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ORIGINAL RESEARCH

Immune checkpoint inhibitor associated vitiligo and its impact on survival in patients with metastatic melanoma: an Italian Melanoma Intergroup study

M. Guida[1*], S. Strippoli[1], M. Maule[2], P. Quaglino[3], A. Ramondetta[3], V. Chiaron Sileni[4], G. Antonini Cappellini[5], P. Queirolo[6], L. Ridolfi[7], M. Del Vecchio[8], E. Cocorocchio[9], A. M. Di Giacomo[10], L. Festino[11], B. Merelli[12], M. Occelli[13], S. Brugnara[14], A. Minisini[15], S. Sava[16], S. Tommasi[17] & S. De Summa[17], on behalf of Italian Melanoma Intergroup

[1]Rare Tumors and Melanoma Unit, IRCCS Istituto Tumori 'Giovanni Paolo II', Bari; [2]Cancer Epidemiology Unit, Department of Medical Sciences, University of Turin, Turin; [3]Dermatologic Clinic, Department of Medical Sciences, University of Turin Medical School, Turin; [4]Veneto Institute of Oncology IOV-IRCCS, Padova; [5]Department of Oncology and Dermatological Oncology, IDI-IRCCS, Rome; [6]Skin Cancer Unit, IRCCS Ospedale Policlinico San Martino, Genova; [7]Medical Oncology Department, IRCCS National Cancer Research Centre, Meldola (FO); [8]Medical Oncology Unit, Department of Medical Oncology and Hematology, Fondazione IRCCS, Milan; [9]Division of Medical Oncology for Melanoma, Sarcoma, and Rare Tumors, IEO, European Institute of Oncology IRCCS, Milan; [10]Center for Immuno-Oncology University Hospital of Siena, Siena; [11]Unit of Melanoma, Cancer Immunotherapy and Development Therapeutics, Istituto Nazionale, Naples; [12]Department of Medical Oncology, ASST Papa Giovanni XXIII, Bergamo; [13]Department of Medicine, Clinical Oncology and Translational Research, Azienda Ospedaliera Santa Croce and Carle University Teaching Hospital, Cuneo; [14]Oncology Unit, S. Chiara Hospital, Trento; [15]Department of Oncology, ASUIUD University Hospital of Udine, Udine; [16]Medical Oncology Unit, A.O. Papardo & Department of Human Pathology, University of Messina, Messina; [17]Molecular Diagnostic and Pharmacogenetics Laboratory, IRCCS Istituto Tumori 'Giovanni Paolo II', Bari, Italy

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Background: Checkpoint inhibitors in melanoma can lead to self-immune side-effects such as vitiligo-like depigmentation (VLD). Beyond the reported association with favorable prognosis, there are limited data regarding VLD patient features and their echo on the therapeutic outcomes.

Methods: To assess the association between VLD and a series of clinical and biological features as well as therapeutic outcomes, we built an observational cohort study by recruiting patients who developed VLD during checkpoint inhibitors.

Results: A total of 148 patients from 15 centers (101 men, median age 66 years, BRAF mutated 23%, M1c 42%, Eastern Cooperative Oncology Group (ECOG) status 0/1 99%, normal lactate dehydrogenase 74%) were enrolled. VLD was induced by ipilimumab, programmed cell death-1 (PD-1) inhibitors, and their combination in 32%, 56%, and 12%, respectively. The median onset was 26 weeks and it was associated with other skin and nonskin toxicities in 27% and 28%, respectively. After 3 years of VLD onset, 52% (95% confidence interval 39% to 63%) were progression free and 82% (95% confidence interval 70% to 89%) were still alive. The overall response rate was 73% with 26% complete response. Univariable analysis indicated that BRAF V600 mutation was associated with a better overall survival ($P = 0.028$), while in multivariable analysis a longer progression-free survival was associated with BRAF V600 ($P = 0.093$), female sex ($P = 0.008$), and M stage other than 1a ($P = 0.024$). When VLD occurred, there was a significant decrease of white blood cell (WBC) count ($P = 0.05$) and derived WBC-to-lymphocytes ratio (dWLR; $P = 0.003$). A lower monocyte count ($P = 0.02$) and dWLR ($P = 0.01$) were also reported in responder patients.

Conclusions: Among VLD population, some features might help to identify patients with an effective response to immunotherapy, allowing clinicians to make more appropriate choices in terms of therapeutic options and duration.

