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Pretreatment metastatic growth rate determines clinical outcome of advanced melanoma patients treated with anti-PD-1 antibodies: a multicenter cohort study

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ABSTRACT

Background Checkpoint inhibitors revolutionized the treatment of metastatic melanoma patients. Although tumor burden and lactate dehydrogenase (LDH) are associated with overall survival (OS), the impact of tumor growth kinetics remains elusive and in part contradictory. The aims of this study were to develop a novel simple and rapid method that estimates pretreatment metastatic growth rate (MGR) and to investigate its prognostic impact in melanoma patients treated with antiprogrammed death receptor-1 (PD-1) antibodies.

Methods MGR was assessed in three independent cohorts of a total of 337 unselected consecutive metastasized stage IIIB–IV melanoma patients (discovery cohort: n=53, confirmation cohort: n=126, independent multicenter validation cohort: n=158). MGR was computed during the pretreatment period before initiation of therapy with anti-PD-1 antibodies nivolumab or pembrolizumab by measuring the increase of the longest diameter of the largest target lesion. Tumor doubling time served as quality control. Kaplan-Meier analysis and univariable as well as multivariable Cox regression were used to examine the prognostic impact of MGR.

Results Pretreatment MGR >3.9 mm/month was associated with impaired OS in the discovery cohort (HR 6.19, 95% CI 2.92 to 13.10, p<0.0001), in the confirmation cohort (HR 3.62, 95% CI 2.19 to 5.98, p<0.0001) and in the independent validation cohort (HR 2.57, 95% CI 1.56 to 4.25, p=0.00023). Prior lines of systemic treatment did not influence the significance of MGR. Importantly, the prognostic impact of MGR was independent of total tumor burden, diameter of the largest metastasis, number of prior lines of systemic treatment, LDH, as well as liver and brain metastasis (discovery and confirmation cohorts: both p<0.0001). Superiority of MGR compared with these variables was confirmed in the independent multicenter validation cohort (HR 2.92, 95% CI 1.62 to 5.26, p=0.00036).

Conclusions High pretreatment MGR is an independent strong prognostic biomarker associated with unfavorable survival of melanoma patients receiving anti-PD-1 antibodies. Further investigations are warranted to assess the predictive impact of MGR in distinct systemic therapeutic regimens.

BACKGROUND

Immune checkpoint inhibitors (ICI), namely antibodies against programmed death receptor-1 (PD-1), have considerably improved the outcome of patients with advanced melanoma and are capable to induce long-lasting responses in melanoma patients.[1 2] However, primary or acquired resistance against ICI is common and occurs in 50%–60% of the patients.[3] Therefore, prognostic biomarkers are urgently needed that identify patients who might benefit from anti-PD-1 antibodies more than others.

Clinical experience at our institution suggests that patients displaying extensive tumor burden and fast-growing tumors tend be non-responders to ICI.[4] This clinical experience is supported by data from Ribas *et al* indicating that high total tumor burden of more or equal than 102 mm according to Response Evaluation Criteria in Solid Tumors

(RECIST) V.1.1 correlates with lower response rates in patients treated with pembrolizumab.[5] Indirect markers for tumor growth or tumor cell turnover like lactate dehydrogenase (LDH) have been studied extensively in the setting of immunotherapy with anti-PD-1 antibodies and were associated with survival.[4 6–9] Thus, the direct investigation of tumor growth as a prognostic marker seems obvious. As early as in the 1960s to 1990s, tumor growth rate (TGR) by means of tumor doubling time (TDT) has been studied in patients with cancer with pulmonary metastases undergoing surgical resection.[10 11] Only patients with slow-growing pulmonary metastases benefited from surgery and achieved long-term overall survival (OS).[10] However, only little is known about the impact of TGR in the context of systemic therapy. In 2014, a French study demonstrated the superiority of initial metastatic kinetics compared with LDH and American joint committee on cancer (AJCC) stage of disease in patients treated with chemotherapy.[12] A recent study reported on fast growing metastases with an intraindividual broad range of TGR being associated with impaired survival in patients treated with BRAF inhibitors (BRAFi).[13] The group around Hartung *et al* determined pretreatment disease kinetics by measuring every metastasis in each patient. The discovery of hyperprogressive disease in patients receiving ICI brought pretreatment TGR again into a broader focus. However, the results in respect of the prognostic impact of pretreatment TGR were conflicting.[14–17] Champiat and colleagues even found an inverse correlation of TGR with objective response in a single-center study including 131 patients with 21 distinct cancer entities treated with antibodies directed against PD-1 or programmed cell death 1 ligand 1 (PD-L1).[14]

The aim of this study was to analyze the prognostic impact of pretreatment TGR and total tumor burden on OS in melanoma patients receiving anti-PD-1 antibodies. Moreover, we aimed at developing a feasible method of approximating pretreatment TGR that could replace the ineffective and time-consuming measurement of each metastasis. We hypothesized that high metastatic growth rate (MGR) correlates with inferior survival and lower response rates to anti-PD-1 therapy in patients with metastatic melanoma.

