacanthoma, pyrexia, arthralgia, rash, fatigue, alopecia, nausea and diarrhea for vemurafenib (33). Compared to vemurafenib, dabrafenib has a lower frequency reported for photosensitivity and a higher frequency for pyrexia (34). Trametenib inhibits the MAPK-pathway further downstream on the MEK-enzyme (35). Common adverse events include rash, diarrhea, peripheral edema and fatigue. Cardiac toxicity leading to decreased ejection fraction or left ventricular dysfunction has been reported in some patients treated with trametinib. Furthermore, ocular events most commonly leading to blurred vision has been reported (36). Pyrexia is the most common and troublesome adverse event in clinical practice often leading treatment interruptions and dose adjustments according to practicing physicians at the Department of Oncology, Sahlgrenska University Hospital.

Combination therapy with dabrafenib plus trametinib was evaluated in the COMBI-d and COMBI-v trials and has been proven superior to dabrafenib or vemurafenib in monotherapy regarding OS and PFS with no increase in toxicity (37, 38). This led to an introduction of this regime in clinical routine 2016 (30). However, patients with an eastern cooperative oncology group (ECOG) performance status (PS) >1 (Appendix) and/or certain cardiovascular diseases or risk factors were not eligible for these trials. Neither were patients with untreated brain metastases (37, 38). With that in mind we wanted to investigate if results from previous trials were applicable on the present population treated in clinical routine at Sahlgrenska University Hospital and in parallel with a quite dramatic change in standard of care in first line therapy with the introduction of PD1-inhibitors.

S100b

The S100 proteins is a family of several known calcium binding proteins which together has

multiple both intra- and extracellular functions including regulation of calcium homeostasis, enzyme activity, protein phosphorylation, cytoskeleton components, cell survival, proliferation and differentiation. Other functions include chemoattraction for leucocytes and macrophages, stimulation of neurite outgrowth and induction of apoptosis (39).

S100b is a protein mainly expressed in melanocytes, glial cells, chondrocytes and adipocytes (40). Elevation of serum S100b concentrations is seen in some patients with metastatic melanoma but can also occur in patients with brain, liver or kidney injury (40, 41). In vitro studies have shown that S100b inhibits phosphorylation of the tumour suppressor p53, suggesting that overexpression in melanoma cells may promote tumour growth (42).

To our knowledge, no study has been made regarding S100b as a prognostic marker for treatment response in patients treated with BRAF- and MEK-inhibitors. It has however been made studies regarding S100b as a prognostic marker in patients undergoing immunotherapy. Wagner et al. demonstrated that patients with elevated S100b in plasma at treatment start had a poorer outcome in OS compared to those with no elevation (43). We wanted to evaluate S100b as a prognostic marker for treatment response, disease progression, OS and PFS in patients with metastatic melanoma treated with dabrafenib and trametinib.

Aim

The overall aim was to describe treatment response, efficacy and toxicity in patients treated with dabrafenib and trametinib for metastatic melanoma in a clinical setting at the Department of Oncology, Sahlgrenska University Hospital. Furthermore, we wanted to investigate S100b as a biomarker for treatment response and disease progression.

Specific objectives

Calculate and describe the clinical response rate (CRR), best overall response rate (BOR), overall survival (OS), progression free survival (PFS) and duration of response (DOR). Describe the frequency of adverse events with specific attention to pyrexia. Is there a correlation between baseline characteristics and overall survival (OS) or progression free survival (PFS)? Does S100b correlate with treatment response and/or disease progression?

Method

Study design

A retrospective descriptive data analysis of patients treated with dabrafenib (Tafinlar®) and trametinib (Mekinist®) for inoperable stage III melanoma or stage IV melanoma at the Department of Oncology, Sahlgrenska University Hospital. Inclusion criteria were combination treatment with dabrafenib and trametinib, BRAF-mutated inoperable stage III melanoma or stage IV melanoma and treatment start between 1st of February 2016 to 31st of January 2018. Patients who had received a previous line of treatment with BRAF- and/or MEK-inhibitors were analysed in a separate ("rechallenge") cohort. Cut-off for data collection was set to 31st of August 2018. Patients were identified via medical records at the Department of Oncology, Sahlgrenska University hospital. Data were transferred manually from patient records to a database constructed in Microsoft Excel.

Collected variables

OS was defined as length in time from treatment start to date of death or censored to the 31st of August 2018, whichever occurred first. PFS was defined as length in time from treatment start to disease progression, shift in treatment, death or censored to the 31st of August 2018, whichever occurred first. DOR was defined as length in time from treatment response to

disease progression, shift in treatment, death or censored to the 31st of August 2018, whichever occurred first. Treatment response was defied as shrinkage of any tumour visualised by radiological exam or shrinkage of any tumour at palpable sites. Disease progression was defined as tumour growth under treatment visualised by radiological exam or growth of tumours at palpable sites. BOR was defined as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). CRR was defined as CR plus PR. CR was defined as complete tumour eradication at all tumour sites at radiological exam or at palpable sites. PR was defined as any shrinkage of any tumour at radiological exam or at palpable sites. SD was defined as no growth nor shrinkage of all tumours at radiological exam or at palpable sites. PD was defined as growth of any tumour at radiological exam or at palpable sites. Tumour stage was defined using TNM-classification system according to the American joint committee on cancer (AJCC) staging manual 8th edition (16). The 0-5 grade PS developed by ECOG was used (appendix). Pyrexia was defined as a body temperature ≥ 38° Celsius. LDH concentrations was defined as > upper limit normal (>ULN) and <ULN. ULN was set to 3.4 μkat/L for patients < 70 years old and 4.2 μkat /L for patients > 70 years old. S100b concentrations >0.1 μg/L were considered as elevated. Therapy change due to resistance (Yes/No) was collected in the rechallenge cohort, yes meaning disease progression on previous BRAF- and/or MEK-inhibition treatment and no meaning no disease progression on previous BRAF- and/or MEK-inhibition treatment. Active brain metastases were defined as untreated or progressive brain metastases.

Other baseline variables collected was patient sex and age, year when primary tumour was resected, pathological features of primary tumour (Breslow depth, ulceration, histotype and site), body mass index (BMI), BRAF-mutation status, number of lesion sites, previous lines of systemic treatment, anatomical site(s) of progression, date of last treatment, reason for discontinuation, follow up duration, diseased (yes/no), date of treatment start, date of death,

date of response, time to response, date of disease progression, date of last treatment, time on treatment, reason for discontinuation, date of last follow-up, adverse event(s).