Key words: melanoma, immunotherapy, vitiligo, checkpoint inhibitors, white blood cells, monocytes, immune-related toxicity

INTRODUCTION

Since the approval of the immunomodulatory antibody ipilimumab as second-line therapy in metastatic melanoma (MM) in 2011, this new class of drugs, comprising both cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death-1 (PD-1)/PD-1 ligand-1 (PD-L1) inhibitors, utilized alone or in combination, has profoundly changed oncology practice.[1] This Copernican revolution has not proved to be without challenges. While the ability to achieve long-lasting response in a subset of patients is a well-known effect of checkpoint inhibitors, no well-defined consensus has been made for duration of therapy, predictive biomarkers, response criteria, and significance of

*Correspondence to: Dr. Michele Guida, Rare Tumors and Melanoma Unit, IRCCS Istituto Tumori 'Giovanni Paolo II', viale Orazio Flacco, 65, Bari, Italy. Tel/Fax: +39-080-5555-136

E-mail: micguida57@gmal.com (M. Guida).

toxicity. The latest challenge is a complex paradigm change enforced by this therapeutic course: by removing key immune inhibitors (CTLA-4 and PD-1) to restore active T-cell response against tumor cells, these agents could also break out a new class of side-effects as a result of overstimulation of the immune system, known as immune-related adverse events (irAEs).[2-4] Among the irAEs, skin reactions, colitis, pneumonitis, and endocrinopathies occur more commonly.[1-3]

Although the knowledge of the self-immune nature of this toxicity led to the development of a standardized management protocol,[4] unresolved issues remain to be clarified, particularly, regarding the various types of side-effects and their correlation to clinical outcomes. It is likely that irAEs could be related to the disease and the checkpoint inhibitor used.[5] The most striking case in this scenario is the vitiligo-like depigmentation (VLD) induced by checkpoint inhibitors in MM patients. This skin toxicity mirrors CD (cluster of differentiation) 8^{+} reactivity against antigens coexpressed in melanoma cells and normal melanocytes, evident from the examination of clonotypically identical cytotoxic T cells infiltrating the tumor lesions and skin depigmented areas.[6] Some studies support that VLD induced by any kind of therapy is a prognostic factor associated with a better overall survival (OS) in patients with stage III and IV melanoma.[7-10] Data from a systematic review and meta-analysis reported an incidence of this skin toxicity in only 3.4% of melanoma patients treated with immunotherapy.[11] More recent data indicate a higher incidence of approximately 10%-28% among patients treated with checkpoint inhibitors.[12-18] However, owing to the limited number of patients included in these reports, no definitive evidence could be gathered about the significance of VLD arising from the use of checkpoint inhibitors as well as the clinical and biological features associated with VLD.

Here, we report a multi-institutional study within the Italian Melanoma Intergroup (IMI) centers, comprising a large population of 148 MM patients who developed VLD during treatment with CTLA-4 and PD-1 inhibitors as a single agent or in combination. We outline the profile of the patients with VLD and define their therapeutic outcomes. Moreover, we performed univariable and multivariable analyses to assess the association between therapy outcomes and a series of clinical and biological features as well as the trend of some peripheral blood parameters.

PATIENTS AND METHODS

Patients, treatment, and assessment

We built an observational cohort study by retrospectively recruiting patients with stage IV melanoma from 15 IMI centers. Patients were considered eligible if they developed VLD during treatment with ipilimumab, or PD-1 inhibitors (pembrolizumab or nivolumab), or the combination of ipilimumab and nivolumab. Patients were treated according to the standard dose and schedule of checkpoint inhibitors. All patients were routinely screened for VLD by a dermatological examination performed once a month during treatment. VLD was defined as the appearance of hypopigmented skin areas and was classified as localized or generalized according to the distribution of the lesions. Generalized vitiligo was defined as a bilateral symmetrical form, including acrofacial vitiligo, diffuse vitiligo vulgaris, and universal vitiligo. Localized vitiligo was defined as a unilateral asymmetrical form, including focal types, segmental types, halo nevi, and perimetastatic types. The mixed types of vitiligo were defined as a mixed distribution pattern of both generalized and localized vitiligo.[10-19]

Patients were eligible if they underwent at least a radiological assessment by RECIST (version 1.1).[20] The radiological assessment was performed in all the centers as per clinical practice every 3/4 months. For all patients, we systematically collected the clinical data such as primary melanoma histology report, anatomic site, TNM stage, timing of main disease events, metastatic sites, treatments, response to therapy, Eastern Cooperative Oncology Group (ECOG), kind and timing of vitiligo onset, lactate dehydrogenase (LDH) value, and white blood cell (WBC) counts before and during immunotherapy. The study was approved by the local Ethics Committee of Istituto Tumori 'Giovanni Paolo II' of Bari (protocol 633/Ethics Committee of 27 June 2017).

Statistical analysis

OS and progression-free survival (PFS) were calculated from vitiligo onset and were estimated with the Kaplan—Meier method. The choice of using VLD diagnosis rather than immunotherapy initiation as the starting point is motivated by the attempt to avoid overestimation of survival time because of the so-called immortal time bias, occurring when time not at risk is erroneously included in the analysis. In our cohort, only patients developing VLD are included in the analysis. Therefore patients, by design, are not at risk of death from the start of the treatment, but from the moment they are diagnosed with VLD. Indeed, the design is based on a biological rationale that the onset of VLD in itself triggers an antitumor response in synergy with checkpoint blockade. For completeness, alternative analyses starting from immunotherapy initiation are shown in Supplementary Material, available at https://doi.org/10.1016/j.esmoop.2021.100064. Survival differences among groups of patients were tested through the log-rank test. To investigate the relationship between covariates and events (death or progression of disease), we fitted both univariable and multivariable Cox proportional hazard models. Considering the small number of events, we built parsimonious models to avoid overfitting. Predictors for the PFS model (48 observed events) were chosen a priori, based on subject matter knowledge, and included stage of metastatic disease (M1a versus M1b, M1c, M1d), BRAF mutation (presence versus absence), the line of therapy (first line versus further line), LDH (above versus below upper limit of normal), type of vitiligo (I versus II and III), sex, and age. In the OS model (18 observed events), we had to perform a stricter selection, and only stage, line of therapy, LDH, and age were