METHODS

Patients

From October 2013 to February 2017, 53 consecutive patients with unresectable melanoma were treated with the anti-PD-1 antibody nivolumab (discovery cohort) and 126 patients with the anti-PD-1 antibody pembrolizumab (confirmation cohort) at the Department of Dermatology, University Hospital Tübingen, Germany and were enrolled retrospectively in this study. A third cohort comprizing 158 melanoma patients treated with either nivolumab or pembrolizumab between February 2013 and September 2019 at 12 distinct clinical sites throughout Austria, Germany and Switzerland (validation cohort) was enrolled as an independent multicentric validation cohort. Online supplemental table 1 summarizes the total number of patients enrolled in the study. Patients were eligible for inclusion if they had a radiographic imaging by CT, MRI, or positron emission tomography-CT (PET-CT) at baseline (T_0) and at least one additional prebaseline staging 28 days or more prior to T_0 (T_{-1}). Patient data, clinical variables, and radiologic reports were obtained from electronic patient records, imaging data were evaluated with the study sites' PACS DICOM viewer. The study was carried out in accordance with the Declaration of Helsinki of 1975 and succeeding amendments.

Treatment and response assessment

Patients received either nivolumab or pembrolizumab in the respective approved dosages or according to the clinical trials' protocols. Clinical response was assessed every 3 months according to RECIST V.1.1.[18]

Determination of MGR

Radiological measurements were evaluated based on radiological images and/or written findings. For each patient, the largest metastasis at baseline was determined using the longest diameter (D) in axial plane. In case of lymph node metastases, the short axis diameter was used. For the determination of MGR, the absolute metastatic growth in millimeters (mm) was determined as the difference between the diameter of the largest lesion at the baseline staging (D_0) and at the prebaseline staging (D_{-1}). This difference was divided by the number of days elapsed between the prebaseline staging and the baseline staging (t). The resulting value was multiplied with 30.4375 days per month to convert mm per day to mm per month (mm/month). The following equation summarizes this relation:

$$MGR = \frac{D_0 - D_{-1}}{t} \times 30.4375 \frac{days}{month}$$

Putatively inactive metastases, for example, curatively irradiated metastases that were constant in size or regressing before anti-PD-1 treatment was commenced, were not considered for MGR determination. In these cases, the next largest metastasis was chosen. However, only a very few lesions qualifying as target lesions had been irradiated before treatment with anti-PD-1 was commenced (discovery cohort: n=3, confirmation cohort: n=1, validation cohort: n=2). Neither their inclusion, nor their omission significantly altered the results. Assessment of clinical response and MGR was performed independently in a blinded fashion. Four experienced reference radiologists (BK, CZ, MP and NR) were involved in this work. Throughout the study, including the 12 independent external study sites, the same methods as well as the same standards to assess radiologic data and radiologic reports were used. As a second measure, tumor growth dynamics was determined by means of the TDT. TDT was determined using the same target lesion utilized for MGR calculation. Tumor volume (V) was approximated by $V = \frac{4}{3}\pi R^3$, where R, the radius of the sphere,

is equal to D/2. The tumor volumes at baseline (V_0) and at prebaseline (V_{-1}) together with the elapsed time in days between these staging examinations (t) were used to calculate TDT using the following equation according to Honda *et al*[19]:

$$TDT = \frac{t \times \log(2)}{\log\left(\frac{V_0}{V_{-1}}\right)}$$

Statistical analysis

Response according to RECIST criteria V.1.1, OS defined as the time from starting anti-PD-1 ICI until death due to any cause or end of follow-up, and progression-free survival (PFS) defined as the time from starting anti-PD-1 treatment until progression or death due to melanoma or end of follow-up were explored in all patients. OS and PFS were analyzed using Kaplan-Meier estimator and two-sided log-rank test as well as with multivariable Cox regression analysis. HR in univariable analyses were determined using univariable Cox regression analysis. The cut-off points for MGR and TDT were determined based on the data of the discovery cohort using a previously described algorithm that minimizes the resulting p value.[20] The obtained cut-off values were applied at all survival analyses throughout the study.

Two-sided Mann-Whitney U test was used to compare MGR according to best overall response. Categorical variables were compared using two-sided Fisher's exact test. Throughout all analyses, p<0.05 were considered statistically significant. All analyses were performed using R V.4.0.2 and the 'survival' and 'maxstat' packages.[21]

RESULTS

Patient characteristics

Three hundred and thirty-seven patients with unresectable metastatic melanoma were included in this study (53 patients in the discovery cohort, 126 patients in the confirmation cohort and 158 patients in the independent validation cohort). Detailed clinical characteristics are summarized in table 1. Most patients started immunotherapy at stage M1c (AJCC classification from 2009) disease (79.2%, 78.6%, and 71.5%, respectively) and had visceral metastases other than lung metastases (73.6%, 66.7%, and 58.2%, respectively). Central nervous system (CNS) metastases were present in 28.3% of the patients in the discovery cohort, in 35.7% in the confirmation cohort, and in 20.3% in the validation cohort. Liver metastases were present in 32.1%, 27.8%, and 29.7.%, respectively. Anti-PD-1 immune checkpoint blockade was implemented as first line treatment in 22.6%, 38.1%, and 54.4% of the patients, respectively. Median OS in the three cohorts was 16.7 months (95% CI 12.9 to not reached), 23.4 months (95% CI 16.8 to not reached), and 38.8 months (95% CI 31.2 to not reached), respectively.