Statistical methods

The statistical analyses were made using GraphPad Prism 7.0. OS, PFS and DOR was measured using the Kaplan-Meier method for all patients and subgroups. OS was measured as months from date of treatment start to date of death or censored to 31st of august 2018, whichever occurred first. PFS was measured as months from date of treatment start to date of progression, shift in treatment, death or censored to 31st of august 2018, whichever occurred first. DOR was measured as months from date of treatment response to disease progression, shift in treatment, death or censored to 31st of August 2018, whichever occurred first. The hazard ratio and p-value was calculated using Log-rank test (Mantel-Cox). The confidence interval was set to 95% and a p-value < 0,05 were considered statistically significant. Univariable analyses were used for subgroup comparisons.

Ethical considerations

Data were handled unidentified using a specific code for each of the patients which key known only by the author and authors supervisor. Before the study started an ethical application were sent to the Swedish board of ethics which were approved 27th of June 2018, registration number 477-18. Access to patient records was granted after approval by the Head of department, Department of Oncology, Sahlgrenska University Hospital.

Results

A total of 67 patients were identified via medical records in which three were excluded due to treatment start outside of the set time interval. Fifty-nine patients were analysed in the primary cohort. Twelve patients were analysed in the rechallenge cohort in which seven also were included in the primary cohort. Baseline characteristic for the primary cohort are shown in table 1. The median follow-up time was 12.2 months (range: 0.9-26.6). The median time on treatment was 6.4 months (range: 2.1-26.6). 57.6% (*n*=34) patients were diseased at the time for data cut-off. 15.3% (*n*=9) patients were on treatment ≥12 months.

Response to treatment

The CRR and frequency of BOR are shown in table 3. The CRR was 83.1%. Patients with brain metastases had a CRR at 73.1%.

Table 1. Baseline characteristics in the primary cohort

Characteristic		***n* = 59**	**(%)**
Age			
	Median (range)	61 (28-87)	
Age – no. (%)			
	<65	32	54.2
	≥65	27	45.8
Sex – no. (%)			
	Male	34	57.6
	Female	25	42.4
PS(ECOG)* - no. (%)			
	0-1	47	79.7
	≥2	12	20.3
BMI – no. (%)			
	<30	46	78.0
	≥30	10	16.9
	Unknown	3	5.1
Metastatic stage** – no. (%)			
	M0	1	1.7
	M1a	3	5.1
	M1b	3	5.1
	M1c	26	44.1
	M1d	26	44.1
	M1d with active brain metastases	23	39.0
Number of lesion sites – no. (%)			
	<3	23	39.0
	≥3	36	61.0
LDH – no. (%)			
	<ULN	22	37.3
	>ULN	37	62.7
BRAF mutation – no. (%)			
	V600E	46	78.0
	Other	8	13.6
	Unknown	5	8.5
Previous lines of systemic therapy			
	0	48	81.4
	1	11	18.6
Previous systemic treatment – no. (%)			
	nivolumab/pembrolizumab	10	16.9
	Chemotherapy	1	1.7
Baseline S100 elevated - no. (%)			
	Yes	46	78.0
	No	13	22.0

Abbreviations: PS(ECOG), performance status (Eastern Cooperative Oncology Group); BMI, body mass index; ULN, upper limit normal.
*PS(ECOG) grade 0-5, higher grades associated with more severe disability.
**Metastatic stage according to TNM-classification, AJCC staging manual 8th edition (16).

Efficacy

Median OS was 15.0 months and the OS rate at 12 months was 65.0%. Median PFS was 6.5 months and the PFS rate at 6 months was 53.1% and 20.6% at 12 months (Figure 2). Median DOR was 5.1 months.

Baseline characteristics and OS

There was a significant difference (p = 0.03) in OS between patients with brain metastasis (M1d) and patients with metastases on other sites (M0-M1c). Median OS for the M0-M1c subgroup was 19.9 months compared to 9.3 months for the M1d subgroup (HR for death, 2.06; 95% CI, 1.04 to 4.22, p = 0.03) (Figure 3). No significant difference in OS was found in the other baseline subgroups (age, sex, PS, BMI, number of lesion sites, LDH, S100b, BRAF-mutation status or previous lines of treatment).

Table 2. Baseline characteristics in the rechallenge cohort

Characteristic		***n* = 12**	**(%)**
Age			
	Median (range)	55.5 (25-77)	
Age – no. (%)			
	<65	10	83.3
	≥65	2	16.7
Sex – no. (%)			
	Male	6	50.0
	Female	6	50.0
PS(ECOG)* - no. (%)			
	0-1	11	91.7
	≥2	1	8.3
BMI – no. (%)			
	<30	11	91.7
	≥30	1	8.3
Metastatic stage** – no. (%)			
	M1a	1	8.3
	M1c	7	58.3
	M1d	4	33.3
Number of lesion sites – no. (%)			
	<3	5	41.7
	≥3	7	58.3
LDH – no. (%)			
	<ULN	3	25.0
	>ULN	9	75.0
BRAF mutation – no. (%)			
	V600E	9	75.0
	Other	2	16.7
	Unknown	1	8.3
Previous lines of systemic therapy			
	2	7	58.3
	>2	5	41.7
Previous sign of resistance*** - no. (%)			
	Yes	5	41.7
	No	7	58.3
Baseline S100 elevated - no. (%)			
	Yes	10	83.3
	No	1	8.3
	Unknown	1	8.3

Abbreviations: PS(ECOG), performance status (Eastern Cooperative Oncology Group); BMI, body mass index; ULN, upper limit normal.
*PS(ECOG) grade 0-5, higher grades associated with more severe disability.
**Metastatic stage according to TNM-classification, AJCC staging manual 8th edition (16).
***Sign of resistance to therapy i.e. aborted first line BRAF- +/- MEK-inhibition treatment due to disease progression.

