Journal for ImmunoTherapy of Cancer

Prognostic value of von Willebrand factor levels in patients with metastatic melanoma treated by immune checkpoint inhibitors

Julia-Christina Stadler,[1,2,3] Laura Keller ,[2,4] Christian Mess,[1] Alexander T Bauer,[1] Julian Koett,[1,3] Glenn Geidel ,[1] Isabel Heidrich,[1,2] Sabine Vidal-y-Sy,[1] Antje Andreas,[2] Carlotta Stramaglia,[1] Mark Sementsov,[2] Wiebcke Haberstroh,[1] Benjamin Deitert,[2] Inka Lilott Hoehne,[1] Robin Reschke,[1,3] Thomas Haalck,[1] Klaus Pantel,[2,3] Christoffer Gebhardt ,[1,3] Stefan W Schneider[1,3]

To cite: Stadler J-C, Keller L, Mess C, *et al*. Prognostic value of von Willebrand factor levels in patients with metastatic melanoma treated by immune checkpoint inhibitors. *Journal for ImmunoTherapy of Cancer* 2023;**11**:e006456. doi:10.1136/jitc-2022-006456

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2022-006456).

J-CS, LK and CM contributed equally.

Accepted 28 April 2023

For numbered affiliations see end of article.

Correspondence to
Professor Stefan W Schneider; st.schneider@uke.de

Professor Christoffer Gebhardt; ch.gebhardt@uke.de

Professor Klaus Pantel; pantel@uke.de

ABSTRACT

Background An increased incidence of thrombotic complications associated with an increased mortality rate has been observed under immune checkpoint inhibition (ICI). Recent investigations on the coagulation pathways have highlighted the direct role of key coagulatory proteins and platelets in cancer initiation, angiogenesis and progression. The aim of this study was to evaluate the prognostic value of von Willebrand factor (vWF) and its regulatory enzyme a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13), D-dimers and platelets in a cohort of patients with metastatic melanoma receiving ICI.

Methods In a prospective cohort of 83 patients with metastatic melanoma, we measured the systemic levels of vWF-antigen (vWF:Ag), ADAMTS13 activity, D-dimers and platelets, before the beginning of the treatment (baseline), and 6, 12 and 24 weeks after. In parallel, we collected standard biological parameters used in clinical routine to monitor melanoma response (lactate deshydrogenase (LDH), S100). The impact of neutrophil-to-lymphocyte ratio (NLR) and C-reactive protein (CRP) on overall survival (OS) in patients receiving ICI was assessed. Univariable and multivariable Cox proportional models were then used to investigate any potential association of these parameters to clinical progression (progression-free survival (PFS) and OS). Baseline values and variations over therapy course were compared between primary responders and resistant patients.

Results Patients with melanoma present with dysregulated levels of vWF:Ag, ADAMTS13 activity, D-dimers, LDH, S100 and CRP at the beginning of treatment. With a median clinical follow-up of 26 months, vWF:Ag interrogated as a continuous variable was significantly associated with PFS in univariate and multivariate analysis (HR=1.04; p=0.007). Lower values of vWF:Ag at baseline were observed in the primary responders group (median: 29.4 µg/mL vs 32.9 µg/mL; p=0.048) when compared with primary resistant patients. As for OS, we found an association with D-dimers and ADAMTS13 activity in univariate analysis and vWF:Ag in univariate and multivariate analysis including v-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutation and Eastern Cooperative Oncology Group (ECOG) performance status. Follow-up over the course of treatment depicts different evolution profiles for vWF:Ag between the primary response and resistance groups.

Conclusions In this prospective cohort, coagulatory parameters such as ADAMTS13 activity and D-dimers are associated with OS but baseline vWF:Ag levels appeared as the only parameter associated with response and OS

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Over the past few years, multiple determinants of response or resistance to immunotherapy have already been identified but the complex biology behind the immunological tumor response is not yet completely understood. An increased incidence of thrombotic complications associated with an increased mortality rate has been already reported under immune checkpoint inhibition (ICI). Interestingly, coagulation factors involved into the last steps of the coagulation cascade could also interplay with antitumor immunity and provide synergistic effect with ICI. Whether other aspects of the coagulation process like those involving von Willebrand factor (vWF) are also implied into ICI response deserve to be explored.

WHAT THIS STUDY ADDS

⇒ Among the different parameters investigated in this prospective cohort, coagulatory-related parameters (platelets, D-dimers, vWF:Ag and ADAMTS13 activity) and also standard biological parameters used in clinical routine to monitor melanoma response (LDH, S100) or inflammation (NLR, CRP) were included. We demonstrate that vWF:Ag levels measured at the beginning of the treatment provide a prognostic value for response to immunotherapy. We also report different evolution patterns of vWF:Ag levels over the treatment course that differ according to patient response to therapy. Therefore, we unveil a new link between coagulation and ICI where vWF:Ag levels correlate with patient response to treatment.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

The key message is that vWF distinguishes responder from non-responder patients early enough to sustain or change therapy. If validated in a larger study, this finding might be used to improve management of patients with melanoma. Further research on experimental models to understand the molecular basis sustaining these observations are now needed. As malignant melanoma is often used as a 'study model' for immunotherapy, these results open further explorations in other clinical entities where immunotherapy might be beneficial. The results of the present study suggest the combination of anticoagulation and immune checkpoint inhibitors to improve response rates and overall survival of melanoma patients.

to ICI. This highlights a potential role of vWF as a biomarker to monitor ICI response of patients with malignant melanoma.

BACKGROUND

The latest advances in targeting immune checkpoints opened a new promising era in management of patients suffering from melanoma. Immune checkpoint inhibition (ICI) using anti-PD-1/anti-CTLA4 antibodies have significantly extended progression-free (PFS) and overall survival (OS) in patients with unresectable malignant melanoma American Joint Committee on Cancer (AJCC) 2017 stage IV and in early stages (AJCC 2017 stage IIB-C, IIIA-D) patients.[1–5] Together with BRAF-/MEK-inhibitors in the presence of BRAF mutation, ICI is considered as the gold standard in treatment of melanoma in adjuvant and palliative situations. These very encouraging results hide however heterogeneous clinical outcomes: 22% of patients have durable clinical response (median OS not reached at 5 years, suggesting a potential long-term survival in these patients) while 60% of patients present clinical progression within the first year of therapy in the metastatic setting.[2] Moreover, 59% of treated patients experience severe (grade III-IV) side effects under anti-PD-1/anti-CTLA4 combination-therapy.[2] Therefore, there is an urgent but still unmet clinical need for the identification of patients presenting a high risk of early treatment failure and of those who will respond on the long term to the treatment. The development of new biomarkers is therefore critical to better personalize the therapeutic strategy and to limit unnecessary exposure to immune-related adverse events.

