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Intratumoural and systemic inflammation as predictor for treatment response in BRAF-mutated melanoma patients under targeted therapies

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Introduction

The incidences of cutaneous melanoma (CM) are increasing worldwide, with estimated continuous case increases for the next decades. Importantly, more than 55,000 deaths per year can be attributed to CM worldwide. Thus, there is high need for potent and practicable biomarkers predicting the treatment outcome to immune checkpoint inhibitors (ICI) as well as BRAF/MEK inhibitors (BRAFi/MEKi), particularly, considering potential adverse events and high cost. There is growing evidence that systemic inflammatory responses represent significant determinants of tumour progression and impaired survival in many malignancies. *Hence, i*nflammation has emerged as an important factor promoting the development and progression of CM. Several immune-based prognostic scores, such as neutrophil count, lymphocyte count, and neutrophil-lymphocyte ratio, have been developed to predict the prognosis as well as response to treatment in several cancers, including CM as well. With regard to intratumoural inflammatory biomarkers it has been shown that there might be a prototype of intratumoural inflammatory infiltrate depicting beneficial prognosis, which is mainly composed of numerous CD3+ T cells, for example CD3+CD8+ cells, Langerhans cells, and absence of CD20+ B cells and plasma cells. Moreover, COX-2 expression has been proposed to be involved in melanoma development and progression. Indeed, intratumoural as well as systemic inflammation in cutaneous melanoma (CM) has thoroughly been studied in the context of patients treated with ICI but not with BRAFi/MEKi. We aimed to study whether parameters of intratumoural and systemic inflammation correlate with clinical outcome in patients with BRAF-mutant metastatic melanoma treated with (BRAFi/MEKi).

Methods

This study was performed according to the declaration of Helsinki and followed a protocol approved by our institutional ethics review board (#16-5985). We studied 51 CM patients with unresectable stage III or IV who had the indication for BRAFi/MEKi treatment based on confirmed BRAF mutation (**Tab. 1**). Therapy, monitoring, and staging procedures (e.g., imaging) were performed in accordance with national guidelines for the management of CM and interdisciplinary tumour board decisions. BRAFi/MEKi were administered in label.

In brief, we studied the following laboratory parameters before the start of BRAFi/MEKi treatment: Pan immune-inflammation value (PIV) = neutrophils × platelets × monocytes/lymphocytes; systemic immune-inflammation index (SII) = platelets × neutrophils/lymphocytes, lactate dehydrogenase, S100B, and C-reactive protein. Immunohistochemistry was performed for CD8 (mouse monoclonal, ready to use 30 min, room temperature, pretreatment pH 9, DAKO, Hamburg, Germany), CD68 (mouse monoclonal, dilution 1:100 for 30 min, pretreatment pH 9, DAKO), and COX-2 (rabbit polyclonal, dilution 1:50 for 30m min, pretreatment pH 9, abcam, Cambridge, UK) in accordance with the manufacturer's recommendations. For microscopic analysis, stained slides were scanned at 40x magnification using the Nanozoomer Whole Slide Scanner from Hamamatsu (Hamamatsu, Herrsching am Ammersee, Germany). The images were evaluated by using the viewer software NDP.view2 (Hamamatsu Photonics, Germany). The H-score quantification was carried out by multiplying the percentage of intratumoural positive cells (0 - 100%) by the staining intensity (0 = none; 1 = slight; 2 = moderate; 3 = strong) and totalization of data (total range: 0-300).

The MedCalc (Ostende, Belgium) software version 20.009 was used for statistical analysis. Univariable analysis was performed using ROC analyses, including the area under the curve (AUC) and the Youden index for evaluation of optimal cut-off values, the Chi² test, and the Spearman's rank correlation procedure. $P < 0.05$ was considered statistically significant. Multivariable analysis was performed using a binary logistic regression model for the dependent variable objective response. A cox proportional-hazards regression model was used for the dependent variable disease progression and melanoma-specific death, exclusively including significant data obtained from univariable testing. Odds and hazard ratios (OR, HR) including the 95% confidence intervals (CI) were calculated as well. A P-value < 0.05 was considered significant.