Table 1. Clinical and disease features of patients developing vitiligo-like depigmentation during therapy with checkpoint inhibitors

Characteristics (N = 148 patients)	
Sex, *n* (%)	
Female	47 (32)
Male	101 (68)
Age at MM diagnosis, median (25th-75th percentiles)	61 (48-70)
Checkpoint inhibitor, *n* (%)	
Ipilimumab	47 (32)
PD-1 inhibitor	83 (56)
Ipilimumab plus PD-1	18 (12)
Line of therapy during which vitiligo appeared, *n* (%)	
First line	77 (52)
Second line	43 (29)
Third line	22 (15)
Fourth line	6 (4)
Type of melanoma, *n* (%)	
Cutaneous	124 (84)
Mucosal	6 (4)
Unknown origin	18 (12)
Anatomic site of primary melanoma, *n* (%)	
Head and neck	18 (12)
Trunk	51 (34)
Upper limbs	6 (4)
Lower limbs	49 (33)
Mutation status, *n* (%)	
BRAF	34 (23)
Wild type	114 (77)
Stage at initial diagnosis, *n* (%)	
I-II	47 (32)
III	63 (42)
IV	38 (26)
Previous adjuvant therapy, *n* (%)	
Yes	24 (16)
No	124 (84)
Disease-free survival, months (months in range)	14 (0-172)
M stage[a]	
M1a	53 (36)
M1b	25 (17)
M1c	62 (42)
M1d	8 (5)
LDH[a]	
>ULN	37 (25)
<ULN	104 (70)
NA	7 (5)
ECOG[a]	
0-1	146 (99)
>1	2 (1)

ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; MM, metastatic melanoma; NA, not assessed; PD-1, programmed cell death-1; ULN, upper limits of normal.
[a] At therapy-induced vitiligo.

included. Absolute values of lymphocytes, WBCs, and monocytes have been compared at two different time points (at the beginning of therapy causing vitiligo and at vitiligo onset) through paired-sample Wilcoxon test because Shapiro—Wilk normality test indicated that data did not follow the normal distribution. Moreover, we computed a derived WBC-to-lymphocyte ratio (dWLR) with the following formula: dWLR = (WBC − Lymphocytes)/Lymphocytes. Ratio values have also been tested with paired sample Wilcoxon test. All analyses were performed with R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). 'statix' 'ggpubr', and 'tidyverse' R packages have been used. Finally, to test differences in monocytes, lymphocytes, WBC, and dWLR values in terms of type of response (partial and complete response versus absence of radiologic objective response) and the two timepoints, analysis of variance of aligned rank-transformed data, a nonparametric test, was performed, because the normality assumption was not reached, which did not allow the use of two-way analysis of variance. 'ARTtool' R package was used.

RESULTS

Vitiligo features

In the time span from June 2007 to November 2017, 148 [101 (68%) male and 47 (32%) female] stage IV melanoma patients treated with checkpoint inhibitors developed VLD.

The vitiligo-inducing therapy included ipilimumab in 47 (32%) patients, PD-1 inhibitors in 83 (56%) patients, and a combination therapy of ipilimumab and nivolumab in the remaining 18 (12%) patients. The main clinical features of this population and of VLD are summarized in Table 1 and Supplementary Material, available at https://doi.org/10.1016/j.esmoop.2021.100064.

Clinical outcomes

With a median follow up of 46 months, progression was observed in 48 patients, 18 of whom died. Median PFS time was 42 months, with 52% [95% confidence interval (CI) 39% to 63%] of the cohort patients still alive and progression free 3 years after VLD onset (Figure 1A). The 25th percentile of OS time was 42 months, with 82% (95% CI 70% to 89%) of the cohort patients still alive 3 years after VLD onset (Figure 1B).

Regarding the response, we found a global overall response rate of 73% (108), with 26% (38) of complete response. Moreover, stable disease was reported in 20% (n = 30) of patients, and only 7% (n = 10) experienced a progressive disease as best response. The rates of overall response and complete response among the different treatments were 64% (n = 30) and 32% (n = 15) for ipilimumab, 78% (n = 65) and 19% (n = 16) for PD-1 inhibitors, and 72% (n = 13) and 39% (n = 7) for the combination therapy, respectively. Likely due to this similar response rate, there were no statistically significant differences in OS and PFS among the different checkpoint inhibitors.