The great majority of biomarkers developed so far have focused on the tumor itself or on the immune system, mostly with the study of the different immune cell populations and their interaction with the tumor.[6 7] While immune-related events are well-recognized side effects of ICI, an increased incidence for thrombotic complications is associated with increased mortality rates.[8–10] Given the substantial role of coagulation factors in malignancy, factors involved in the coagulation cascade have also been evaluated as potential biomarkers for tumor progression and therapeutic efficacy. So far, elevated levels of P-selectin and D-dimers have been associated with worse prognosis and disease progress in patients with cancer.[11 12] Another promising coagulatory protein to serve as biomarker in tumor progression is the multimeric von Willebrand factor (vWF). Several studies reported that patients with cancer present higher systemic values of vWF compared with healthy donors.[13–16] When a vessel wall is damaged, vWF released in the subendothelial connective tissue binds fibrillar collagen, mediates platelets adhesion to damaged vascular walls and subsequently platelet activation and aggregation, thus promoting coagulation and vessel repair.[17] When secreted from intact, activated endothelial cells into the circulation, vWF is stretched within the blood flow exposing binding sites for circulating platelets[18] and leukocytes[19 20] bridging coagulation and inflammation.[21] Physiologically, ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13) cleaves the active ultra-large vWF (ULVWF) multimers into smaller ones, thereby limiting the prothrombotic action of vWF.[22] Own data obtained from mouse models deficient for ADAMTS13 activity further demonstrate that intraluminal vWF fibers promote tumor progression and metastasis formation.[13 23 24] Notably, the observation that a primary skin melanoma promotes luminal vWF fiber formation and platelet aggregation in distant organs such as the liver, lung and brain may indicate that vWF could trigger cancer-associated thrombosis and inflammation and actively promote metastasis formation.[24]

We therefore hypothesized that variations in the plasmatic levels of platelets and key coagulatory proteins including D-dimers, vWF and its regulatory enzyme ADAMTS13 could serve as potential biomarker to predict the response of patients with metastatic melanoma to immunotherapy.

In this investigation, we explored the systemic levels of platelets, D-dimers, vWF-antigen (vWF:Ag, to differentiate from vWF activity) and the activity of its regulatory enzyme ADAMTS13 in a prospective cohort of 83 patients with metastatic melanoma starting a new line of immunotherapy. We measured the variations of these parameters between the start of therapy (hereafter referred as baseline), and after 6, 12 and 24 weeks of treatment and observed their evolution profiles according to the patient response to therapy. We present our results in relation with the standard blood tumor markers used in clinical routine to monitor melanoma tumor evolution (LDH, S100), and, owing to the close connection between vWF and inflammation, to neutrophil-to-lymphocyte ratio (NLR) and CRP.[21]

MATERIAL AND METHODS

Patients, treatment and clinical evaluation

This prospective study included 83 patients with metastatic melanoma receiving ICI (four infusions of the combination of Ipilimumab 3 mg/kg bodyweight and Nivolumab 1 mg/kg bodyweight every 3 weeks followed by Nivolumab monotherapy 240 mg every 2 weeks, or

480 mg every 4 weeks (Bristol Myers Squibb, BMS); or Pembrolizumab monotherapy 200 mg every 3 weeks, or 400 mg every 6 weeks (Merck & Co., MSD)) at the University Skin Cancer Center Hamburg, Germany. Patients were included between March 2018 and February 2022.

Inclusion criteria were: (i) a confirmed diagnosis of stage IV melanoma according to the 2017 AJCC melanoma staging and classification or non-resectable stage III, (ii) at least 18 years of age and (iii) at least 6 months of clinical follow-up. All histological types of melanoma, including mucosal melanoma were eligible for inclusion. Exclusion criteria were the presence of an autoimmune disease, HIV, hepatitis B or C, pregnancy or concomitant systemic therapy for melanoma. Pretreated brain metastases were allowed to be included (table 1).

Treatment efficacy was assessed using contrast-enhanced CT of the whole body, MRI or positron emission tomography (PET-CT) at week 12 after the first ICI infusion and every 12 weeks. Clinical response was defined based on immune-related response criteria using the recently

Table 1 Patients clinical characteristics at baseline

	Low vWF:Ag (N=42)	High vWF:Ag (N=41)	Total (N=83)	P value
Age				>0.05
Mean (SD)	62.27 (16.62)	67.63 (14.42)	64.95 (15.70)	
Sex				>0.05
Female	12 (28.6%)	16 (39.0%)	28 (337%)	
Male	30 (71.4%)	25 (61.0%)	55 (66.3%)	
ECOG				=0.039
0	36 (85.7%)	26 (65.0%)	62 (75.6%)	
1+2	6 (14.3%)	14 (35.0%)	20 (24.4%)	
Primary melanoma site				>0.05
CUP	7 (16.7%)	8 (19.5%)	15 (18.1%)	
Cutaneous	28 (66.7%)	28 (68.3%)	56 (67.5%)	
Mucosal	5 (11.9 %)	3 (7.3%)	8 (9.6%)	
Uveal	2 (4.8%)	2 (4.9%)	4 (4.8%)	
AJCC				>0.05
III	3 (7.1%)	4 (9.8%)	7 (8.4%)	
IV	39 (92.9%)	37 (90.2%)	76 (91.6%)	
Metastatic classification				>0.05
M0	6 (14.3%)	4 (9.8%)	10 (12%)	
M1a	5 (11.9%)	5 (12.2%)	10 (12%)	
M1b	13 (31.0%)	9 (22.0%)	22 (26.5%)	
M1c	9 (21.4%)	15 (36.6%)	24 (28.9%)	
M1d	9 (21.4%)	8 (19.5%)	17 (20.5%)	
Prior Therapies				>0.05
No	30 (71.4%)	24 (58.5%)	54 (65.1%)	
Yes	12 (28.6%)	17 (41.5%)	29 (34.9%)	
Mutation status				=0.045
BRAF	20 (47.6%)	10 (24.4%)	23 (40.4%)	
NRAS	5 (11.9%)	12 (29.3%)	16 (28.1%)	
None	17 (40.5%)	19 (46.3%)	18 (31.6%)	
Baseline therapy				>0.05
Anti-PD1	12 (28.6%)	12 (28.6%)	24 (28.9%)	
Combination	30 (71.4%)	29 (70.7%)	59 (71.1%)	

Clinical characteristics are reported for the entire cohort but also for the patients of each median group of vWF:Ag levels. P values of Fisher test for categorical variables and Wilcoxon test for continuous variables between low and high vWF:Ag groups are reported.
AJCC, American Joint Committee on Cancer; CNS, central nervous system; ECOG, Eastern Cooperative Oncology Group; ULN, upper limit of normal.

proposed iRECIST criteria[25] that are based on RECIST 1.1 and revised according to tumor board decision.

Collection of peripheral blood samples

Citrate (S-Monovette 2.9 mL Starstedt) and EDTA (S-Monovette 7.5 mL Starstedt) plasma and serum (S-Monovette 7.5 mL Starstedt) samples were collected the same day but few hours before each infusion of ICI. Given different treatment regimens among the patients, evaluated time-points are defined by the number of weeks of treatment as follows: baseline refers to the sample taken before the first therapy infusion, after 6 weeks of treatment, after 12 weeks of treatment, after 24 weeks of treatment. Plasma and serum samples were centrifuged within 2 hours after venipuncture at 1800g during 10 min and aliquoted before being stored at −80°C.

Analysis of peripheral blood samples

Counts for platelets, lymphocytes and neutrophils used to define NLR were measured in the peripheral blood by routine clinical laboratory analysis using an ADVIA 2120i System analyzer (Siemens). LDH was measured by spectrophotometry (Atellica Solution CH930), S100 by chemiluminescence (Liaison XL), D-dimers and CRP by turbidimetry (Atellica Coag 360 and Atellica Solution CH930, respectively). The following counts/measurements were considered as normal: platelets (150–300×10^9/L), LDH (120–246 U/L), S100 (<0.150 µg/L) D-dimers (0.21–0.52 mg/L), CRP (<5 mg/L).

vWF ELISA assay

vWF:Ag levels from citrate plasma samples were analyzed as previously described.[13 16 26] Briefly, a sandwich ELISA was established by using a polyclonal rabbit anti-vWF antibody (Dako, Copenhagen, Denmark) and a polyclonal rabbit peroxidase-labeled antihuman vWF antibody (Dako, Copenhagen, Denmark). A standard curve was generated using Standard Human Plasma (Behring, Marburg, Germany) with a defined vWF content (2nd International Standard 87/718, National Institute for Biological Standards and Control, London).