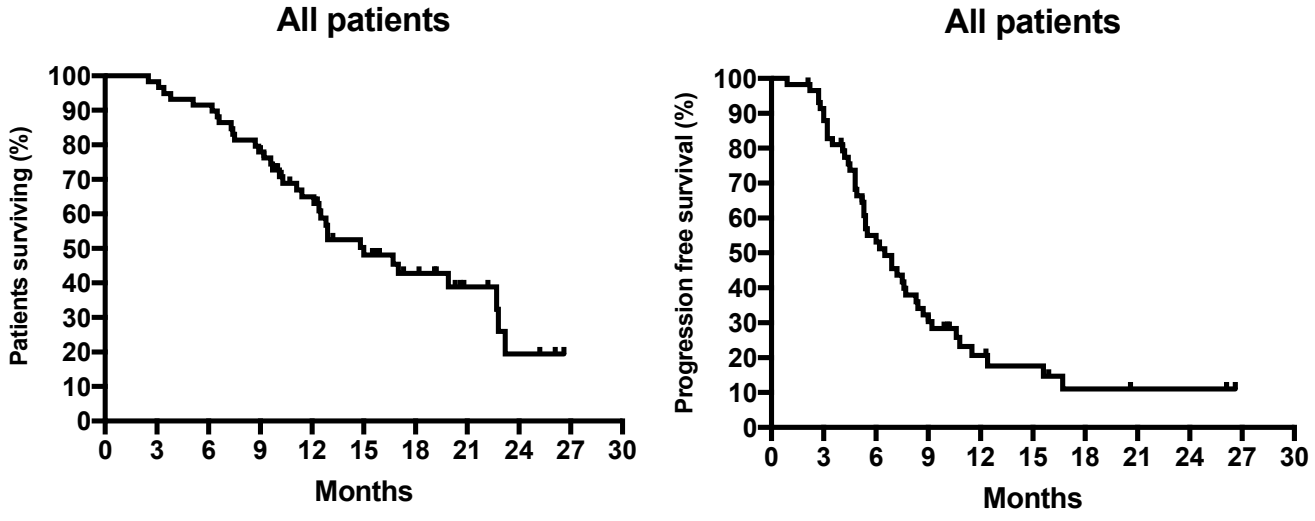
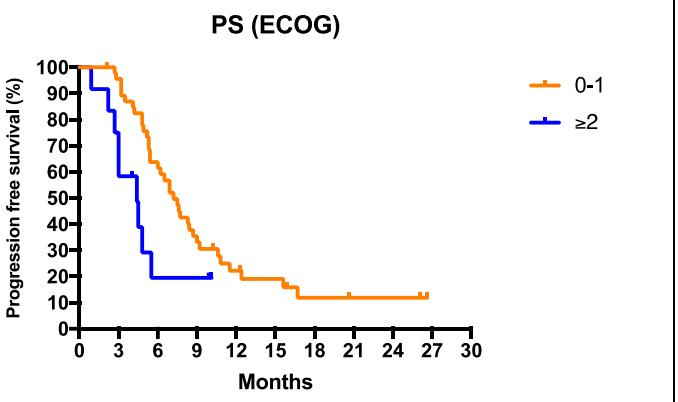
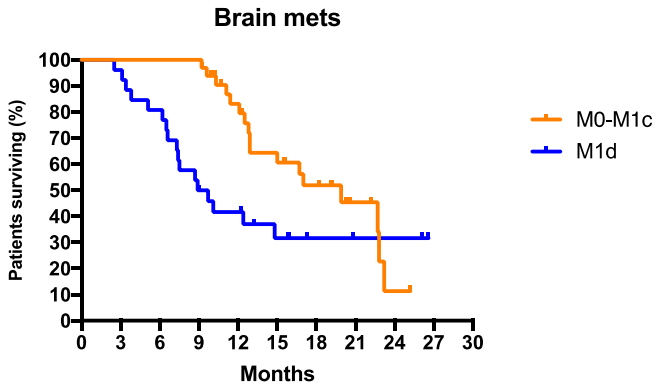


Figure 2. Kaplan-Meier estimates of OS (left) and PFS (right) amongst all patients in the primary cohort. Median OS was 15.0 months. Median PFS was 6.5 months. Rate of OS was 65% at 12 months. Rate of PFS was 53.1% at 6 months and 20.6% at 12 months.
Abbreviations: OS, overall survival; PFS, progression free survival.



HR, 2.21 (95% CI, 0.84 to 5.81) p=0.03



HR, 2.06 (95% CI 1.01 to 4.22) p= 0.03

Figure 3. Kaplan-Meier estimates showing differences in progression free survival (PFS) (left) and overall survival (OS) (right) in performance status (PS) eastern cooperative oncology group (ECOG)* (left) and metastatic stage** (right) subgroups.
Abbreviations: PFS, progression free survival; OS, overall survival; HR, hazard ratio; p, p-value; PS, performance status; ECOG, eastern cooperative oncology group.
*PS ECOG grade 0-5, high grades associated with more severe disability. 0 = Fully active, able to carry on all pre-disease performance without restriction. 1 = Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work. 2 = Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours. 3 = Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours. 4 = Completely disabled; cannot carry on any selfcare; totally confined to bed or chair. 5 = Dead.
**Metastatic stage according to TNM-classification, AJCC staging manual 8th edition (16): M0 = No evidence of distant metastasis. M1a = Distant metastasis to skin, soft tissue including muscle, and/or nonregional lymph node. M1b = Distant metastasis to lung with or without M1a sites of disease. M1c = Distant metastasis to non-central nervous system visceral sites with or without M1a or M1b sites of disease. M1d = Distant metastasis to central nervous system with or without M1a, M1b or M1c sites of disease.

Table 3. Clinical response rate and frequency of best overall response in primary and rechallenge cohort

	Primary cohort - %. (*n*)	Rechallenge* resistance ** - %. (*n*)	Rechallenge no resistance *** - %. (*n*)
CRR	83.1 (49)	60.0 (3)	100.0 (7)
CR	6.8 (4)	0 (0)	0 (0)
PR	76.3 (45)	60.0 (3)	100.0 (7)
SD	10.2 (6)	0 (0)	0 (0)
PD	6.8 (4)	40.0 (2)	0 (0)

Abbreviations: CRR, clinical response rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.
*Patients previously treated with a line of BRAF- and/or MEK-inhibitors.
**Switched to other treatment with sign of disease progression i.e. resistance to treatment under a previous line of BRAF- +/- MEK-inhibition treatment.
***Switched to other treatment without sign of disease progression under a previous line BRAF +/- MEK-inhibition treatment.

Baseline characteristic and PFS

There was a significant difference in PFS in the ECOG PS (p = 0,03) and BMI (p = 0.03) subgroups. Median PFS in the ECOG PS 0-1 subgroup was 7.2 months and 4.4 months in the ECOG PS ≥2 subgroup (HR for progression, 2.21; 95% CI, 0.84 to 5.81, p = 0.03) (Figure 3). Median PFS in the BMI < 30 subgroup was 7.5 months and 4.5 months in the BMI ≥30 subgroup (HR for progression, 2.2; 95% CI, 0.79 to 6.19, p = 0.03). No significant difference in PFS was found in the other baseline subgroups (age, sex, M-stage, number of lesion sites, LDH, S100b, BRAF-mutation status or previous lines of treatment).

Safety

The frequency of adverse events is shown in table 4. 78.0% (*n*= 46) had any type of adverse event. The most common event was pyrexia affecting 54.2% (*n*= 32) of the patients. There was no significant benefit in OS or PFS for patients with pyrexia.