Table 1 Characteristics of the study population

	Discovery cohort (n=53) No (%)	Confirmation cohort (n=126) No (%)	Validation cohort (n=158) No (%)
Age (years)			
≤60	31 (58)	45 (35.7)	45 (28.5)
>60	22 (42)	81 (64.3)	113 (71.5)
Gender			
Female	21 (40)	49 (38.9)	53 (33.5)
Male	32 (60)	77 (61.1)	105 (66.5)
BRAF mutational status			
Negative	37 (70)	77 (61.1)	100 (63.3)
Positive	16 (30)	46 (36.5)	57 (36.1)
Unknown	0 (0)	3 (2.4)	1 (0.6)
No of organs involved			
1	7 (13)	15 (11.9)	32 (20.3)
2	15 (28)	40 (31.7)	35 (22.2)
3	14 (26)	26 (20.6)	43 (27.2)
4	7 (13)	19 (15.1)	34 (21.5)
5	7 (13)	14 (11.1)	6 (3.8)
6	2 (4)	7 (5.6)	7 (4.4)
≥7	1 (2)	5 (4.0)	1 (0.6)
AJCC M stage (AJCC 2009)			
M0	1 (2)	3 (2.4)	8 (5.1)
M1a	2 (4)	4 (3.2)	10 (6.3)
M1b	8 (15)	20 (15.9)	27 (17.1)
M1c	42 (79)	99 (78.6)	113 (71.5)
Visceral metastasis			
No	14 (26)	42 (33.3)	66 (41.8)
Yes	39 (74)	84 (66.7)	92 (58.2)
CNS metastasis			
No	38 (72)	81 (64.3)	126 (79.7)
Yes	15 (28)	45 (35.7)	32 (20.3)
Liver metastasis			
No	36 (68)	91 (72.2)	111 (70.3)
Yes	17 (32)	35 (27.8)	47 (29.7)
Prior treatment regimens			
Anti-CTLA-4	36 (68)	54 (42.9)	51 (32.3)
Anti-PD-1	0 (0)	4 (3.2)	11 (7.0)
BRAFi±MEKi	10 (19)	34 (27.0)	29 (18.4)
MEKi	3 (6)	0 (0.0)	4 (2.5)
Chemotherapy	9 (17)	28 (22.2)	15 (9.5)
Radiotherapy	29 (55)	53 (42.1)	40 (25.3)
Adjuvant interferon	17 (32)	49 (38.9)	31 (19.6)
Other	1 (2)	1 (0.8)	1 (0.6)
Line of treatment			
First line	12 (23)	48 (38.1)	87 (55.1)
Second line	24 (45)	40 (31.7)	39 (24.7)
≥Third line	17 (32)	38 (30.2)	32 (20.3)

Continued

Table 1 Continued

	Discovery cohort (n=53) No (%)	Confirmation cohort (n=126) No (%)	Validation cohort (n=158) No (%)

AJCC 2009 refers to the AJCC staging guideline for melanoma from 2009.
AJCC, American Joint Committee on Cancer; BRAFi, BRAF inhibitors; CNS, central nervous system; CNS, central nervous system; PD-1, programmed death receptor-1.

MGR is strongly associated with OS and predicts response to anti-PD-1 antibodies

Figure 1 graphically depicts MGR determination and illustrates generic CT image examples of slow-growing and fast-growing metastases. Univariable analysis of OS in the discovery cohort revealed significantly impaired OS in patients with MGR >3.9 mm/month compared with the remaining patients (HR 6.19, 95% CI 2.92 to 13.10, p<0.0001) (figure 2A). Two-year OS was 8.0% (2.1%–30.2%) vs 64.3% (48.8%–84.7%). This result was confirmed in the confirmation cohort (HR 3.62, 95% CI 2.19 to 5.98, p<0.0001, 2 years OS: 24.9% (95% CI 15.2% to 40.9%) vs 62.3% (95% CI 51.3% to 75.7%)) (figure 2B), and in the validation cohort (HR 2.57, 95% CI 1.56 to 4.25, p=0.00023, 2 years OS: 41.4% (95% CI 29.4% to 58.4%) vs 80.8% (95% CI 72.7% to 89.8%)) (figure 2C). TDT less than 37 days was also associated with unfavorable OS (HR 1.83, 95% CI 1.11 to 3.03, p=0.018), but its impact was inferior compared with MGR (online supplemental figure 1). Analysis of PFS showed similar results for the three cohorts with MGR being strongly associated with reduced PFS (online supplemental figure 2). Importantly, the results for MGR and OS remained highly significant after exclusion of mucosal and uveal melanomas (online supplemental figure 3). In the pooled subgroup of mucosal melanoma, MGR only showed a non-significant trend (HR 1.86, 95% CI 0.52 to 6.63, p=0.34), but in uveal melanoma patients, high MGR significantly correlated with impaired OS (HR 5.87, 95% CI 1.04 to 32.97, p=0.045) (online supplemental figure 4).

Analysis of best objective response according to MGR showed a significant surplus of patients with progressive disease (PD) in the subgroup with MGR exceeding 3.9 mm/month in the discovery cohort (OR 28.4, 95% CI 5.9 to 187.5, p<0.0001), in the confirmation cohort (OR 5.1, 95% CI 2.2 to 12.5, p<0.0001), and in the validation cohort (OR 3.7, 95% CI 1.7 to 8.1, p=0.00036) (table 2). Median MGR was significantly higher in patients reaching PD as best objective response compared with patients with stable disease, partial response or complete response (online supplemental figure 5).

Multivariable Cox regression analysis of OS including MGR, diameter of the largest target lesion, sum of target lesions according to RECIST V.1.1 criteria, liver metastasis, CNS metastasis, LDH, and line of treatment revealed MGR as the only factor being significantly associated with OS in all three cohorts (discovery cohort: HR 9.1, 95% CI 3.2 to 25.4, p<0.0001; confirmation cohort: HR 3.8, 95% CI 2.1 to 6.7, p<0.0001; validation cohort: HR 2.9, 95% CI 1.6 to 5.3, p=0.00036) (table 3).