All measurements were done in technical triplicates and all time points from the same patient were analyzed on the same plate.

ADAMTS13 activity ELISA assay

ADAMTS13 activity was measured in citrate plasma samples by a commercially available kit according to the manufacturer's instructions (Technoclone GmbH).

Statistical analysis

Biological data below the lower limit of sensitivity were considered equal to the half of the limit by convention. Continuous variables were described with the mean and the SD. Categorical variables were described by absolute number and percentages and differences between groups were assessed with Fischer test.

For continuous variables, Pearson's correlation analyses were performed to assess the relationship between variables in the entire cohort.

Differences between response groups at all time points were assessed with a Student t-test if n>30 or non-parametric two-sided Mann-Whitney U test if N<30. Within each group of response, differences between time-point were assessed with a non-parametric Wilcoxson matched paired test. No formal adjustment of significance level was performed due to the explorative nature of this pilot study (online supplemental table 1).

Progression-free survival (PFS) and OS were described via the Kaplan-Meier estimator from the start of therapy to the date of first reported PD or death. Univariable regression analysis considered biological data as continuous variables. Multivariable regression analysis included covariables that came out significant with univariable analysis unless otherwise mentioned in the text: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. Analyses were carried out using Graphpad Prism 9 and R 4.2.2.

RESULTS

Patients characteristics and response to therapy

In total, 83 patients were included in this prospective study. The scheme of our study is presented in figure 1 and patient characteristics are listed in table 1 for the entire cohort and according to baseline levels of vWF:Ag. The majority of patients presented with cutaneous melanoma (67.5%) and were receiving ICI as a first line (65%). Previous treatment included anti-PD1, anti-MEK, anti-BRAF, IFNalpha or chemotherapy. The median time of clinical follow-up, defined as the median time to censoring for survived patients, is 26 months. Because tumor response is re-evaluated at each clinical evaluation, we decided to group the patients according the tumor response definition from the Society for Immunotherapy of Cancer (SITC) Cancer Immune Responsiveness Task Force[27] that defines primary resistance and primary response. Primary resistance was defined as disease progression after receiving at least 6 weeks of exposure to immune checkpoint inhibitor but no more than 6 months. Secondary resistance was defined as disease progression after 6 months of response to therapy.

Overall, 36% of the patients responded to the therapy while 62% of the patients were classified as resistant (50% primary and 12% secondary resistance).

Patients with metastatic melanoma present with dysregulation of coagulatory parameters before the start of ICI

Mean values of all the parameters investigated before the start of ICI (hereafter referred as baseline) in our cohort of patients are reported in table 2. LDH, CRP and S100 were in average above the superior limit of reference intervals. NLR was in average inferior to 5, a cut-off commonly used to define elevation in the metastatic setting.[28] Among the coagulatory parameters investigated

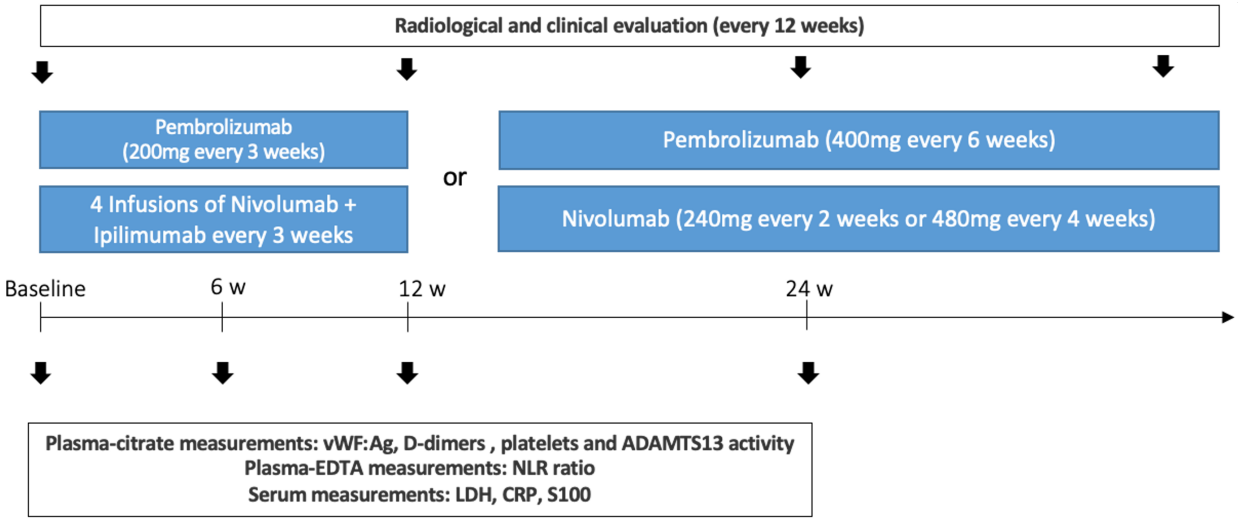

Figure 1 Scheme of the study. Scheme of the study describing the different ICI treatments, clinical follow-up and the different timepoints at which the patients were sampled. ICI, immune checkpoint inhibition; NLM, neutrophil-to-lymphocyte ratio; .

in this study, platelets counts fell within reference values while D-dimers levels were in average, above the upper limit of their reference interval. The average of vWF:Ag levels among healthy donors is 14.077±4.91 µg/mL (mean±SD), and ADAMTS13 activity is 95.92%±13.28% (mean±SD).[13 16] In our cohort, vWF:Ag levels varied between 17.30 and 62.41 µg/mL, with an average of 31.68±7.82 µg/mL (mean±SD). ADAMTS13 activity was comprised between 18.4% and 91.9% with an average of 60.18%±16.42% (mean±SD). Therefore, D-dimers, vWF:Ag and ADAMTS13 activity are dysregulated in patients with metastatic melanoma before the start of ICI.

In order to further explore the relation of vWF:Ag, ADAMTS13 activity, D-dimers and platelet counts with standard biological parameters measured in daily clinical routine to monitor tumor evolution (LDH, S100) or that are related to inflammation (CRP and NLR) and described as poor prognostic indicator in patients with melanoma, Pearson correlations were also investigated and positive correlations are figured in online supplemental table 2. CRP values were correlated to D-dimers values (R=0.52, p<0.0001) and less strongly to LDH (R=0.32, p=0.009) and NLR (R=0.3, p=0.037). LDH values were also correlated to D-dimers (R=0.29, p=0.026). Of note, we did not observe any significant correlation between vWF:Ag levels and all the other parameters in this cohort.

Baseline vWF:Ag level is associated to clinical response to ICI

A univariate Cox proportional hazard analysis to investigate any potential association to clinical progression of each blood-based parameter measured at the beginning of the treatment was conducted (table 3). Biological parameters were first interrogated as continuous variables. CRP, D-dimers and vWF:Ag levels were associated to a significant risk of progression (table 3).