However, among VLD patients, a longer PFS was found in the Cox multivariable regression analysis in women with respect to men (hazard ratio 0.34, 95% CI 0.16-0.76, P = 0.008) and for M stage other than M1a (hazard ratio 0.45, 95% CI 0.22-0.90, P = 0.024). Moreover, the presence of BRAF V600 mutation was associated with a better OS, with no deaths occurring in the mutation carrier (P = 0.028; Figure 1C). The Cox univariable and multivariable analyses results are summarized in Tables 2 and 3.

WBC trend and VLD

The values of WBC, lymphocytes, monocytes, and dWLR at beginning of treatment and onset of VLD were available for 88 patients (60%). When vitiligo occurred, we found a significant lowering of WBC count (P = 0.05) and dWLR (P = 0.003; Figure 2A; Table S1 in Supplementary Material,

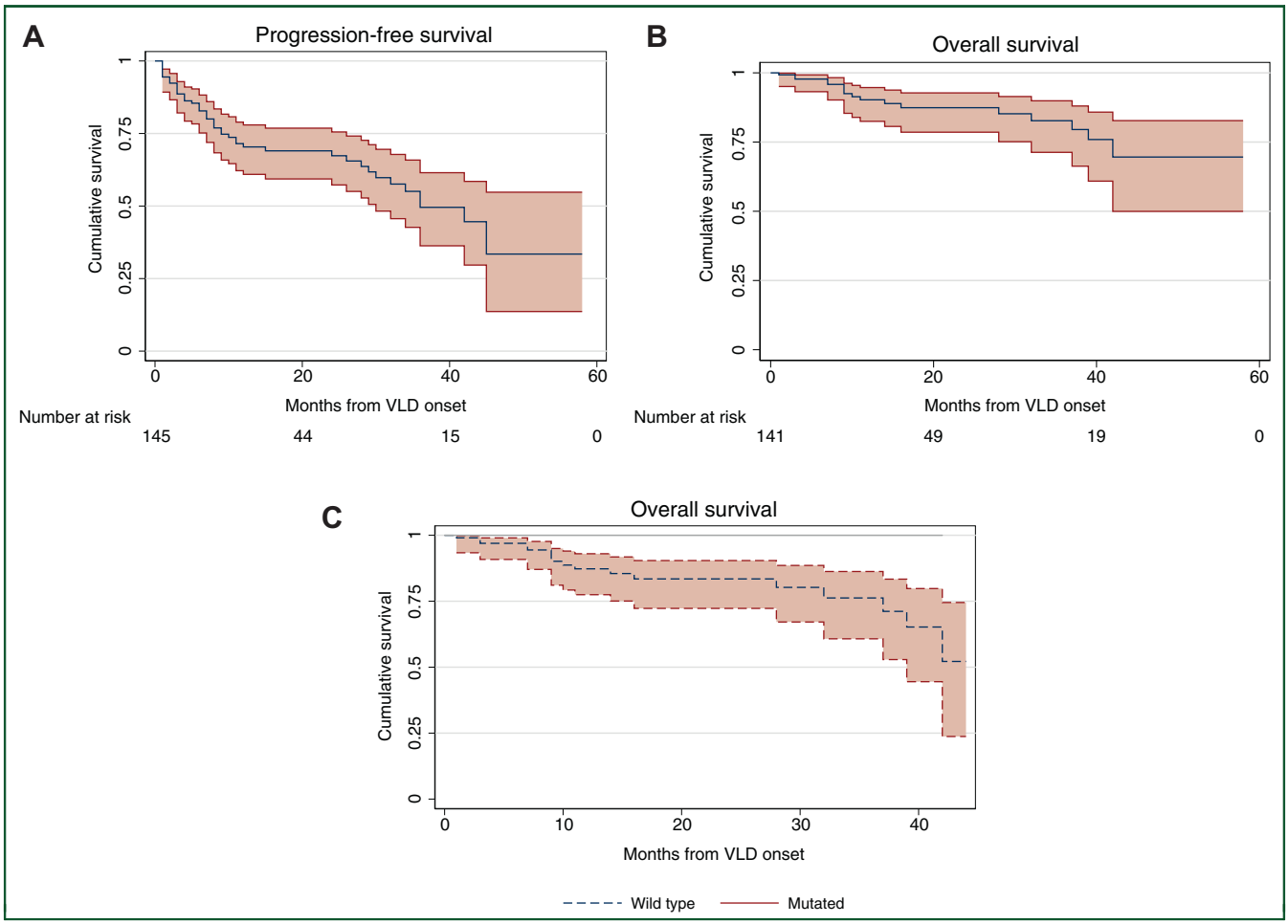

Figure 1. **Kaplan–Meier curves of (A) progression-free survival and (B) overall survival (OS) in the entire population of 148 patients developing vitiligo-like depigmentation (VLD) during treatment with checkpoint inhibitors. (C) OS by BRAF status ($P = 0.028$).**

available at https://doi.org/10.1016/j.esmoop.2021.100064). Then, we test the variability of absolute values of these hematological parameters across VLD patient groups with and without RECIST response. We found that in responder patients there was a significant lowering of monocyte count and dWLR ($F_{1,173} = 5.34$, $P = 0.02$, $F_{1,173} = 6.03$, $P = 0.01$, respectively; Figure 2B).