Based on the unexpected minor importance of LDH in multivariable analysis, the respective impact of LDH and MGR on OS was assessed in a combined Kaplan-Meier analysis of the pooled cohorts (figure 3). While LDH barely separated the MGR low and MGR high subgroups (MGR low: HR 1.30, 95% CI 1.02 to 1.65, p=0.036; MGR high: HR 1.21, 95% CI 0.98 to 1.49, p=0.076), the differences according to MGR was highly significant both in the LDH low (upper limit of normal, ≤ULN) and LDH high (>ULN) subgroups (LDH low: HR 3.75, 95% CI 2.33 to 6.02, p<0.0001; LDH high: HR 2.69, 95% CI 1.74 to 4.18, p<0.0001). These results were underlined by the comparison of LDH low—MGR high with LDH high—MGR low patients (HR 0.51, 95% CI 0.32 to 0.83, p=0.0061).

To investigate whether MGR was prognostic for OS independently of prior therapies, subgroup analyses comprizing patients with any prior systemic therapy, treatment-naïve patients, patients with prior BRAFi therapy, and patients with prior anti-CTLA-4 therapy were conducted. The prognostic impact of MGR on OS (figure 4) and PFS (online supplemental figure 6) was comparable and highly significant (p<0.001, each) throughout these subgroups. The predictive capacity of MGR also remained high in subgroup analysis comprizing either patients who received prior radiotherapy (HR 3.85, 95% CI 2.35 to 6.31, p<0.0001) or being radiotherapy-naïve (HR 2.84, 95% CI 1.86 to 4.35, p<0.0001) (online supplemental figure 7).

DISCUSSION

Indirect serum biomarkers of tumor burden and tumor cell turnover like LDH are commonly used for prediction of response and monitoring of the course of disease.[5 6 8 22] However, they are prone to failure due to limited specificity and their surrogate nature.[23 24] Therefore, direct approaches for measuring tumor burden and disease kinetics are needed. In the present study, pretreatment MGR was found to represent a reliable prognostic marker strongly correlating with OS and PFS of melanoma patients treated with anti-PD-1 antibodies. In multivariable analysis, pretreatment MGR was clearly superior to the established prognostic factors tumor burden, LDH, liver metastasis and CNS metastasis.

In the last decade, TGR has been of increasing interest due to limitations of the established criteria to evaluate response to anticancer therapy. Several studies could show that the variation of on-treatment compared with pretreatment TGR is superior to the determination of objective response based on RECIST and that a significant number of patients classified with PD showed decreasing TGR under therapy.[15 25 26] Moreover, with the introduction of ICI, TGR was discovered as a measure to identify patients showing hyperprogressive disease.[14 16 17 27 28] Interestingly, the results of our study are in sharp contrast