Each unit increase in vWF:Ag or CRP levels was associated with a 4% increase of risk for progression. Each unit increase in D-dimers levels leads to a 10% increase of risk for progression. To assess the relevance of D-dimers and vWF for tumor progression under ICI treatment, PFS

Table 2 Baseline values of the different biological parameters in the cohort of patients with metastatic melanoma

	Low vWF:Ag (N=42)	High vWF:Ag (N=41)	Total (N=83)	P value
vWF:Ag (µg/ml)	25.76 (3.90)	37.75 (5.95)	31.68 (7.82)	<0.0001
ADAMTS13 activity (%)	65.47 (12.09)	55.87 (18.36)	60.18 (16.42)	=0.04
D-dimers (mg/L)	1.38 (1.84)	3.04 (6.15)	2.16 (4.46)	>0.05
Platelets (10^9/L)	322.55 (120.60)	308.00 (106.85)	315.46 (113.61)	>0.05
LDH (U/L)	396.79 (449.38)	432.16 (333.31)	413.35 (397.17)	>0.05
S100 (µg/L)	0.40 (0.77)	1.25 (2.19)	0.81 (1.66)	=0.026
NLR	3.26 (1.48)	4.00 (2.07)	3.63 (182)	>0.05
CRP (mg/L)	16.58 (24.66)	33.42 (48.96)	24.66 (38.96)	>0.05

Biological parameters are reported for the entire cohort but also for the different vWF:Ag low and high groups (median cut-off). Data represent mean (±SD). P values of Kruskal-Wallis test between low and high groups are reported.
NLR, neutrophil-to-lymphocyte ratio; vWF, von Willebrand factor.

Table 3 PFS and OS univariable analysis of the clinical and blood-based parameters at baseline

	Progression-free survival		Overall survival	
	HR (95% CI)	P value	HR (95% CI)	P value
Clinical data				
Age	1 (0.98 to 1)	0.876	1 (1 to 1)	0.063
ECOG (0 vs ≥1)	1.9 (1.1 to 3.3)	0.031	2.9 (1.4 to 5.3)	0.002
Brain metastasis (yes vs no)	1.5 (0.89 to 2.6)	0.128	2 (1 to 3.8)	0.048
Number of prior therapies (0 vs ≥1)	1.4 (0.79 to 2.3)	0.26	1.1 (0.55 to 2.1)	0.843
BRAF mutation (yes vs no)	0.52 (0.29 to 0.94)	0.031	0.45 (0.21 to 0.98)	0.043
Treatment (combi-therapy vs monotherapy)	1.3 (0.73 to 2.4)	0.36	1.1 (0.55 to 2.4)	0.724
Biological data				
LDH	1 (1 to 1)	0.25	1 (1 to 1)	0.09
S100	1 (0.9 to 1.2)	0.57	0.88 (0.67 to 1.2)	0.381
CRP	1.04 (1 to 1.1)	0.007	1.04 (1 to 1)	0.004
NLR	1 (0.86 to 1.3)	0.686	0.99 (0.8 to 1.2)	0.956
Platelets	1 (1 to 1)	0.741	1 (1 to 1)	0.93
D-dimers	1.1 (1 to 1.2)	<0.001	1.1 (1 to 1.2)	0.006
ADAMTS13 activity	0.99 (0.97 to 1)	0.263	0.97 (0.94 to 1)	0.022
vWF:Ag	1.04 (1 to 1.1)	0.007	1.04 (1 to 1.1)	0.043

Age and biological parameters are interrogated as continuous variables.
ECOG, Eastern Cooperative Oncology Group; NLR, neutrophil-to-lymphocyte ratio; vWF, von Willebrand factor.

was analyzed by Kaplan-Meier estimates. For D-dimers, no statistical differences could be observed in Kaplan-Meier analysis when grouping patients according to the median (data not shown) or according to an optimized cut-point (0.36 mg/L) in this cohort (figure 2A). By contrast, the median of vWF:Ag-baseline measurements allowed to identify two patients groups (low<30.8 µg/mL and high>30.8 µg/mL) with significantly different evolutions (figure 2B, HR (95% CI): 1.82 (1.06 to 3.13) ; log-rank test: p=0.026). The median time of progression for patients with high levels of vWF:Ag baseline was 5.06 months (95% CI 2.85 to 9.19 months) while the median time of progression was 7.88 months (95% CI 5.84 to NR months) for patients with low vWF:Ag baseline levels (p=0.029).

Next, we verified the discriminant ability of baseline D-dimers and vWF:Ag levels to detect a progression by estimating the area under the curve (AUC) value of the receiver operating characteristic (ROC) curve. For D-dimers, AUC was equal to 0.50 while it was equal to 0.70 (95% CI 0.58 to 0.81) for vWF:Ag baseline levels (figure 2C,D). Youden-index discrimination of the best vWF-antigen baseline cut-off value was determined at 28.66 µg/mL. vWF:Ag baseline measurement test performances to detect non-responders were determined accordingly. Sensitivity was 80%, specificity was 59%, positive predictive value was 72% and negative predictive value was 63%.

We then interrogated vWF:Ag levels impact on clinical progression in multivariate analysis. Age, Eastern Cooperative Oncology Group (ECOG) status, the presence of brain metastasis, the type of treatment and the number of prior therapies were defined as the main clinical parameters with a potential influence on clinical outcome. The number of therapies received before ICI had no significant impact on PFS (table 3 and online supplemental figure 1A). ECOG performance status was associated with a higher risk of progression while carrying a BRAF mutation was associated with a lower risk of progression (table 3). In addition, patients with low and high levels of baseline vWF:Ag differ as well for these two clinical parameters (table 1). Importantly, vWF:Ag levels remained independently associated to clinical relapse (HR=1.04; 95% CI 1.01 to 1.1, p=0.007) when implementing these two clinical features in a multivariable model (online supplemental figure 2A). We also tested vWF:Ag in a multivariable model including LDH and S100, as vWF:Ag patients groups had significantly different levels of S100. vWF:Ag levels remained independently associated to clinical relapse (HR=1.04; 95% CI 1.01 to 1.1, p=0.006) when implementing these two clinical features in a multivariable model (online supplemental figure 2B). When ECOG and BRAF mutation status were included, D-dimers impact on PFS remained significant (online supplemental figure 2E).

Since primary and secondary responses to ICI actually represent different clinical scenario,[27] we investigated if vWF:Ag levels differed between primary response and primary resistance groups. In contrast to D-dimers (figure 2E), vWF:Ag levels were significantly higher in the primary resistance group (mean: 29.4 µg/mL vs 32.9 µg/mL; p=0.048, figure 2F). Of note, LDH baseline values