Table 4. Adverse events in primary cohort

Event	***n* (%)**
Any event	46 (78.0)
Pyrexia	32 (54.2)
Nausea	8 (13.6)
Fatigue	6 (10.2)
Exanthema	5 (8.5)
Edema	3 (5.1)
Vertigo	3 (5.1)
Artalgia	2 (3.4)
Cough	2 (3.4)
Diarrhea	2 (3.4)
Rosacea	2 (3.4)
Acne	1 (1.7)
Blurred vision	1 (1.7)
Bradycardia	1 (1.7)
Headache	1 (1.7)
Rhabdomyolysis	1 (1.7)

S100b and response to treatment

All patients (*n*=59) had a registered S100b concentration at baseline. Out of these 78.0% (*n*=46) had an elevated concentration at baseline. Amongst patients responding to treatment (CR and PR) (*n*=49), 75.5% (*n*=37) had an elevated S100b concentration at baseline. Amongst these 94.6% (*n*=35) had an observed descending concentration within 6 weeks. 51.4% (*n*=19) had a normalised (<0,1 μg/L) S100b concentration within 6 weeks. Amongst patients with SD (*n*=6), 66.7% (*n*=4) had an elevated S100b concentration at baseline. 50% (*n*=2) had an observed descending concentration within 6 weeks. 50% (*n*=2) had a normalised (<0,1 μg/L) concentration within 6 weeks. Amongst patients with PD (*n*=4), 100% (*n*=4) had an elevated S100b concentration at baseline. 50% (*n*=2) had an observed descending concentration within 6 weeks. 50% (*n*=2) had a normalised (<0,1 μg/L) concentration within 6 weeks. Figure 4 illustrates relative changes in S100b concentrations from treatment start in patients with elevated S100b concentrations.

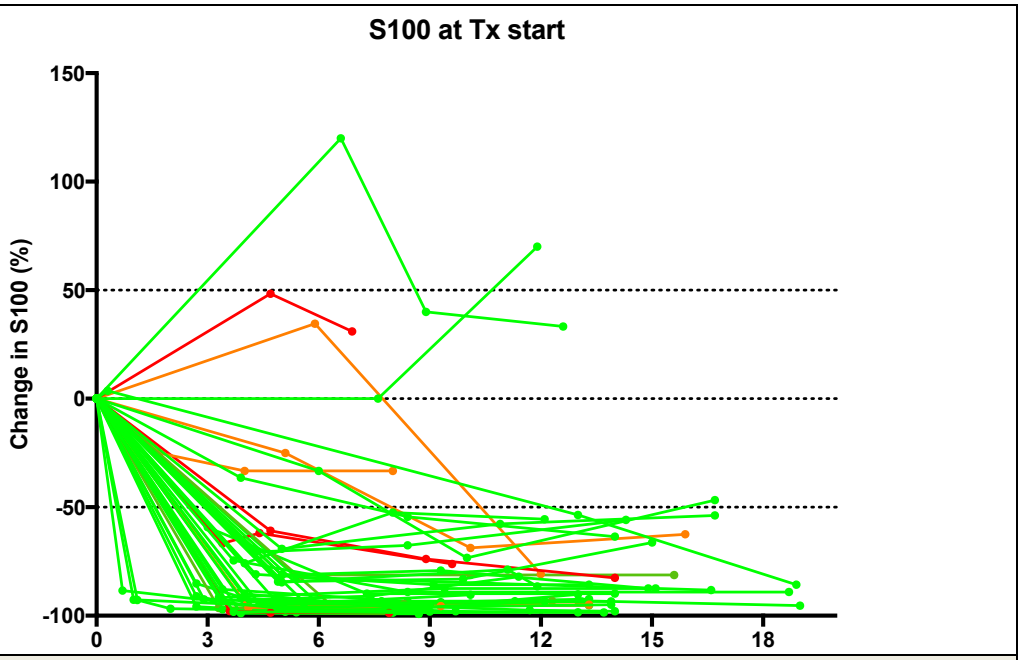


Figure 4. Spider plot illustrating relative changes in S100b from tx start over 19 weeks in patients with elevated S100b. Colours representing BOR (Green= CR+PR, orange = SD, red = PD).
Abbreviations: Tx, treatment; BOR, best overall response; CR, complete response; PR, partial response; SD, stable disease; PR, progressive disease.

S100b and disease progression

76.3% (*n*=45) of the patients progressed in their disease. Out of these 84.4% (*n*=38) had an observed S100b concentration at date of progression (+/- 1 week), all of these patients also had at least one observed concentration 2-14 weeks prior to progression.

60.5% (*n*=23) had an elevated concentration at date of progression (+/- 1 week) and 57.9% (*n*=22) had at least one elevated concentration 2-14 weeks prior to progression. 44.7% (*n*=17) had an ≥100% increase in S100b concentration 2-14 weeks prior to progression to date of progression. Figure 5 illustrates changes in S100b concentrations weeks prior to progression in absolute and relative concentrations.