DISCUSSION

In melanoma patients, VLD is a dermatological, spontaneous, or treatment-induced phenomenon characterized by a loss of epidermis melanocytes due to antitumor immunity, with the pathogenesis likely based on both antibody and $CD8^+$ activation against antigens shared by melanoma and melanocytes.[21] Thus, it is expected that this kind of vitiligo, as a surrogate of robust antimelanoma immunity, could be associated with improved survival. At present, beyond case reports and small single-center experiences, there are limited data regarding the feature profile of patients with this irAE and its influence on the therapeutic outcomes with these drugs. To clarify this, we performed the largest observational cohort study of 148 patients with VLD induced by CTLA-4 and PD-1 inhibitors.

A previous meta-analysis by Teulings et al.[11] focused on the incidence of VLD in a large population of stage III and IV melanoma patients from 139 studies of immunotherapy including 28 studies with CTLA-4 and PD-1 blockade. The pooled cumulative incidence of VLD was 3.4% (total patients: 304) with 2% due to checkpoint inhibitors (74 patients). However, this review reported PFS and OS data from only 35 and 18 patients, respectively. Although a significant survival benefit was shown with a doubling of PFS and a quadruplication of OS in patients who developed VLD compared with those who did not, the analysis accounted for only four patients treated with checkpoint inhibitor.

In our analysis, we reported 36-month PFS and OS rates of 52% and 82%, which were much longer than those previously reported.[22-24] Likewise, VLD was also associated with a remarkable response rate of 73% with 26% of complete response, which is much higher than those reported in registrative trials.[22-24] Thus, we found no proper comparison with unselected melanoma population in a real-word setting due to the late occurrence of VLD (median onset about 6 months) and the consequent selection of a long-lasting responder population. Of note, similar results were reported in five small retrospective analyses by Quach et al.,[12] Nakamura et al.,[16] Freeman-Keller et al.,[17]

Table 2. Univariable and multivariable Cox regression analyses of PFS in melanoma patients treated with checkpoint inhibitors and who developed VLD

Variable	HR (95% CI)	*P* value
PFS univariate analysis		
Sex		
Male	1	
Female	0.42 (0.21-0.85)	**0.016**
BRAF status		
Wild-type	1	
Mutation carrier	0.45 (0.19-1.05)	0.065
Site of primary tumor		
Trunk	1	0.536
Upper limb	0.98 (0.13-7.47)	
Lower limb	1.05 (0.54-2.06)	
Unknown origin	0.36 (0.11-1.25)	
Mucosal	1.22 (0.28-5.31)	
Head	1.41 (0.60-3.30)	
Age at MM diagnosis	1.02 (1.00-1.04)	0.114
Line of therapy		
First	1	
Second or further	0.57 (0.32-1.02)	0.060
Stage at initial diagnosis		
I	1	0.669
II	0.51 (0.18-1.51)	
III	0.91 (0.49-1.67)	
IV	1.12 (0.26-4.86)	
M Stage at treatment (binary)		
M1a	1	
M1b, M1c, M1d	0.83 (0.47-1.48)	0.527
LDH (binary)		
<ULN	1	
>ULN	1.52 (0.81-2.82)	0.189
Type of VLD		
I	1	0.625
II	1.36 (0.73-2.53)	
III	1.11 (0.46-2.66)	
Type of VLD (binary)		
I	1	0.400
II, III	1.29 (0.72-2.31)	
PFS multivariate analysis		
Sex		
Male	1	**0.008**
Female	0.34 (0.16-0.76)	
BRAF status		
Wild type	1	**0.093**
Mutation carrier	0.46 (0.19-1.14)	
Age at MM diagnosis	1.01 (0.98-1.03)	0.574
Line of therapy		
First	1	0.206
Second or further	0.67 (0.36-1.24)	
M stage (binary)		
M1a	1	**0.024**
M1b, c, d	0.45 (0.22-0.90)	
LDH		
<ULN	1	0.111
>ULN	1.78 (0.88-3.61)	
Type of VLD (binary)		
I	1	0.664
II, III	1.15 (0.62-2.11)	

Bold entries are statistically significance findings.
CI, confidence interval; HR, hazards ratio; LDH, lactate dehydrogenase; MM, metastatic melanoma; PFS, progression-free survival; ULN, upper limits of normal; VLD, vitiligo-like depigmentation.