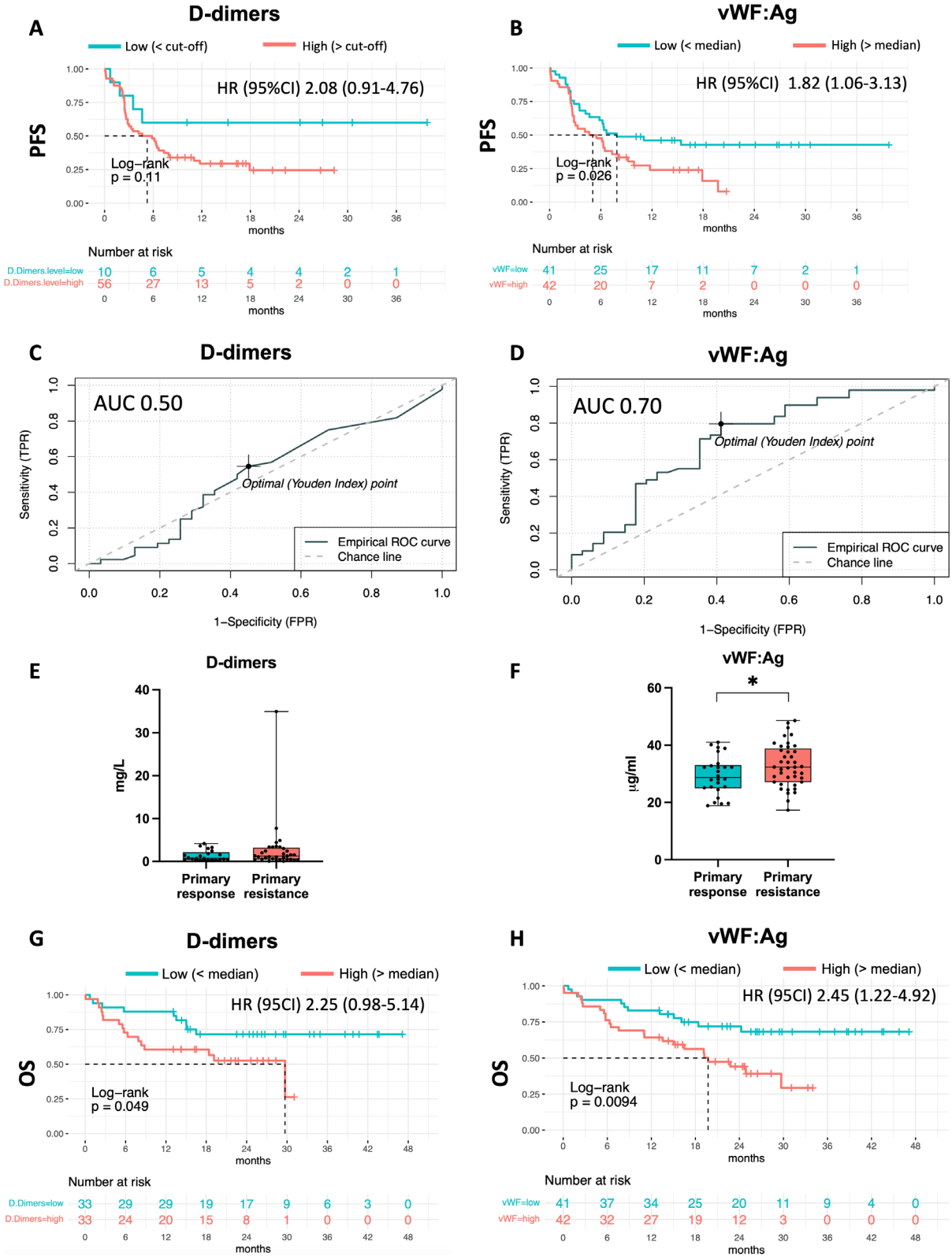

Figure 2 vWF:Ag at baseline are associated with clinical response, PFS and OS . (A,B) Kaplan Meier function for PFS according to D-dimers levels (A) and vWF:Ag (B) at baseline. 'Low' and 'high' patient groups are defined according to the best cut-point of D-dimers (0.36 mg/L) and according to median value of vWF:Ag in the entire cohort. Censoring of data is indicated by vertical bars. (C,D) ROC curves of D-dimers and vWF:Ag for progression association. (E,F) Comparison of primary resistance and primary response groups at baseline for D-dimers and vWF:Ag levels. (G,H) Kaplan Meier function for OS according to D-dimers and vWF:Ag levels at baseline. 'Low' and 'high' patient groups are defined according to the median value of D-dimers and vWF:Ag in the entire cohort. Censoring of data is indicated by vertical bars. OS, overall survival; PFS, progression-free survival; vWF, von Willebrand factor.

were also statistically different between these two groups of patients (mean: 288 U/L vs 468 U/L; p=0.039, online supplemental figure 3A). No significant difference was observed for other parameters (platelets, ADAMTS13 activity, S100, CRP and NLR) (online supplemental figure 3B–F).

Altogether, these results demonstrate that among coagulatory parameters, plasmatic vWF:Ag, measured before the beginning of the treatment, is the most robust biomarker associated with clinical response in patients treated with immunotherapy.

Association of coagulatory parameters levels at baseline with overall survival

Cox proportional hazard model analyses were conducted to investigate any potential association of blood-based parameters to OS (table 3). Here, univariate analyses revealed that ADAMT13 activity, D-dimers, CRP and vWF:Ag levels were associated to a significant increase in the risk of death (table 3). In line with the results obtained for PFS, each unit increase in vWF:Ag and D-dimers levels lead to a 4% and 10% increase of risk for recurrence or death, respectively. For ADAMTS13 activity, no differences could be observed in Kaplan-Meier analysis when grouping patients according to the median or tertile values of these parameters (data not shown). Kaplan-Meier estimates were used to produce OS curves using the median value of D-dimers and vWF:Ag baseline measurements (figure 2G,H). Using the D-dimers median as cutpoint resulted in a slightly significant difference in OS (HR(95% CI): 2.25 (0.98 to 5.14) ; log-rank test: p=0.049). Importantly, the vWF:Ag median split the patient cohort into two groups with highly significant differences for OS (HR(95% CI): 2.45 (1.22 to 4.92) ; log-rank test: p=0.0094). The median time of death for patients with high vWF:Ag baseline levels was 19.15 months (95% CI 11.53 to NR months) while the median time of progression was not reached for patients with low vWF:Ag baseline levels (p=0.012). In multivariate Cox proportional hazards models, adjusting for brain metastasis and ECOG performance status (table 3), neither vWF:Ag nor D-dimers levels were significantly associated to an increase in risk of death (data not shown). When adjusting for ECOG and BRAF mutation status (table 3), D-dimers baseline levels were not independently associated to OS (online supplemental figure 2F) while vWF:Ag baseline levels remained associated to OS (online supplemental figure 2C). LDH and S100 levels tested together with vWF:Ag did not alter vWF:Ag association to OS (online supplemental figure 2D). Therefore, we conclude that the plasmatic vWF:Ag level is a promising biomarker candidate associated to OS in patients with melanoma.

Evolution profiles during the course of ICI

In addition to baseline measurements, we assessed the evolution of the coagulatory markers (vWF:Ag, ADAMTS13 activity, platelets, D-dimers) together with daily clinical routine blood metrics (LDH, S100, CRP, NLR) during the course of therapy (ie, after 6, 12 or 24 weeks) within each primary response group (figure 3 and online supplemental figure 4). Due to the explorative nature of this study, p values were not adjusted to multiple comparisons between the different time-points.

No statistically significant variation was observed for LDH and NLR over the course of therapy according to the type of response (online supplemental figure 4B,G,H). However, we could observe significantly lower levels of LDH between the primary responder and primary resistant groups after 12 and 24 weeks of treatment (median: 260 vs 317 U/L; p=0.044 and 261 vs 305 U/L; p=0.0035) (online supplemental figure 4A). For CRP, a significant difference between primary responders and primary progressors was observed at 12 weeks of treatment (median: 4 vs 11 mg/L; p=0.008) (online supplemental figure 4E). S100 levels decreased within the primary response group at 6 and 12 weeks (online supplemental figure 4D), translating into statistical difference with the primary resistance group over the course of therapy (online supplemental figure 4C).

For ADAMTS13, no differences in the enzymatic activity were observed at the different timepoints of ICI treatment. In addition, no significant differences between primary responders and primary progressors were detected (figure 3A,B). By contrast, D-dimer levels significantly decreased between baseline and 24 weeks and between 6 weeks and 24 weeks in the primary response group (median of differences: −3.03 mg/L; p=0.029 and median of differences: −0.33 mg/L; p=0.001) (figure 3D). Statistically lower levels could be observed at 12 and 24 weeks when comparing the primary response and the primary resistance groups (median: 0.58 vs 1.08 mg/L ; p=0.037 and median: 0.51 vs 1.21 mg/L; p=0.029) (figure 3C). The systemic platelet count also decreased during treatment course in primary responders and primary progressors but always remained above upper limit of reference (figure 3E,F). Remarkably, while vWF:Ag levels increased during the first 24 weeks of treatment in primary progressive patients (median of differences: 5.22 µg/mL; p=0.021), vWF:Ag levels peaked at 6 weeks in primary responders (median of differences: 2.98 µg/mL; p=0.005) but decreased to baseline levels after 24 weeks (median of differences: −3.74 µg/mL; p=0.04) (figure 3H). As a consequence, a statistical difference in vWF:Ag levels between primary response and primary resistance was found also at 24 weeks (median: 31.9 vs 36.9 µg/mL; p=0.016) (figure 3G).