Results

Patient characteristics and clinical outcomes have been detailed in **Tab. 1**. The objective response rate (ORR) was 43.1%. Disease progression was observed in 38/51 (74.5%) patients after a median progression-free survival (PFS) time of 8 months (range: 1-90 months). Melanoma-specific deaths were observed in 31/51 (60.8%) patients. The melanoma-specific survival time was 17 months (range: 4-90). The parameters for intratumoural inflammation assessed did not correlate with the systemic immune-inflammation markers studied. Adverse events (AEs) did not correlate with clinical outcome. On univariable analysis, intratumoural protein expression of C8 and CD68 and baseline levels of PIV, did not significantly correlate with the above-mentioned clinical outcome measures. However, univariable analyses revealed that lower intratumoural COX-2 expression ($P < 0.0001$), lower SII ($P = 0.0053$), elevated CRP, and stage IV significantly correlated with objective response to targeted therapy. Disease progression was significantly associated with elevated CRP ($P = 0.026$), elevated S100B ($P < 0.0001$), and stage III ($P = 0.0053$). Melanoma-specific death significantly correlated with stage IV (P 0.010) and elevated LDH ($P = 0.027$) and S100B ($P < 0.0001$). On multivariable analyses, lower intratumoural COX-2 expression (OR 33.9, 95% CI 3.2 to 356.8) and lower SII (OR 6.3, 95% CI 1.1 to 34.8) proved to be significant independent predictors for objective response to targeted therapy. Elevated S100B (HR 1.2, 95% CI 1.03 to 1.3) was a significant predictor for progressive disease, whereas stage III (HR 0.28, 95% CI 0.11 to 0.36) turned out to be a protective factor. Elevated S100B (HR 1.37, 95% CI 1.14 to 1.65) and LDH (HR 1.002, 95% CI 1.0001 to 1.003) were significant independent predictors for melanoma-specific death, whereas again stage III (HR 0.24, 95% CI 0.084 to 0.68) turned out to be a protective factor.

Tab. 1. Overview on baseline characteristics and clinical outcome of patients (n = 51) with advanced BRAF-mutated melanoma under therapy BRAF/MEK inhibitors (BRAFi/MEKi).

Parameters	Data of 51 patients in total
Age	
median (range)	60 (17-84) years
Gender	
Female/male	24 (47.1%)/27(52.9%)
Primary vertical tumour thickness	
median (range) mm	2.8 (0.3-38)
Ulceration: no/yes	35 (68.6%)/16 (31.4%)
IMelanoma stage, S100B, and LDH* prior to initiation of BRAFi/MEKi therapy	
Unresectable stage III	
Stage IV	15 (29.4%)
S100B elevated: no/yes	36 (70.6%)
LDH elevated: no/yes	22 (43.1%)/29 (56.9%)
	18 (35.3%)/33 (64.7%)
Median (range) time on BRAFi/MEKi therapy	8 (1-36) months
BRAFi-mono/BRAFi/MEKi-combi	12 (23.5%)/39 (76.5%)
AEs** (no/yes)	22 (43.1%)/29 (56.9%)
Objective response (rate)	22 (43.1%)
Progressive disease	38 (74.5%)
Median (range) progression-free survival	8 (1-90) months
Melanoma-specific deaths	31 (60.8%)
Median (range) melanoma-specific survival	17 (4-90) months