Table 3. Univariable and multivariable Cox regression analyses of OS in melanoma patients treated with checkpoint inhibitors and who developed vitiligo-like depigmentation

Variable	HR (95% CI)	*P* value
OS univariate analysis		
Sex		
Male	1	
Female	0.87 (0.33-2.33)	0.787
BRAF status		
Wild-type	NE	
Mutation carrier		
Site of primary tumor		
Trunk	1	0.318
Limbs	1.99 (0.62-6.37)	
Other	0.94 (0.23-3.80)	
Age at MM diagnosis	1.02 (0.99-1.06)	0.203
Line of therapy		
First	1	
Second or further	0.60 (0.23-1.54)	0.285
Stage at diagnosis		
I	1	0.534
II	0.34 (0.04-2.71)	
III	0.57 (0.21-1.53)	
IV	1.30 (0.16-10.38)	
Stage M		
M1A	1	
Others	0.56 (0.22-1.42)	0.222
LDH (binary)		
<ULN	1	
>ULN	1.61 (0.61-4.68)	0.308
Type of vitiligo		
I	1	0.943
II	1.20 (0.43-3.35)	
III	1.08 (0.28-4.24)	
Type vitiligo (binary)		
I	1	
II, III	1.16 (0.44-3.04)	0.757
OS multivariate analysis		
Age at MM diagnosis	1.02 (0.98-1.05)	0.364
Line of therapy		
First	1	
Second or further	0.56 (0.19-1.64)	0.293
M stage		
M1a	1	0.145
Others	0.46 (0.16-1.3)	
LDH (binary)		
<ULN	1	
>ULN	1.92 (0.65-5.69)	0.242

CI, confidence interval; HR, hazards ratio; LDH, lactate dehydrogenase; MM, metastatic melanoma; NE, not estimable; OS, overall survival; ULN, upper limits of normal.

Bottlaender et al.,[25] and Nakano et al.[26] and in one prospective study by Hua et al.[14] However, a limited number of patients were included in these reports ($N = 10$, 19, 9, 16, 30, and 17, respectively). Because of these small sample sizes, some data are conflicting and no correlation was found with other clinical or biological characteristics. Nakamura et al.[16] reported a response rate of only 41% in VLD patients, which was the same as that of patients without VLD, and Hua et al.[14] did not find a statistically significant advantage in OS between the two groups after correction for time load bias. Similar data were reported in a larger retrospective study of the French pharmacovigilance database that described the outcomes of 94 melanoma patients with VLD associated with checkpoint inhibitors.[27] Although the response rate and the detailed features of the patient population were not reported, a median PFS of 22 and 20 months for pembrolizumab and nivolumab, respectively, and an OS rate of 65% in the entire population at 33 months of median follow-up were documented.[27]