DISCUSSION

In this prospective cohort of patients with metastatic melanoma dysregulated levels of LDH, S100, CRP, D-dimers, vWF:Ag and ADAMTS13 activity were observed at the beginning of treatment. Among all coagulatory parameters evaluated in this study (eg, D-dimers or ADAMTS13 activity), baseline vWF:Ag levels measured before ICI treatment appeared to be the most robust prognostic

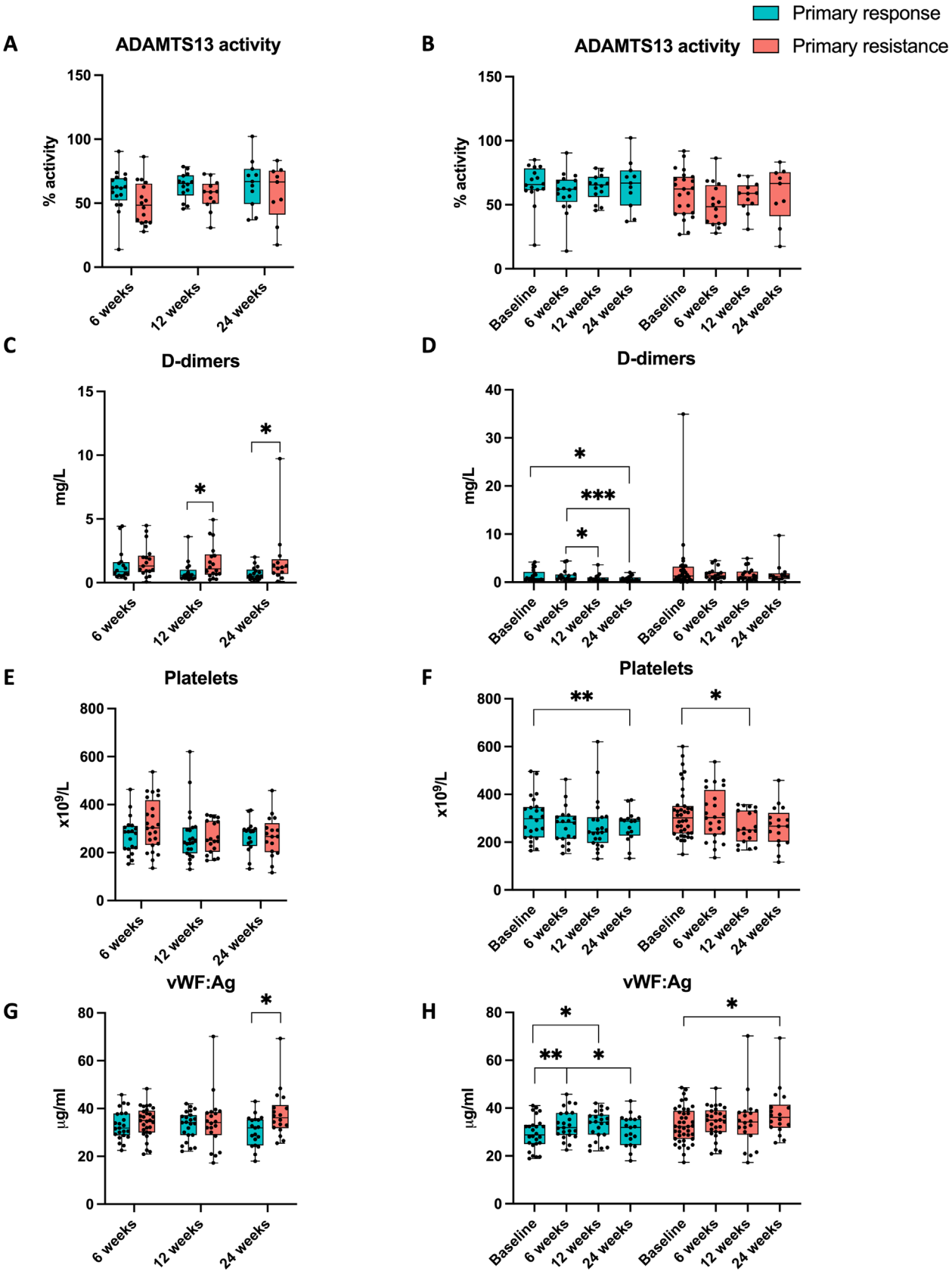

Figure 3 Evolution profiles of vWF-antigen and related coagulatory parameters during the course of ICI. (A,C,E,G) Comparison of primary resistance and primary response groups at 6, 12 and 24 weeks for ADAMTS13 activity (A) D-dimers, (C), platelets (E) and vWF:Ag (G). Median, minimum and maximum values are displayed. (B,D,F,H) ADAMTS13 activity (B), D-dimers (D), platelets (F) and vWF:Ag (H) measurements within primary resistance and primary response groups at baseline, 6, 12 and 24 weeks. Median, minimum and maximum values are displayed. ICI, immune checkpoint inhibition; vWF, von Willebrand factor.

biomarker associated with response to ICI therapy in this cohort of patients with melanoma. The lowest levels of vWF:Ag were associated to longer PFS and to longer OS in patients with metastatic melanoma treated by ICI in univariate and multivariate analysis.

The observations of the present study about the relationship of vWF:Ag levels at baseline and response to ICI sustain the hypothesis that a procoagulatory milieu might affect T cell activity or support the immune suppressor properties of the tumor microenvironment by orchestrating its cellular composition. Intraluminal vWF fibers could promote the extravasation of particular immune cells population with inhibitory properties on effector T cells but also recruit platelets.[19 20] Indeed, it was shown that platelets provide several mechanisms to inhibit T cell functions.[29 30] The increased baseline levels of vWF:Ag observed in non-responder patients could result from a pronounced endothelial cell activation and may explain the detrimental effects on ICI response. In this line, high levels of vascular endothelial growth factor-A (VEGF-A), a potent activator of the endothelium followed by the secretion of vWF, has been associated to a shorter PFS in a cohort of patients with metastatic melanoma treated by anti-CTLA4.[31 32] Mechanistically, a strong activation of the vascular endothelium could also have consequences on the antitumor immunity, by creating a sort of immune barrier.[33–37] Therefore, our observations of higher levels of vWF:Ag in non-responders set a rationale for further studies to decipher the role of endothelial cell activation in tumor infiltration by leucocytes.

With a comprehensive monitoring at 6, 12 and 24 weeks of treatment, we describe different vWF:Ag levels evolution profiles according to tumor response. While vWF:Ag levels increase over the course of therapy in primary resistant patients, patients with primary response presented an early increase of vWF:Ag levels between baseline and 6 weeks followed by a progressive decrease until 24 weeks. The trigger for this increase is unknown but might be related to early flare-response in CRP or other proteins that have been recently reported and observed in patients with a favorable outcome to ICI in melanoma or other cancer entities.[38 39] This preliminary observation raises the hypothesis of an activable, non-exhausted endothelium, associated to a favorable therapy response that would be worth investigating in experimental models and confirmed on a higher number of patients.