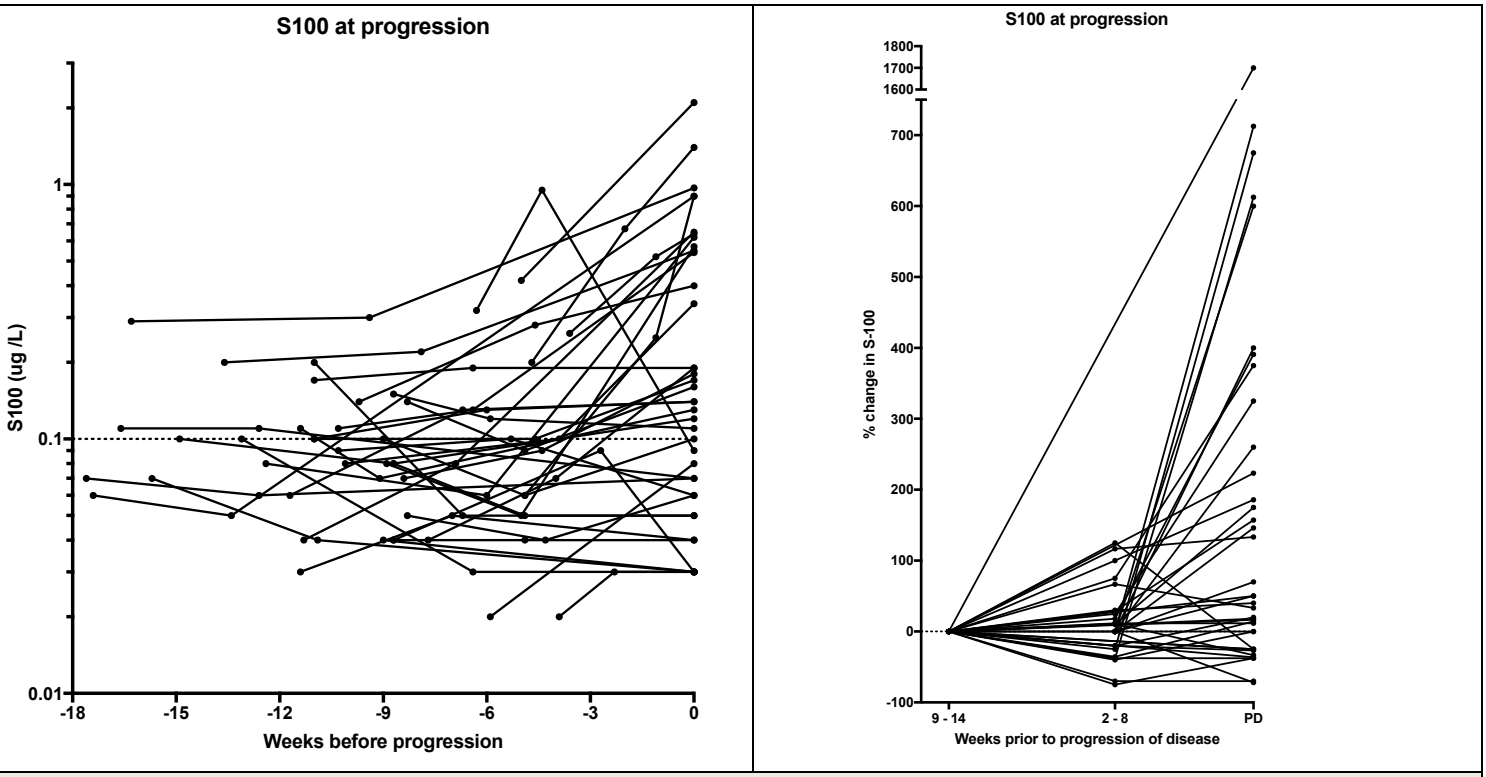


Figure 5. Plots illustrating changes in S100b concentrations weeks prior to progression. Left plot illustrates absolute concentrations with y-axis in a logarithmic scale, x-axis represents weeks in a continuum, dotted line at cut-off for elevation. Right plot illustrates relative changes in y-axis, x axis represents grouped intervals 2-8 weeks prior to progression, 9-14 weeks prior to progression and at date of progression (+/- 1 week).
Abbreviation: PD, progression date.

Rechallenge cohort

Baseline characteristics for the rechallenge cohort are shown in table 2. The CRR was 83.3% in the rechallenge cohort. The CRR was 50% for the patients with prior resistance to BRAF- +/- MEK-inhibition treatment and 100% for patients with no prior resistance to BRAF- +/- MEK-inhibition treatment (Table 3). Median PFS was 2.4 months in patients with prior resistance to BRAF- +/- MEK-inhibition treatment and 8.5 months in patients with no prior resistance to BRAF- +/- MEK-inhibition treatment. Median DOR was 1.9 months in patients with prior resistance to BRAF- +/- MEK-inhibition treatment and 5.3 months in patients with no prior resistance to BRAF- +/- MEK-inhibition treatment. Median OS was 5.5 months in patients with prior resistance to BRAF- +/- MEK-inhibition treatment and 25.3 months in patients with no prior resistance to BRAF- +/- MEK-inhibition treatment (Figure 6).

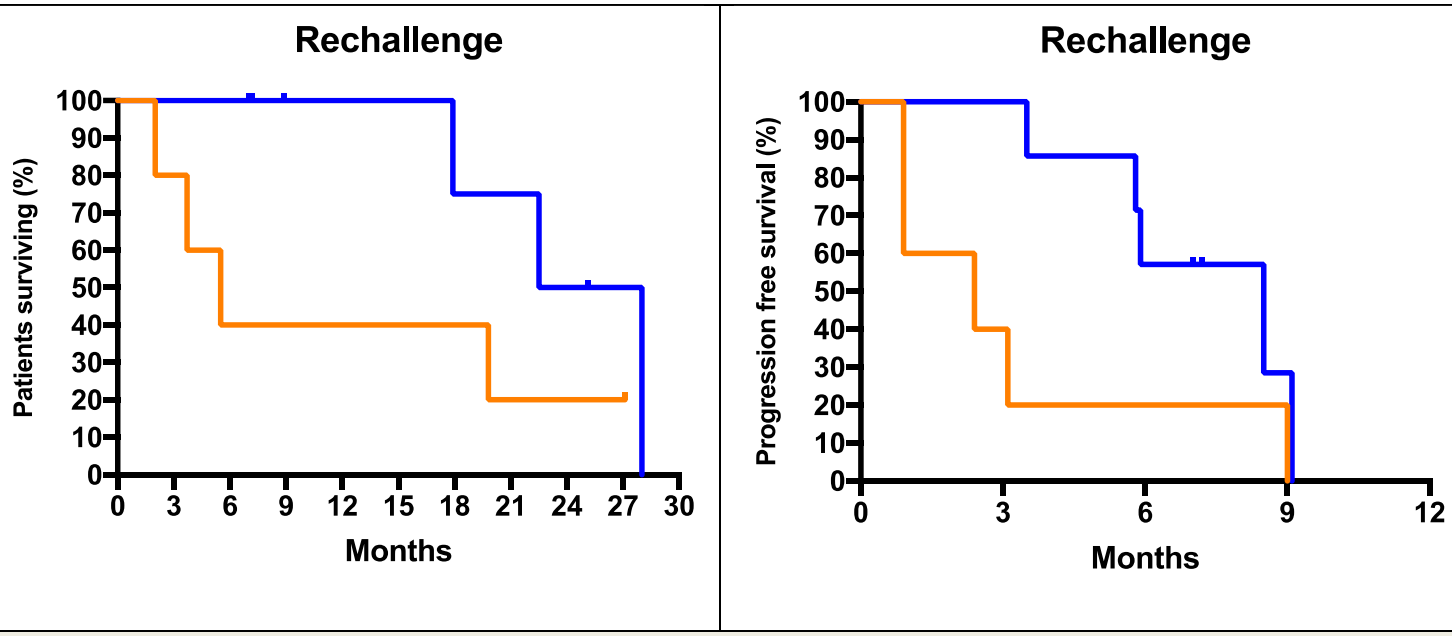


Figure 6. Kaplan-Meier estimates of OS (left) and PFS (right) in the rechallenge* cohort. Orange lines represents patients with prior resistance** (*n* =5) and blue lines patients with no prior resistance (*n*=7). Differences are not statistically significant.
*Patients previously treated with a line of BRAF- and/or MEK-inhibitors.
**Disease progression on previous line of BRAF- and/or MEK-inhibition treatment.
Abbreviations: OS, overall survival; PFS, progression free survival.