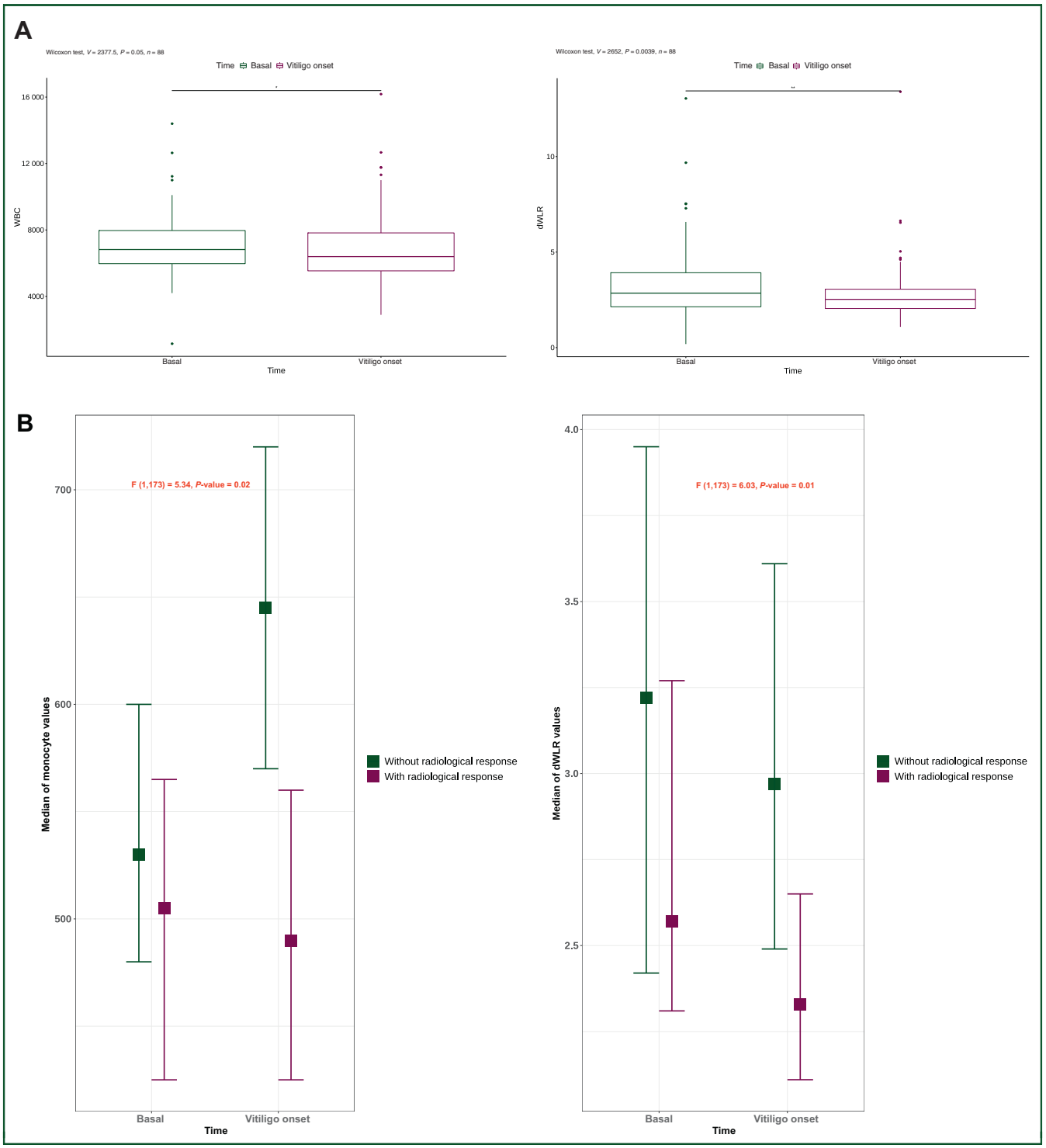

Figure 2. **(A) Boxplots showing a significant variation of absolute value of white blood cells (WBCs) and WBC to lymphocytes ratio between the beginning of immunotherapy and the vitiligo-like depigmentation onset. (B) At those time-points, a lower monocyte count ($P = 0.02$) and dWLR were reported in responder versus nonresponder patients.**

In our population, the main features of VLD were similar to those previously reported. The skin depigmentation onset occurred on photoexposed areas and was not associated with the Koebner phenomenon, which normally characterizes common vitiligo, as reported in eight patients of the prospective study by Larsabal et al.[13] In our population, the onset of VLD was earlier in the combination therapy than in single checkpoint inhibitor, which was consistent with previously reported data of phase III trial CheckMate 067[22] as well as a single-center retrospective analysis.[18,26,27] Interestingly, we showed that after VLD onset, there was no statistical difference in clinical

outcomes regardless of whether the checkpoint was utilized as a single agent or in combination. This could be proof of the independent strength of the antimelanoma immunity accompanying this skin manifestation. A further hint in favor of the positive intrinsic and independent prognostic values of VLD could be deduced by the fact that in both univariable and multivariable survival analyses of this population, there were very few other clinical and biological characteristics that were able to positively influence clinical outcomes. Ultimately, it is conceivable that incidence of VLD identifies a homogeneous population of patients already with a favorable prognosis, which is further emphasized by the immunological therapy. This hypothesis could explain the unexpected data of a longer PFS in M stage other than M1a. Intriguingly, we found a positive correlation with a probability of a longer PFS in female patients, which is in contrast to a recent meta-analysis that revealed that the magnitude of benefit to checkpoint inhibitors is sex dependent with a significant advantage for male patients.[28] However, this sex difference arises from a heterogeneous spectrum of studies on different type of cancers.

Another compelling result is the evidence of better survival in the presence of the BRAF V600 mutation (Figure 2). Better clinical outcomes in BRAF-mutated patients compared with the wild type were previously reported in CheckMate 067 for all kinds of immunotherapy.[22] Moreover, a genetic signature eliciting a deeper immunogenicity has long been described in BRAF-mutated melanoma.[29] Of interest, 60% of our patients with BRAF mutation had been pre-treated with BRAF/MEK inhibitor drugs. As already known, this treatment could induce an antigen and immunological modulation, which could reactivate the melanoma immune response,[30,31] thereby making these patients more responsive to immunotherapy with checkpoint inhibitors.

Finally, among the blood profile, we found a lowering of WBC and dWLR when VLD occurs compared with the beginning of immunotherapy. Even if we looked at late variation, as the median time of vitiligo onset is about 6 months from the beginning of immunotherapy, these findings could be a confirmation of previous several reports that documented earlier differences in lymphocyte, neutrophils-to-lymphocytes ratio, or monocyte as predictive biomarkers.[32-35] We chose to investigate the ratio (dWLR) that accounts for the sum of neutrophils and monocytes in the numerator as these cells are well-known markers of inflammatory effector functions and regulatory properties essential for malignancy growth and immune escape.[32,36-38] We also found a decrease of this ratio as well as of monocyte count at VLD onset in patients with partial or complete response compared with nonresponder patients. Such findings might mirror a major recruitment of immunosuppressive cellular actors in the tumor microenvironment of nonresponder patients for which the main source is circulating monocytes. The negative prognostic significance of monocytes and monocytic myeloid-derived suppressor cells has been reported in patients with diffuse large B-cell lymphoma under R-CHOP therapy,[39] whereas no data are available in solid tumors.