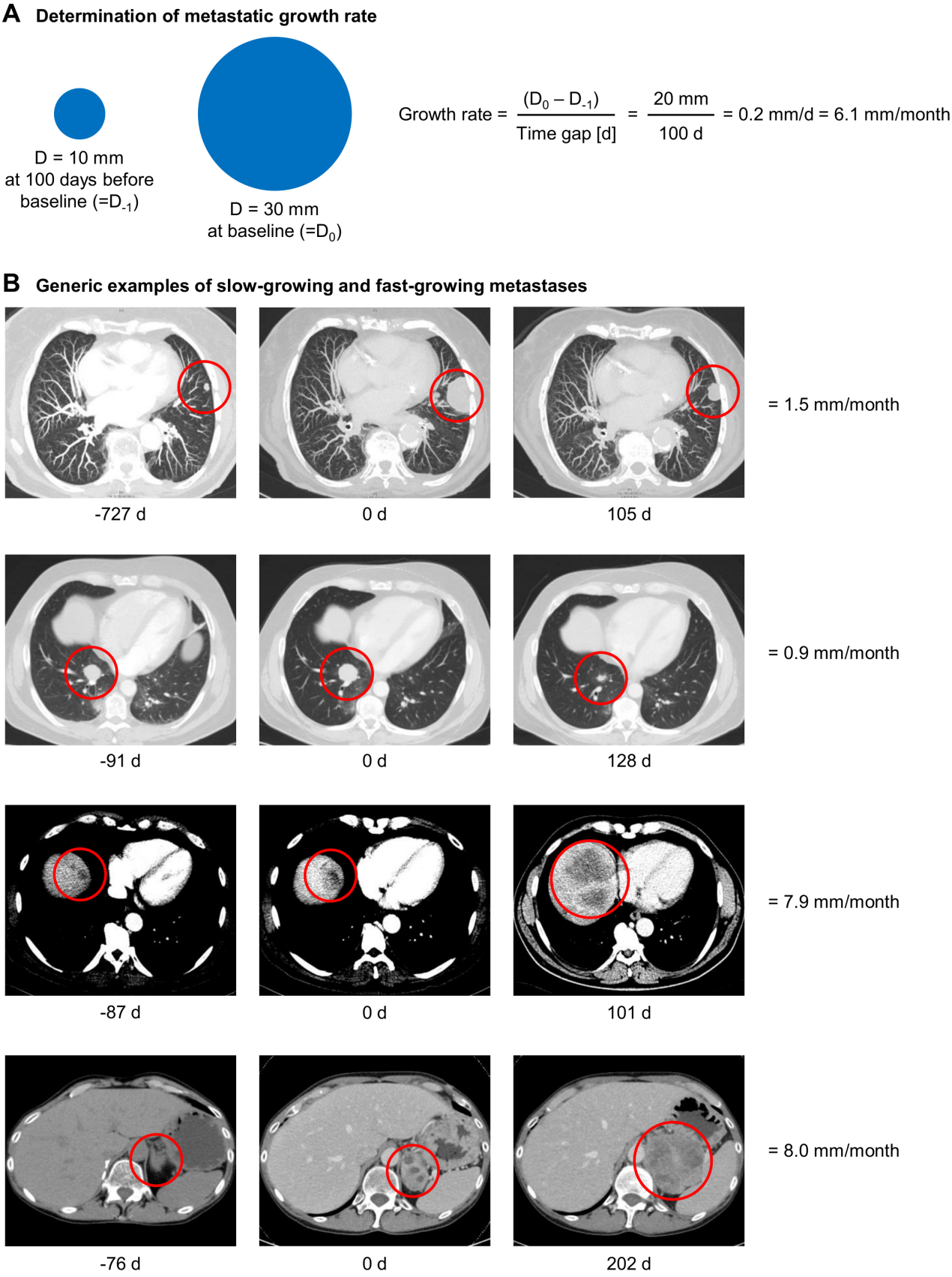


Figure 1 Determination of metastatic growth rate (MGR). (A) Schematic of MGR calculation based on two CT-/MRI-based measurements of metastatic diameters of the largest target lesion. D_0 is the diameter of the largest target lesion at baseline (T_0), whereas D_{-1} is the diameter of the identical target lesion at the last staging prior to baseline (T_{-1}). (B) Example CT images of the largest target lesions of two patients with low MGR (upper two rows) and of two distinct patients with high MGR (lower two rows) at the indicated time points. d, days.

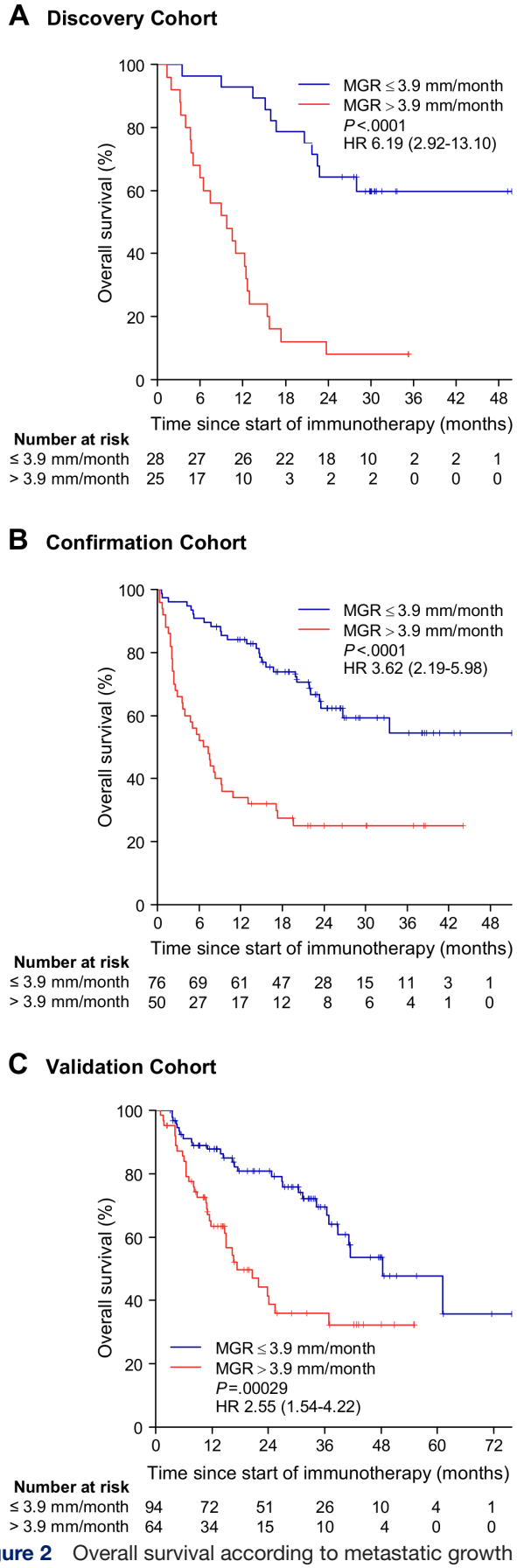


Figure 2 Overall survival according to metastatic growth rate (MGR). Kaplan-Meier curves depicting overall survival according to MGR. PD-1, programmed death receptor-1.

to findings of Champiat and colleagues who found an inverse correlation between pretreatment TGR and the percentual change of the sum of RECIST target lesions under therapy with anti-PD-1 or anti-PD-L1 antibodies in a single-cohort study on a heterogeneous set of 131 patients with 21 distinct tumor entities.[14] The authors drew the conclusion that unlike in targeted therapy, ICI showed improved efficacy in patients exhibiting faster TGR. Importantly, the authors missed to notice the confounding effect of their heterogeneous cohort composed of fast-growing cancers like high-grade glioma (median TDT: 63.4 days)[29] or melanoma (median TDT: 61 days)[30] and slow-growing cancers like adenocarcinoma of the lung (median TDT: 258 days)[19] or clear cell renal cell carcinoma (median TDT: 826 days).[31] However, anti-PD-1 therapy induced response rates and PFS largely differ between these entities (melanoma: objective response rate (ORR) 32%, median PFS 6.9 months; renal cell carcinoma: ORR 25%, median PFS 4.6 months; esophagogastric cancer: ORR 12%, median PFS 1.4 months).[22 32 33]

The question whether pretreatment MGR is prognostic in general or specifically predictive for outcome in anti-PD-1-treated patients cannot be answered by our study. Although some studies implicated a strong general prognostic impact on survival of cancer patients,[10–12] the extent of MGR's discriminatory power was unexpectedly impressive in our data. Moreover, besides OS, high MGR was also clearly associated with unfavorable PFS and failure to achieve disease control or an objective response. In multivariable analysis of all three independent cohorts, MGR was most clearly associated with OS compared with the diameter of the largest target lesion, sum of RECIST target lesions, LDH, presence of liver metastasis, and presence of brain metastasis. Superiority of MGR over LDH was confirmed by the combined Kaplan-Meier analysis of both biomarkers that demonstrated the pronounced prognostic impact of MGR compared with LDH.