*, lactate dehydrogenase; **, adverse events

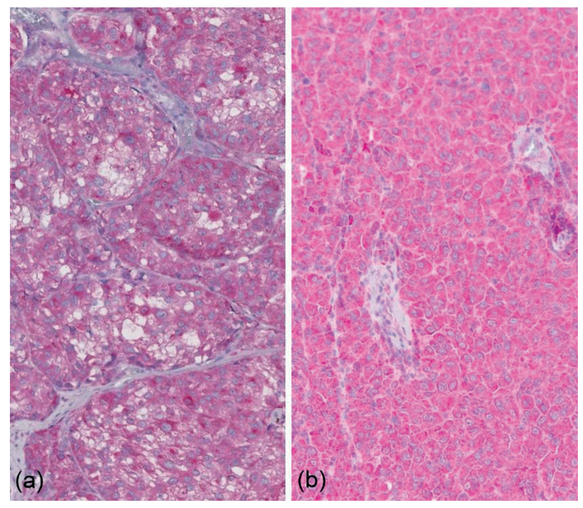

Fig. 1. Exemplarily showing intratumoural COX-2 immunostaining (H-score associated criterion for response < 179) of a melanoma patient with a COX-2 H-score of 146 who responded to targeted therapy (**a**). A melanoma patient with higher intratumoural COX-2 staining (H-score: 229) did not respond to targeted therapy (**b**).

Discussion

Inflammatory reactions play a crucial role in shaping solid cancers development in a lot of aspects, ranging from tumour initiation to progression and metastatic disease. Inflammatory related peripheral cells (e.g., neutrophils, lymphocytes) derived from the peripheral blood were significantly associated with tumour progression in a variety of cancers. According to the data of Mesti et al., high pre-ICI therapy levels of SII are associated with higher risk of melanoma progression and death. Interestingly, subgroup analysis of patients with high SII according to immune-related AEs revealed that patients with immune-related-AEs had higher PFS when compared to those who did not. An association which we did not observe in our BRAFi/MEKi-treated patients. In the present study, disease progression and melanoma-specific death were predicted by the classic biomarkers such as AJCC 8th stage, S100B, and LDH. However, we showed that lower pre-BRAFi/MEKi SII levels were significantly associated with treatment response. Fucà et al. showed that a high baseline PIV was independently associated with poor PFS and overall survival. However, we could not detect significant correlations between PIV and clinical outcome measures in the present and a previous study with ICI-treated patients.[9] This discordance between the studies may be explained by different study population characteristics. For example, Fuca et al. exclusively studied stage IV CM patients.

In melanoma COX-2 expression has been detected in human specimens and murine models. COX-2 expression has been proposed to be involved in melanoma development and progression. High COX-2 expression strongly correlates with a deeper Breslow index and a higher rate of lymph node involvement. Hence, COX-2 is an independent prognostic biomarker of lower survival outcomes in melanoma. Meyer et al. observed a significantly decreased PFS and a tendency to invasion in patients with high levels of COX-2, albeit only in primary metastatic tumours. By contrast, a recent study evaluated the COX-2 expression in melanoma lymph node metastases. Notably, high COX-2 expression reduced PFS by approximately 3 years without an association with BRAF/NRAS mutations. Nevertheless, it seems that BRAF-mutant melanomas are linked to increased COX-2 and PD-L1 expression via interleukin-1 upregulation suggesting that high levels of COX-2 represent a negative prognostic factor in metastatic melanoma. The COX-2 pathway induces PD-L1 expression *via* PI3K/AKT/mTOR, NF-κB and STAT3 activation. Hence, COX-2 is also a resistance factor against antigen-specific T lymphocyte cytotoxicity and thus of importance regarding response to ICIs. We studied for the first time intratumoural COX-2 expression in metastases of patients with BRAF-mutant melanomas who were treated with BRAFi/MEKi. Whereas we did not find a correlation between COX-2 expression and survival, low intratumoural COX-2 turned out to be a strong predictor for response to BRAFi/MEKi therapy. Nevertheless, limitations of the present study include the retrospective design and the small study sample as well.

Conclusions

The present study indicates that low SII and low intratumoural COX-2 protein expression are significant independent predictors for treatment response to BRAFi/MEKi but not for disease progression and melanoma-specific death.

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