Discussion

OS and PFS

Patients treated with dabrafenib and trametinib in this clinical evaluation have lower survival rates compared to previous trials and other observational data. The primary cohort of this clinical evaluation presented a median OS at 15 months with a rate of OS at 65.0% after 12 months compared to a median OS not reached and a rate of OS at 72% after 12 months in the COMBI-v trial (38). Another 5- year follow-up trial of patients treated with dabrafenib and trametinib presented a median OS at 25 months (44). Median PFS was 6.5 months in the primary cohort of this clinical evaluation compared to 11.4 months in COMBI-v trial and 9 months in the 5-year follow-up trial (38, 44). Median DOR was 5.1 months in the primary cohort of this evaluation which indicates that most patients respond to treatment not long after treatment start.

These differences can most likely be explained by differences in baseline characteristics between the treated cohorts. Trials mention above (38, 44) excluded patients with ECOG PS >1 and patients with certain cardiovascular disease and/or risk factors. Furthermore, patients with M1d disease were eligible only if they had undergone local treatment (i.e. surgery and/or radiotherapy) for brain metastases with no increase in lesion size for a minimum of 12 weeks. In the primary cohort of this clinical evaluation 20.3% (*n*=12) of the patients had an ECOG PS ≥2, 44.1% (*n*=26) had M1d disease and 39% (*n*=23) had M1d disease with active brain metastases. However, more trials and evaluations from other clinics are needed to confirm or discard these results.

The COMBI-mb trial studied outcomes in patients with brain metastases treated with dabrafenib and trametinib. Patients with M1d disease in the primary cohort of this clinical evaluation presented a median OS at 9.3 months and a median PFS at 5.4 months. Results

similar to those presented in COMBI-mb where median OS was 10.8 months and median PFS was 5.6 months for patients with M1d disease with no previous local treatment and an ECOG PS of 0 or 1 (45). Considering that 44.1% of the patients in the primary cohort of this clinical evaluation had M1d disease it is reasonable to assumes this subcategory of patients had a large impact on OS and PFS in the primary cohort of this clinical evaluation.

Patients with M1d disease displayed a significantly impaired OS compared to patients with M0-M1c disease in the primary cohort of this clinical evaluation. A bit surprisingly though, no significant difference was shown regarding PFS between these subcategory of patients. There was however a relatively large numerical difference (median 5.4 months compared to 7.2 months).

Another aspect is the fact that immunotherapy has been introduced as first choice in treating metastatic melanoma for most patients at the clinic and that after the COMBI-v and other phase 3 trials were conducted. This means that mainly patients with symptomatic disease receive treatment with dabrafenib and trametinib in contrast to mostly asymptomatic patients in COMBI-v and other trials. This is predominantly patients with great burden of disease, elevated LDH, high ECOG PS and M-stage. All prognostic factors predicting a poorer survival outcome (46-48). In the primary cohort of this clinical evaluation 88.2% (*n*=52) had M1c-M1d disease, 62.7% (*n*=37) had elevated LDH, 61.0% (*n*=36) had >3 lesion sites and 20.3% (*n*=12) had an ECOG PS ≥2 at baseline. This probably affected survival outcomes negatively.

Response to treatment

The CRR for all patients in the primary cohort of this evaluation was 83.1% compared to the ORR of 64% in the COMBI-v trial (38). Patients with M1d disease in the primary cohort of this evaluation had an CRR of 73.1% compared to the ORR of 58% for patients with M1d

disease with no previous local treatment and an ECOG PS of 1 or 2 in the COMBI-mb trial (45). These differences in response are likely to depend on differences in assessing response. The treating physician's definition of response was used in this evaluation, meaning any shrinkage of baseline tumours on radiological exam or at palpable sites. Patients in the COMBI-v and COMBI-mb trials used the response evaluation criteria for solid tumours (RECIST) version 1.1 for assessment of response (49). RECIST defines response stricter and this should be taken into account comparing our response rates with COMBI-v and COMBI-mb. However, data on clinical response rates still shreds light on an important aspect of this treatment, namely which response rates to expect in clinical practice. Although, more research is needed from other clinical institutions to compare these results regarding CRR more accurately.

Subgroups comparisons

Differences between M-stage subgroups is discussed above. A previous large analysis for factors predicting treatment efficacy in patients treated with dabrafenib and trametinib identified LDH, ECOG PS and number of lesions sites as important factors predicting OS and PFS (48). In this subgroup analysis neither LDH levels nor number of lesion sites displayed significant differences. There was however, large numerical differences in both OS and PFS between patients with LDH concentrations <ULN and >ULN. 22.7 months compared to 12.8 months for OS and 8.7 months compared to 5.3 months for PFS.

There was a large numerical difference in OS between patients with ≥3 lesion sites and <3 lesion sites in the primary cohort of this evaluation (12.9 months compared to 19.9 months). There was however no large numerical difference in PFS between the same subgroups. Why OS did not fell out as significant might be an effect of post-protocol treatment meaning patients often get other systemic treatments after ending treatment with dabrafenib and trametinib. PFS is harder to explain but an explanation might be that PFS is overall short for

all subgroups in primary cohort of this evaluation. Also the population size is most likely to small to detect minor differences.

Patients with BMI <30 had a significantly longer PFS compared to patients with BMI >30 contradicting a previous meta-analysis suggesting that obese patients benefited both in OS and PFS when treated with dabrafenib and trametinib (50). However, the CI for this difference is wide and there might be confounding factors that has not been taken into account.

The same analysis mentioned above also identified male sex, age and BRAF-mutation status as minor negative predictors for OS and PFS in patients treated with dabrafenib and trametinib (48). This subgroup analysis could not detect any significant differences for those subgroups. The population size is most likely to small to detect such minor differences.

Safety

The panorama of adverse events in the primary cohort of this evaluation is similar to previous conducted studies on patients treated with dabrafenib and trametinib with pyrexia as the most common event affecting more than half of all treated patients (38). A slight difference is the fact that no cardiac events leading to decreased ejection fraction or left ventricular dysfunction was reported. Neither were any ocular events. Screening patients for those events with UCG and ocular examinations is however not an implemented routine at the clinic why such events may have been undetected. Regarding pyrexia as a prognostic factor for survival outcome, there was no survival benefit in patients with pyrexia in the primary cohort of this evaluation consistent with previous results (51).

Pyrexia still remains a paramount factor for treatment tolerability often leading to treatment interruptions and dose adjustments. However, a clinical trial with the BRAF- and MEK-inhibitors encorafenib and binimetinib has shown promising results with survival rates

comparable to dabrafenib and trametinib with an overall better tolerability and lower rates of pyrexia (52).