In comparison to the studies published so far, the main strength of our study is the inclusion of three independent cohorts comprizing a multicenter external validation cohort and 337 patients in total. Therefore, we can conclude with certainty that MGR constitutes a powerful and valuable novel prognostic marker for patients treated with anti-PD-1 antibodies.

Our data suggest that anti-PD-1 antibodies are not capable to inhibit rapidly growing metastases. Recently, Huang *et al* delineated the ratio of T-cell invigoration to tumor burden as being closely associated with response to anti-PD-1 checkpoint blockade.[34] Patients with low ratios of Ki67 positive PD-1 positive T-cells to tumor burden exhibited low ORR and impaired survival. In line are recent findings of our group that showed that the interruption of interferon-induced senescence leads to an uncontrolled growth of melanoma metastases.[35] These results indicate that a disequilibrium between unleashed tumor growth and T-cells leads to fatal outcomes.

In the recent past, several efforts have been made to identify novel prognostic markers in the setting of ICI

Table 2 Best objective response according to MGR

	Discovery cohort n (%)			Confirmation cohort n (%)			Validation cohort n (%)		
	PD	SD	PR/CR	PD	SD	PR/CR	PD	SD	PR/CR
MGR ≤3.9 mm/month	4 (14)	9 (32)	15 (54)	23 (31)	13 (17)	39 (52)	18 (19)	23 (24)	53 (56)
MGR >3.9 mm/month	21 (84)	1 (4)	3 (12)	32 (70)	3 (7)	11 (24)	30 (47)	11 (17)	23 (36)
	OR*: 28.4 (5.9 to 187.5) p<0.0001			OR: 5.1 (2.2 to 12.5) p<0.0001			OR: 3.7 (1.7 to 8.1) p=0.00036		

Best objective response was assessed according to Response Evaluation Criteria in Solid Tumors V.1.1.
*ORs and p values were determined utilizing two-sided Fisher's exact test comparing PD versus SD/PR/CR.
CR, complete response; MGR, metastatic growth rate; PD, progressive disease; PR, partial response; SD, stable disease.

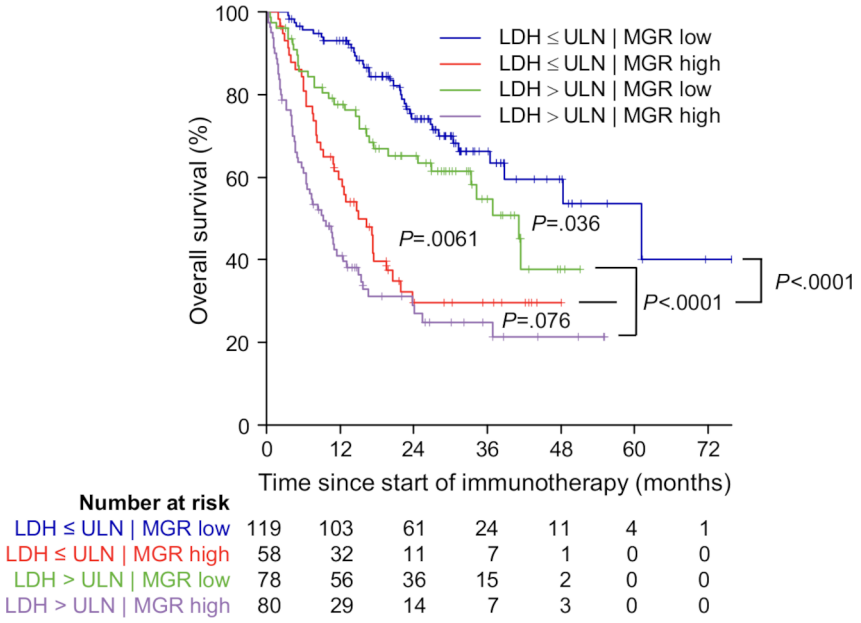
with PD-1 antibodies. Most promising, but also controversial is the utilization of PD-L1 status as a predictor of response.[36 37] Although several studies have highlighted the predictive impact of PD-L1 expression in tumor tissue, several limitations deserve cautious appraisal of this molecular biomarker.[2 37–40] A major disadvantage of PD-L1 status concerns the prerequisite of surgically accessible metastases and its high effort. Moreover, technical issues like PD-L1 expression heterogeneity within the microenvironment hampers the evaluation of immunohistochemistry.[36]

In previous studies, TGR was either computed based on all 1–5 RECIST target lesions, 1–10 iRECIST target lesions, or by measuring the total number of each measurable metastasis.[12–17 25–27] The approach used by Gaudy-Marqueste *et al* as well as Hartung *et al* implicated the