The low values of ADAMTS13 activity observed in our cohort when compared with healthy donors suggest that patients with melanoma exhibit ULVWF multimers in the vasculature, further promoting tumor progression.[16] Even if we did not observe any association of ADAMTS13 activity levels with PFS or clinical response, lower values of ADAMTS13 activity were associated with shorter OS, suggesting thereby a potential implication of large vWF fibers into the onset of thrombotic complications. In addition, we observed a positive correlation between elevated levels of D-dimers measured at the beginning of treatment and shorter PFS univariate and multivariate analysis and OS in univariate analysis only. This result might be also related to several clinical studies providing evidence for an association between immunotherapy and thrombotic complications occurrence affecting OS.[9 10 40 41]

As for the other biological parameters measured on this study, we did not find any association with PFS and OS for LDH, S100 and NLR. These biomarkers were mostly evaluated in ICI monotherapy cohorts (either anti-CTLA-4 or anti-PD-1) and conflicting results on the association of these parameters with clinical outcome were reported mainly due to a low average in sample size.[42] In the specific context of patients with melanoma treated with combination ICI, PFS was not evaluated but NLR was found to be associated with worse OS in one study and LDH with OS as well in one out of two studies.[42–44] In line with a previous examination, CRP was significantly associated with PFS and OS in this cohort.[45]

Some limitations of our study should be mentioned. Here, we performed a monocentric explorative prospective study and further analyses on a larger number of patients are warranted to confirm our observations. In melanoma, a cohort of patients treated with targeted therapy would be interesting to investigate if the role of vWF is relevant only in the context of ICI and could hold a predictive biomarker value. As melanoma is frequently considered as an adequate model to study immunotherapy, it would be also worth investigating if our observations are reproduced in other clinical entities where ICI is also approved. Finally, it remains to be explored whether high levels of vWF are predictive for the occurrence of cancer-associated thrombosis (CAT) affecting immunotherapy in our patient cohort. Because it was recently demonstrated that high vWF plasma levels are associated with increased risk of long-term venous thromboembolism,[46] a prolonged follow-up of the patients would be interesting to investigate this question.

Overall, our results suggest that an alteration of vWF:Ag levels in patients with metastatic melanoma might have a detrimental effect on the outcome to ICI. vWF measurement presents the advantage to be performed via a simple and inexpensive assay and could therefore be readily and easily implemented in many medical laboratories in a routine basis. Thus, our study strengthens a rational basis for new therapy approaches based on the combination of anticoagulatory molecules and immune checkpoint inhibitors to improve OS of patients with metastatic melanoma.[47 48]

Author affiliations
[1]Department of Dermatology and Venereology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[2]Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[3]Fleur Hiege Center for Skin Cancer Research, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[4]Centre de Recherche en Cancerologie de Toulouse, Toulouse, France

Twitter Robin Reschke @DrRobinReschke

Acknowledgements The authors would like to thank all patients and their families who have accepted to participate in this study. The authors would also warmly thank Thomas Filleron for critical reading of the manuscript. The authors acknowledge financial support from the Open Access Publication Fund of UKE – Universitätsklinikum Hamburg-Eppendorf – and DFG – German Research Foundation.

Contributors Concept and design: CG, KP and SWS; acquisition, analysis, or interpretation of data: JCS, LK, CM, ATB, JK; drafting of the manuscript: LK, ATB; critical revision of the manuscript for important intellectual content: all authors; statistical analysis: CM; administrative, technical, or material support: ATB, JK, SVS, AA, WH, MS, CS, BD, ILH, RR, IH, GG, KP, CG and SWS; supervision: KP, CG and SWS; guarantor: SWS; KP, CG, SWS are joint last authors;

Funding This project was partially funded by Hiege Stiftung (JCS, LK, KP, CG, SWS), Roggenbuck Stiftung (LK), and Novartis TIM project (S2TAF-058). JCS, JK, GG and IH received partial funding of a one-year Clinical Scientist fellowship by the Mildred Scheel Nachwuchszentrum, UKE Hamburg (HaTriCS4). BD was financially supported by IPRime Scholarship, UKE, Hamburg. LK was financially supported by ITMO Cancer 'Plan Cancer 2014-2019' and received funding from Fondation de France. LK and KP were financially supported by KMU-innovativ-23 n°031B0843D. RR was supported by Deutsche Forschungsgemeinschaft (RE 4468/2-1).

Competing interests J-CS, LK, ATB, CM, AA, SVS, MS, WH, CS, BD, ILH, RR, TH, JK, IH, GG, KP and SWS declare no competing interest. CG is a member of the advisory board and has received honoraria and travel expenses from Almirall, Amgen, Beiersdorf, BioNTech, Bristol-Myers Squibb, Immunocore, Janssen, MSD Sharp & Dohme, Novartis, Pierre-Fabre, Roche, Sanofi Genzyme, SUN Pharma, Sysmex/Inostics, outside the submitted work. CG holds shares of Dermagnostix.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Hamburger Ärztekammer (PV5392). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The data sets used during the study are available from the corresponding author on reasonable request.

ORCID iDs
Laura Keller http://orcid.org/0000-0002-1786-9760
Glenn Geidel http://orcid.org/0009-0008-3999-5902
Christoffer Gebhardt http://orcid.org/0000-0001-7090-9584