Rechallenge cohort

A majority of patients who underwent rechallenge with dabrafenib and trametinib responded to treatment. Patients with prior resistance to treatment had an CRR at 50.0% and patients with no prior resistance to treatment had an CRR at 100%. Previous studies displayed lower response rates for this subcategory of patients (32% and 43%) but they did however use RECIST v. 1.1 for assessing response in contrast to this evaluations clinical assessment.

Median PFS in the rechallenge cohort of this clinical evaluation was 2.4 months for patients with prior resistance to treatment and 8.5 months for patients with no prior resistance to treatment. Median OS was 5.5 months for patients with prior resistance to treatment and 25.3 months for patients with no prior resistance treatment. Previous studies presented a median PFS at 5.0 months and 4.9 months and a median OS at 9.8 months and 19.9 months. These studies did however not subcategorize patients into prior resistance to treatment and no prior resistance to treatment (53, 54).

No vast conclusion can be drawn from the OS and PFS numbers in the rechallenge cohort of this evaluation considering that the population size was small. However, results in the rechallenge cohort is somewhat comparable to those of previous studies. The most important finding for this subcategory of patients is that many of the patients respond to treatment. Although, more studies with larger population sizes are needed to make better conclusions in what to expect from rechallenge with dabrafenib and trametinib.

S100b

The majority of patients with an elevated S100b concentration at treatment start had decreased concentrations short after treatment start. Almost all patients responding to

treatment (CR and PR) (94.6%) had a decreased concentration within 6 weeks. However, that was also the case for patients not responding to treatment (SD and PD). 66.7% of patients with SD and 50 % of patients with PD had a decrease in S100b concentration within 6 weeks. Furthermore, 50% of the patients with SD and PD had normalised (<0,1 μg/L) concentration within 6 weeks suggesting that S100b is an uncertain marker for predicting treatment response.

Many (84.4%) patient who progressed in their disease had an elevated S100b concentration at progression date (+/- 1 week) and some (60.5%) had an elevated concentration 2-14 weeks prior to progression. Less than half (44.7%) of the patients had an ≥100% increase in S100b concentration from 2-14 weeks prior to progression to date of progression (+/- 1 week). Thus, indicating that S100b can detect progression early in some patients but in far from all. Consequently, it cannot be used alone in the purpose of progression screening but can be a valuable tool together with clinical and radiological examinations.

Regarding S100b as a prognostic marker for survival, patients with normal S100b concentrations at baseline had no significant benefit in OS or PFS compared to patients with elevated concentrations. There was however a larger numerical difference in PFS, 10.8 months compared to 6 months in favour for patients with normal concentrations. Wagner et al. demonstrated a significant benefit in OS favouring patients with low concentrations (43). However, they used >0.3 μg/L as cut-off for elevation compared to the >0.1 μg/L standard at the Department of Oncology, Sahlgrenska University Hospital and involved exclusively patients treated with immunotherapy. This may have affected the outcome and should be taken in consideration comparing with the results of this evaluation.

In conclusion S100b serves as an uncertain marker for treatment response and disease progression in this clinical evaluation but further investigation may explore the possibility for other cut-offs for elevation in patients with metastatic melanoma.

Strengths and limitations

This clinical evaluation has an unselected population displaying the current population currently treated in clinical practice with dabrafenib and trametinib for metastatic melanoma. This generated different results comparing to pivotal clinical trials which should be seen as a strength, giving new perspectives on treatment expectations for this category of patients.

A retrospective approach means difficulties in data collection, data not expressed in medical records and lab analysis not made in a standardized way leads to data loss and different quantity sets of data between patients. This was most pronounced analysing S100b at progression where many patients did not have data at date of progression and/or weeks imminent to progression. Survival data is however solid and does not change in retrospect.

The population size in this clinical evaluation was most likely not powered to detect minor differences between subgroups hence the probability for type 1 and 2 errors should be taken in consideration interpreting these results. Furthermore, no multivariable analysis was made adjusting for covariation between subgroups.

The clinical approach in assessing response could be seen as limitation in regards of lack of comparability to other trials using RECIST version 1.1. On the other hand, this data on response reflects patients with clinical response which yields another perspective on the matter.

Conclusion

Patients treated with dabrafenib and trametinib for metastatic melanoma in clinical practice constitutes a different population compared to populations in previous pivotal clinical trials. A population composed of a large number of patients with negative prognostic factors generating lower survival rates than expected. This may mainly be due to a large subgroup of patients with brain metastases which had a significantly lower OS compared to other patients. The majority of patients respond and benefit from treatment initially but for most of the patients this time is limited, consolidating most physicians current view of the treatment. Still treatment stands superior to chemotherapy historically used in the majority of all patients with metastatic melanoma.

Pyrexia constitutes the most troublesome adverse event in clinical practice leading to treatment interruptions and dose adjustments. Fortunately, new BRAF- and MEK-inhibitors with lower rates of pyrexia are under evaluation.

S100b serves an unreliable biomarker for treatment response and disease progression. The majority of patients who responded to treatment had an associated decrease in S100b concentration. That was also the case for patients not responding to treatment. Furthermore, not all patients had an elevated concentration at treatment start. Regarding S100b as a biomarker for disease progression, elevated concentrations is seen in some patients weeks prior to progression but in far from all. Concluding that S100b should be interpreted only in a context together with clinical and radiological examinations.

Many patients receiving rechallenge with dabrafenib and trametinib respond to treatment. This also include patients with prior resistance to treatment. Suggesting it can be used as a late palliative option for patients with metastatic melanoma when other treatments have

failed. However, the population size for this subcategory of patients was small and no vast conclusions can be drawn from it.

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Populärvetenskaplig sammanfattning

Utvärdering av målinriktad terapi vid spridd melanomsjukdom

Malignt melanom är en cancersjukdom som utgår från hudens pigmentceller. I Sverige drabbas ca 4 000 människor av malignt melanom årligen vilket gör det till Sveriges femte vanligaste cancersjukdom. Fler och fler människor drabbas av sjukdomen varje år och det även om man tar hänsyn till en växande och åldrande befolkning. Solexponering, ljus hudfärg och ärftlighet är alla riskfaktorer för att drabbas av sjukdomen. Om tumören upptäcks i ett tidigt skede begränsad till huden botas och överlever de allra flesta genom att denna avlägsnas kirurgiskt från huden. Om tumören hunnit sprida sig till ett eller flera av kroppens organ behandlas däremot sjukdom med läkemedel. Historisk sett har behandlingen vid spridd sjukdom varit begränsad till cellgifter i symptomlindrande syfte. Denna behandling påverkar inte sjukdomens förlopp och de flesta drabbade avled därför till följd av sjukdomen inom cirka ett år.