Table 3 Multivariable Cox regression analysis of overall survival

	Discovery cohort (n=53)		Confirmation cohort (n=126)		Validation cohort (n=158)	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
MGR						
≤3.9 mm/month	1		1		1	
>3.9 mm/month	9.1 (3.2 to 25.4)	<0.0001	3.8 (2.1 to 6.7)	<0.0001	2.9 (1.6 to 5.3)	0.00036
Diameter of largest TL						
≤Median	1		1		1	
>Median	0.3 (0.1 to 0.9)	0.022	1.3 (0.6 to 3.0)	0.47	0.7 (0.3 to 1.5)	0.34
Sum of RECIST TLs						
≤Median	1		1		1	
>Median	3.1 (1.0 to 9.6)	0.046	0.6 (0.3 to 1.3)	0.16	1.3 (0.6 to 2.7)	0.45
Liver metastasis						
No	1		1		1	
Yes	2.4 (0.9 to 6.5)	0.081	2.0 (1.1 to 3.4)	0.016	2.0 (1.2 to 3.3)	0.0088
CNS metastasis						
No	1		1		1	
Yes	2.9 (1.1 to 8.2)	0.039	2.2 (1.3 to 3.8)	0.0058	1.3 (0.7 to 2.4)	0.40
LDH						
≤ULN	1		1		1	
>ULN	1.6 (0.7 to 3.8)	0.28	1.7 (0.9 to 3.1)	0.081	1.5 (0.9 to 2.5)	0.16
Line of treatment						
First line	1		1		1	
Second line	0.8 (0.3 to 2.4)	0.73	0.7 (0.4 to 1.3)	0.28	2.0 (1.1 to 3.9)	0.033
≥Third line	0.9 (0.2 to 3.1)	0.84	1.0 (0.5 to 1.9)	0.96	2.3 (1.2 to 4.3)	0.012

CNS, central nervous system; LDH, lactate dehydrogenase; MGR, metastatic growth rate; RECIST, response evaluation criteria in solid tumors version 1; ULN, upper limit of normal.



Number at risk

LDH ≤ ULN | MGR low	119	103	61	24	11	4	1
LDH ≤ ULN | MGR high	58	32	11	7	1	0	0
LDH > ULN | MGR low	78	56	36	15	2	0	0
LDH > ULN | MGR high	80	29	14	7	3	0	0

Figure 3 Overall survival according to lactate dehydrogenase (LDH) and metastatic growth rate (MGR) Kaplan-Meier curves depicting overall survival in the pooled entire cohort according to LDH below or above upper limit of normal (ULN) and pretreatment MGR below or above 3.9 mm/month.

measurement of all metastases with a diameter of at least 10 mm in three dimensions.[12 13] Especially in patients harboring hundreds of metastases, this approach is very time-consuming and limits sample size.[13] Data derived from chest roentgenograms had shown little intraindividual variation in TDT of multiple metastatic lesions suggesting that measuring only one target lesion could be sufficient to reflect disease kinetics.[41 42] This justifies our rationale for measuring only one target lesion which seems suitable to reflect tumor growth dynamics. Different models of tumor growth have been developed, comprizing exponential, exponential-linear, Gompertz function, or logistic growth, and there is a long-lasting discussion on what is the best to describe this dynamic process.[43–45] To take the classical exponential model into account, our study includes the TDT used by many authors.[19 43 46 47] In addition, we introduce a novel approach that, like the logistic or Gompertz models, considers the declining growth rate of larger metastases when angiogenesis, nutrient and oxygen depletion, as well as tumor cell necrosis increasingly play a role.[44 45 48] While TDT is constant at 30 days per doubling for two lesions that increase from 10 to 20 mm (diameter) within 90 days, and from 40 to 80 mm within 90 days, respectively, MGR reflects this more dramatic absolute growth of the larger lesion with calculated growth rates of 3.4 mm/month and 13.5 mm/month, respectively. Murphy *et al* presented in detail with impressive examples that all models of tumor growth encounter their limits under certain conditions.[48] Despite these considerations, the herewith introduced MGR, although not claiming to represent a theoretical mathematical model of tumor growth, was capable to discriminate the patients more efficiently than TDT.

We are aware of several limitations of our study. The retrospective design makes it susceptible for a patient selection bias. However, we included all consecutive patients receiving nivolumab or pembrolizumab who had at least one prebaseline staging at our center. Moreover, a third independent cohort of patients enrolled at 12 participating centers and assessed by 12 independent and experienced dermato-oncologists and radiologists confirmed the results observed in the two monocentric cohorts. Thereby, site-specific treatment procedures and patient selection bias could be minimized. To the best of our knowledge, this is by far the largest set of patients analyzed concerning pretreatment tumor growth kinetics.

Another putative limitation constitutes the impact of prior treatment regimens on MGR. In the discovery cohort 36 of the 53 patients had been previously treated with ipilimumab. It seems suggestive that patients showing stable or even slightly decreasing target lesions on treatment with ipilimumab might benefit from an anti-PD-1 antibody or tend to benefit from any ICI. However, MGR remained a strong prognostic factor in subgroup analysis of patients with any prior systemic therapy, prior BRAFi, prior ipilimumab, and treatment-naïve patients.

The probably most important limitation of the concept of pretreatment tumor growth dynamics as baseline prognostic factor is the prerequisite of at least one prebaseline staging. In a personal statement by Jean Jacques Grob, Georgina Long, Dirk Schadendorf and Keith Flaherty published in 2015, the expert authors had discussed the option of postponing the start of therapy to achieve this premise.[11 12 31] However, ethical reservations should be discussed carefully when deciding about treatment delays owed to diagnostic procedures. Given a median MGR