REFERENCES

1 Wolchok JD, Chiarion-Sileni V, Gonzalez R, *et al*. Overall survival with combined Nivolumab and Ipilimumab in advanced Melanoma. *N Engl J Med* 2017;377:1345–56.
2 Larkin J, Chiarion-Sileni V, Gonzalez R, *et al*. Five-year survival with combined Nivolumab and Ipilimumab in advanced Melanoma. *N Engl J Med* 2019;381:1535–46.
3 Weber J, Mandala M, Del Vecchio M, *et al*. Adjuvant Nivolumab versus Ipilimumab in resected stage III or IV Melanoma. *N Engl J Med* 2017;377:1824–35.
4 Eggermont AMM, Blank CU, Mandala M, *et al*. Adjuvant Pembrolizumab versus placebo in resected stage III Melanoma. *N Engl J Med* 2018;378:1789–801.
5 Luke JJ, Rutkowski P, Queirolo P, *et al*. Pembrolizumab versus placebo as adjuvant therapy in completely resected stage IIB or IIC Melanoma (KEYNOTE-716): a randomised, double-blind, phase 3 trial. *Lancet* 2022;399:1718–29.
6 Kalbasi A, Ribas A. Tumour-intrinsic resistance to immune checkpoint blockade. *Nat Rev Immunol* 2020;20:25–39.
7 Kalaora S, Nagler A, Wargo JA, *et al*. Mechanisms of immune activation and regulation: lessons from Melanoma. *Nat Rev Cancer* 2022;22:195–207.
8 Moik F, Colling M, Mahé I, *et al*. Extended anticoagulation treatment for cancer-associated thrombosis-rates of recurrence and bleeding beyond 6 months: a systematic review. *J Thromb Haemost* 2022;20:619–34.
9 Bauer AT, Gorzelanny C, Gebhardt C, *et al*. Interplay between coagulation and inflammation in cancer: limitations and therapeutic opportunities. *Cancer Treat Rev* 2022;102:102322.
10 Sussman TA, Li H, Hobbs B, *et al*. Incidence of thromboembolism in patients with Melanoma on immune checkpoint inhibitor therapy and its adverse association with survival. *J Immunother Cancer* 2021;9:e001719.
11 Ay C, Simanek R, Vormittag R, *et al*. High plasma levels of soluble P-Selectin are predictive of venous thromboembolism in cancer patients: results from the Vienna cancer and thrombosis study (CATS). *Blood* 2008;112:2703–8.
12 Desch A, Gebhardt C, Utikal J, *et al*. D-dimers in malignant Melanoma: association with prognosis and dynamic variation in disease progress. *Int J Cancer* 2017;140:914–21.
13 Bauer AT, Suckau J, Frank K, *et al*. Von Willebrand factor fibers promote cancer-associated platelet aggregation in malignant Melanoma of mice and humans. *Blood* 2015;125:3153–63.
14 Obermeier HL, Riedl J, Ay C, *et al*. The role of ADAMTS-13 and von Willebrand factor in cancer patients: results from the Vienna cancer and thrombosis study. *Res Pract Thromb Haemost* 2019;3:503–14.
15 Franchini M, Frattini F, Crestani S, *et al*. Von Willebrand factor and cancer: a renewed interest. *Thromb Res* 2013;131:290–2.
16 Karampinis I, Nowak K, Koett J, *et al*. Von Willebrand factor in the plasma and in the tumor tissue predicts Cancerassociated thrombosis and mortality. *Haematol* 2022;108:261–6.
17 Brass S. Cardiovascular biology. Platelets and proteases. *Nature* 2001;413:26–7.
18 Schneider SW, Nuschele S, Wixforth A, *et al*. Shear-induced unfolding triggers adhesion of von Willebrand factor fibers. *Proc Natl Acad Sci USA* 2007;104:7899–903.
19 Petri B, Broermann A, Li H, *et al*. Von Willebrand factor promotes Leukocyte extravasation. *Blood* 2010;116:4712–9.
20 Pendu R, Terraube V, Christophe OD, *et al*. P-Selectin glycoprotein ligand 1 and Beta2-integrins cooperate in the adhesion of leukocytes to von Willebrand factor. *Blood* 2006;108:3746–52.
21 Kawecki C, Lenting PJ, Denis CV. Von Willebrand factor and inflammation. *J Thromb Haemost* 2017;15:1285–94.
22 Stockschlaeder M, Schneppenheim R, Budde U. Update on von Willebrand factor Multimers: focus on high-molecular-weight Multimers and their role in hemostasis. *Blood Coagul Fibrinolysis* 2014;25:206–16.
23 Feinauer MJ, Schneider SW, Berghoff AS, *et al*. Local blood coagulation drives cancer cell arrest and brain metastasis in a mouse model. *Blood* 2021;137:1219–32.
24 Goertz L, Schneider SW, Desch A, *et al*. Heparins that block VEGF-A-mediated von Willebrand factor fiber generation are potent inhibitors of Hematogenous but not Lymphatic metastasis. *Oncotarget* 2016;7:68527–45.
25 Seymour L, Bogaerts J, Perrone A, *et al*. iRECIST: guidelines for response criteria for use in trials testing Immunotherapeutics. *Lancet Oncol* 2017;18:e143–52.
26 Kerk N, Strozyk EA, Pöppelmann B, *et al*. The mechanism of Melanoma-associated thrombin activity and von Willebrand factor release from endothelial cells. *J Invest Dermatol* 2010;130:2259–68.
27 Kluger HM, Tawbi HA, Ascierto ML, *et al*. Defining tumor resistance to PD-1 pathway blockade: recommendations from the first meeting of the SITC Immunotherapy resistance Taskforce. *J Immunother Cancer* 2020;8:e000398.
28 Templeton AJ, McNamara MG, Šeruga B, *et al*. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst* 2014;106.
29 Rolfes V, Idel C, Pries R, *et al*. PD-L1 is expressed on human platelets and is affected by immune checkpoint therapy. *Oncotarget* 2018;9:27460–70.
30 Rachidi S, Metelli A, Riesenberg B, *et al*. Platelets subvert T cell immunity against cancer via GARP-TGFβ axis. *Sci Immunol* 2017;2.
31 Yuan J, Zhou J, Dong Z, *et al*. Pretreatment serum VEGF is associated with clinical response and overall survival in advanced

Melanoma patients treated with Ipilimumab. *Cancer Immunol Res* 2014;2:127–32.
32 Babačić H, Lehtiö J, Pico de Coaña Y, *et al*. In-depth plasma proteomics reveals increase in circulating PD-1 during anti-PD-1 Immunotherapy in patients with metastatic cutaneous Melanoma. *J Immunother Cancer* 2020;8:e000204.
33 Klein D. The tumor vascular Endothelium as decision maker in cancer therapy. *Front Oncol* 2018;8:367.
34 Weishaupt C, Goerge T, Loser K. Activated Melanoma vessels: a sticky point for successful Immunotherapy. *Exp Dermatol* 2020;29:1046–54.
35 Motz GT, Santoro SP, Wang L-P, *et al*. Tumor Endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. *Nat Med* 2014;20:607–15.
36 Hellebrekers DMEI, Castermans K, Viré E, *et al*. Epigenetic regulation of tumor endothelial cell Anergy: silencing of Intercellular adhesion Molecule-1 by Histone modifications. *Cancer Res* 2006;66:10770–7.
37 Dirkx AEM, Oude Egbrink MGA, Kuijpers MJE, *et al*. Tumor angiogenesis modulates Leukocyte-vessel wall interactions in vivo by reducing endothelial adhesion molecule expression. *Cancer Res* 2003;63:2322–9.
38 Klümper N, Saal J, Berner F, *et al*. C reactive protein flare predicts response to checkpoint inhibitor treatment in non-small cell lung cancer. *J Immunother Cancer* 2022;10:e004024.
39 Mehta A, Rucevic M, Sprecher E, *et al*. Plasma proteomic biomarkers identify non-responders and reveal biological insights about the tumor microenvironment in melanoma patients after PD1 blockade. *Cancer Biology* [Preprint].
40 Sato R, Imamura K, Sakata S, *et al*. Disorder of coagulation-Fibrinolysis system: an emerging toxicity of anti-PD-1/PD-L1 Monoclonal antibodies. *JCM* 2019;8:762.
41 Boutros C, Scoazec J-Y, Mateus C, *et al*. Arterial thrombosis and anti-PD-1 blockade. *Eur J Cancer* 2018;91:164–6.
42 Baltussen JC, Welters MJP, Verdegaal EME, *et al*. Predictive biomarkers for outcomes of Immune Checkpoint Inhibitors (ICIs) in Melanoma: a systematic review. *Cancers (Basel)* 2021;13:6366.
43 Capone M, Giannarelli D, Mallardo D, *et al*. Baseline neutrophil-to-lymphocyte ratio (NLR) and derived NLR could predict overall survival in patients with advanced Melanoma treated with Nivolumab. *J Immunother Cancer* 2018;6:74.
44 Wagner NB, Forschner A, Leiter U, *et al*. S100B and LDH as early prognostic markers for response and overall survival in Melanoma patients treated with anti-PD-1 or combined anti-PD-1 plus anti-CTLA-4 antibodies. *Br J Cancer* 2018;119:339–46.
45 Laino AS, Woods D, Vassallo M, *et al*. Serum Interleukin-6 and C-reactive protein are associated with survival in Melanoma patients receiving immune Checkpoint inhibition. *J Immunother Cancer* 2020;8:e000842.
46 Edvardsen MS, Hindberg K, Hansen E-S, *et al*. Plasma levels of von Willebrand factor and future risk of incident venous thromboembolism. *Blood Adv* 2021;5:224–32.
47 Haist M, Stege H, Pemler S, *et al*. Anticoagulation with factor Xa inhibitors is associated with improved overall response and progression-free survival in patients with metastatic malignant Melanoma receiving immune Checkpoint inhibitors-A retrospective, real-world cohort study. *Cancers* 2021;13:5103.
48 Wang S-J, Khullar K, Kim S, *et al*. Effect of Cyclo-oxygenase inhibitor use during Checkpoint blockade Immunotherapy in patients with metastatic Melanoma and non-small cell lung cancer. *J Immunother Cancer* 2020;8:e000889.