På senare tid har flertalet nya läkemedel tagits fram verksamma mot spridd melanomsjukdom. Läkemedlen kan i huvudsak delas in i två huvudtyper med skilda verkningsmekanismer: 1. läkemedel som modulerar immunsystemet så att det bekämpar tumören på ett mer effektivt sätt och 2. läkemedel som verkar mot specifika egenskaper unika för tumören, så kallad målinriktad terapi. År 2012 godkändes det första läkemedlet i kategorin målinriktad terapi i Sverige tätt följt av nya immunmodulerande läkemedel. Dessa läkemedel har tillsammans bidragit till att förlänga överlevnaden markant hos patienter med spridd melanomsjukdom.

År 2016 godkändes i Sverige en specifik kombinationsbehandling inom kategorin målinriktad terapi. Denna kombinationsbehandling hade tidigare utvärderats i flera stora multinationella studier med goda resultat. Dessa studier exkluderade dock flertalet kategorier av patienter som nu får behandlingen ute på klinikerna(i ”verkligheten”). Detta gäller exempelvis patienter

med ett malignt melanom som spridit sig till hjärnan samt patienter som är väldigt trötta och påverkade av sin sjukdom. Vi ville därför utvärdera behandlingen och se om patienterna som behandlas på onkologiska kliniken, Sahlgrenska universitetssjukhuset hade samma nytta av behandlingen som de i tidigare multinationella studier. Utöver detta ville vi även utvärdera om ett specifikt blodprov kallat S100b kunde användas för att förutse behandlingssvar och eventuell behandlingsresistens i samma patientgrupp.

Totalt 59 patienter påbörjade behandlingen mellan 1 februari 2016 och 31 januari 2018. Det visade sig att behandlingen var mindre effektiv hos dessa patienter jämfört med vad som observerats i tidigare multinationella studier. Detta bedöms främst bero på att runt 40% av patienterna som fått behandlingen hade spridning av sjukdomen till hjärnan. Dessa patienter hade en statistiskt signifikant kortare överlevnad jämfört med alla andra patienter i denna studie och var en patientkategori som inte fått vara med i tidigare multinationella studier.

Immunmodulerande behandling räknas idag som förstahandsbehandling mot spridd malignt melanomsjukdom vilket gör att endast patienter som ej bedöms gynnas av eller klara av denna behandling får behandling med målinriktad terapi. Detta är svårt sjuka patienter med flertalet riskfaktorer för en kortare överlevnad. Detta är också en förklaring till det något sämre resultatet i denna studie.

S100b visade sig uppvisa bristande förutsättning till att användas ensamt för att förutsäga behandlingssvar och behandlingsresistens. Blodprovet kan dock lämpa sig bra tillsammans med andra prover och röntgenundersökningar för att skapa en bättre helhetsbild av behandlingssvar och eventuell resistensutveckling hos varje enskild patient som står på behandling med målinriktad terapi.

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Appendix

Grade	ECOG performance status
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

T Category	Thickness	Ulceration status
TX: primary tumor thickness cannot be assessed (e.g., diagnosis by curettage)	Not applicable	Not applicable
T0: no evidence of primary tumor (e.g., unknown primary or completely regressed melanoma)	Not applicable	Not applicable
Tis (melanoma *in situ*)	Not applicable	Not applicable
T1	≤1.0 mm	Unknown or unspecified
T1a	<0.8 mm	Without ulceration
T1b	<0.8 mm 0.8–1.0 mm	With ulceration With or without ulceration
T2	>1.0–2.0 mm	Unknown or unspecified
T2a	>1.0–2.0 mm	Without ulceration
T2b	>1.0–2.0 mm	With ulceration
T3	>2.0-4.0 mm	Unknown or unspecified
T3a	>2.0–4.0 mm	Without ulceration
T3b	>2.0–4.0 mm	With ulceration
T4	>4.0 mm	Unknown or unspecified
T4a	>4.0 mm	Without ulceration
T4b	>4.0 mm	With ulceration

Extent of regional lymph node and/or lymphatic metastasis		
N Category	Number of tumor-involved regional lymph node	Presence of in-transit, satellite, and/or microsatellite metastases
N2a	Two or three clinically occult (i.e., detected by SLN biopsy)	No
N2b	Two or three, at least one of which was clinically detected	No
N2c	One clinically occult or clinically detected	Yes
N3	Four or more tumor-involved nodes or in-transit, satellite, and/or microsatellite metastases with two or more tumor-involved nodes, or any number of matted nodes without or with in-transit, satellite, and/or microsatellite metastases	
N3a	Four or more clinically occult (i.e., detected by SLN biopsy)	No
N3b	Four or more, at least one of which was clinically detected, or presence of any number of matted nodes	No
N3c	Two or more clinically occult or clinically detected and/or presence of any number of matted nodes	Yes

Extent of regional lymph node and/or lymphatic metastasis		
N Category	Number of tumor-involved regional lymph node	Presence of in-transit, satellite, and/or microsatellite metastases
NX	Regional nodes not assessed (e.g., SLN biopsy not performed, regional nodes previously removed for another reason)	No
	Exception: pathological N category is not required for T1 melanomas, use cN.	
N0	No regional metastases detected	No
N1	One tumor-involved node or in-transit, satellite, and/or microsatellite metastases with no tumor-involved nodes	
N1a	One clinically occult (i.e., detected by SLN biopsy)	No
N1b	One clinically detected	No
N1c	No regional lymph node disease	Yes
N2	Two or three tumor-involved nodes or in-transit, satellite, and/or microsatellite metastases with one tumor-involved node	

	M Criteria	
M Category	Anatomic site	LDH level
M0	No evidence of distant metastasis	Not applicable
M1	Evidence of distant metastasis	See below
M1a	Distant metastasis to skin, soft tissue including muscle, and/or nonregional lymph node	Not recorded or unspecified
M1a(0)		Not elevated
M1a(1)		Elevated
M1b	Distant metastasis to lung with or without M1a sites of disease	Not recorded or unspecified
M1b(0)		Not elevated
M1b(1)		Elevated
M1c	Distant metastasis to non-CNS visceral sites with or without M1a or M1b sites of disease	Not recorded or unspecified
M1c(0)		Not elevated
M1c(1)		Elevated
M1d	Distant metastasis to CNS with or without M1a, M1b, or M1c sites of disease	Not recorded or unspecified
M1d(0)		Normal
M1d(1)		Elevated

Suffixes for M category: (0) LDH not elevated, (1) LDH elevated. No suffix is used if LDH is not recorded or is unspecified.

TNM-classification system for melanoma. Tables collected from the AJCC staging manual eight edition(16).