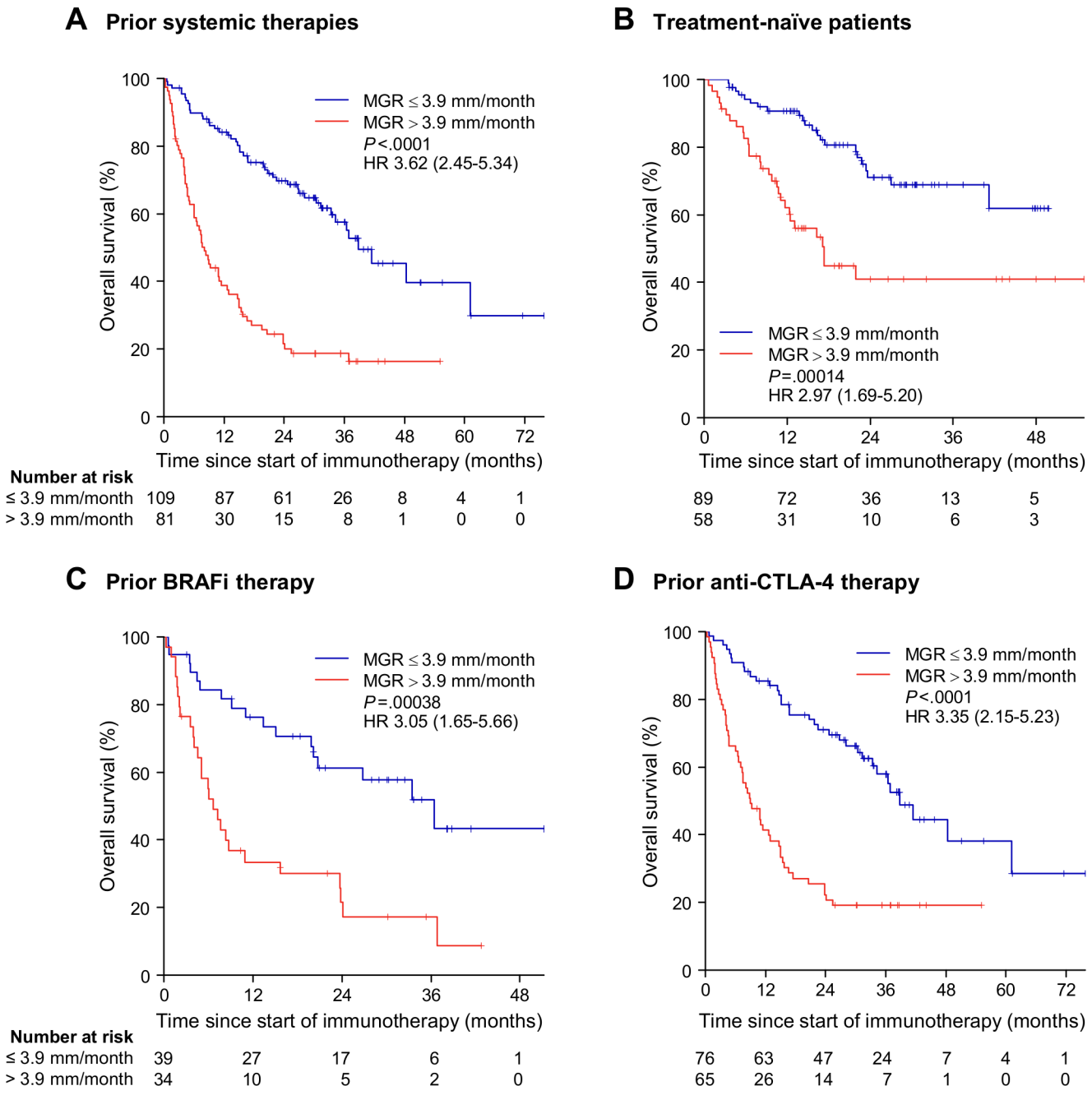


Figure 4 Overall survival according to MGR in regard of prior therapies. Kaplan-Meier curves depicting overall survival according to pretreatment MGR in (A) patients with prior systemic therapy/therapies, (B) treatment-naïve patients, (C) patients with prior BRAFi therapy and (D) patients with prior anti-CTLA-4 therapy. BRAFi, BRAF inhibitor; MGR, metastatic growth rate.

of approximately 3 mm/month and a median doubling time of 41 days, consecutive staging examinations should be separated by at least 1 month to account for measuring inaccuracy. This recommendation for a minimum and an ideal time interval between the two successive measures of target lesions is in accordance with previous suggestions.[12 49] In our validation cohort, the median interval between the pretreatment staging examinations was 86 days (IQR: 61–116 days) indicating a low risk for measuring inaccuracy.

Apart from intentional treatment delays, there can be several other reasons that normally lead to consecutive staging examinations prior to anti-PD-1 therapy. In our study, the most common cause for the existence of two staging examinations before initiation of ICI was the requirement to complete staging information, for example, in cases where only a CT of the abdomen was available or in cases where a PET scan was needed to improve diagnostic sensitivity. The second most common cause were regular on-treatment staging examinations during prior therapies like ipilimumab or BRAFi. Delays between initial staging and start of therapy also led to the necessity to perform an additional staging scan immediately before anti-PD-1 therapy. However, this reason was less common.

CONCLUSIONS

In conclusion, high pretreatment TGR is associated with unfavorable outcome and non-response in advanced melanoma patients treated with anti-PD-1 antibodies. With MGR, we propose a novel measure of tumor growth kinetics that independently predicts survival, superiorly compared with total tumor burden, LDH, site of metastasis and other known factors. As a time-efficient method, determination of MGR can be easily implemented in routine clinical settings and should be explicitly considered prior to therapeutic decisions. Investigation of the predictive impact of MGR in distinct therapeutic regimens like PD-1 blockade, combined ICI with anti-CTLA-4 and anti-PD-1, and small molecule inhibitors targeting the MAPK pathway, is warranted.

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