Despite these interesting findings, some limitations of this analysis deserve to be underlined. First, it is a retrospective study with a long duration of recruitment. In addition, the small number of deaths during follow-up restrains the conclusions along with the need for any associations to be confirmed in prospective studies due to the large number of statistical analysis drawn. Unlike other similar studies[12-17] we did not match our population with a comparison one without VLD. This matching was made difficult by the long period of the accrual and heterogeneity of our VLD population (treated with different checkpoint inhibitors and lines of therapy) as well as due to the enrichment of responder patients. Moreover, our mainly goal is to define the clinical and biological features associated with better outcomes among patients with checkpoint blockade-induced VLD. Thus these areas could be a starting point to validate the detailed molecular and immunological apparatus that supports VLD and orchestrates the effective antitumor reaction. Finally, the identification of antigen-specific immune effector cells which mediated VLD could be the next step to assess and monitor the clinical benefit associated with this irAE.

CONCLUSIONS

Our data clearly show that patients who develop VLD during treatment with checkpoint inhibitors have a longer survival and a higher response rate with respect to those reported in all large controlled clinical studies. In our opinion, it is likely that these beneficial outcomes are due to the selection of a population with an intrinsic capability to implement a powerful antitumor response emphasized by the treatment with checkpoint inhibitors.

Although VLD cannot be used as a predictor of response, as it follows and does not anticipate a response, our data could contribute to better recognition of patients with an effective antimelanoma immunity and may help clinicians in decision making regarding therapeutic options and duration. This could be particularly useful in case of PD-1 blockade wherein the treatment interruption after a complete response remains an unresolved question.

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DISCLOSURE

The authors have declared no conflicts of interest.

REFERENCES

1. Winer A, Bodor JN, Borghaei H. Identifying and managing the adverse effects of immune check-point blockade. *J Thorac*. 2018;10(3):480-489.

2. Topalian SL. Targeting immune check-points in cancer therapy. *J Am Med Assoc*. 2017;318(17):1647-1648.
3. Brahmer JR, Lacchetti C, Thompson JA. Management of immune-related adverse events in patients treated with immune check-point inhibitor therapy: American Society of Clinical Oncology Clinical Practice guideline summary. *J Oncol Pract*. 2018;14(4):247-249.
4. Champiat S, Lambotte O, Barreau E, et al. Management of immune check-point blockade dysimmune toxicities: a collaborative position paper. *Ann Oncol*. 2016;27(4):559-574.
5. Khoja L, Day D, Wei-Wu Chen T, et al. Tumour and class-specific patterns of immune-related adverse events of immune check-point inhibitors: a systematic review. *Ann Oncol*. 2017;28(10):2377-2385.
6. Becker JC, Guldberg P, Zeuthen J, et al. Accumulation of identical T cells in melanoma and vitiligo-like leukoderma. *J Invest Dermatol*. 1999;113(6):1033-1038.
7. Bystryn JC, Rigel D, Friedman RJ, et al. Prognostic significance of hypopigmentation in malignant melanoma. *Arch Dermatol*. 1987;123(8):1053-1055.
8. Gogas H, Ioannovich J, Dafni U, et al. Prognostic significance of autoimmunity during treatment of melanoma with interferon. *N Engl J Med*. 2006;354(7):709-718.
9. Boasberg PD, Hoon DS, Piro LD, et al. Enhanced survival associated with vitiligo expression during maintenance biotherapy for metastatic melanoma. *J Invest Dermatol*. 2006;126(12):2658-2663.
10. Quaglino P, Marenco F, Osella-Abate S, et al. Vitiligo is an independent favourable prognostic factor in stage III and IV metastatic melanoma patients: results from a single-institution hospital-based observational cohort study. *Ann Oncol*. 2010;21(2):409-414.
11. Teulings HE, Limpens J, Jansen SN, et al. Vitiligo-like depigmentation in patients with stage III-IV melanoma receiving immunotherapy and its association with survival: a systematic review and meta-analysis. *J Clin Oncol*. 2015;33(7):773-781.
12. Quach HT, Dewan AK, Davis EJ, et al. Association of anti-programmed cell death 1 cutaneous toxic effects with outcomes in patients with advanced melanoma. *J Am Med Assoc*. 2019;5(6):906-908.
13. Larsabal M, Marti A, Jacquemin C, et al. Vitiligo-like lesions occurring in patients receiving anti-programmed cell death therapies are clinically and biologically distinct from vitiligo. *J Am Acad Dermatol*. 2017;76(5):863-870.
14. Hua C, Boussemart L, Mateus C, et al. Association of vitiligo with tumor response in patients with metastatic melanoma treated with pembrolizumab. *JAMA Dermatol*. 2016;152(1):45-51.
15. Hwang SJ, Carlos G, Wakade D, et al. Cutaneous adverse events (AEs) of anti-programmed cell death (PD)-1 therapy in patients with metastatic melanoma: a single-institution cohort. *J Am Acad Dermatol*. 2016;74(3):455-461.
16. Nakamura Y, Tanaka R, Asami Y, et al. Correlation between vitiligo occurrence and clinical benefit in advanced melanoma patients treated with nivolumab: a multi-institutional retrospective study. *J Dermatol*. 2017;44(2):117-122.
17. Freeman-Keller M, Kim Y, Cronin H, et al. Nivolumab in resected and unresectable metastatic melanoma: characteristics of immune-related adverse events and association with outcomes. *Clin Cancer Res*. 2016;22(4):886-894.
18. Hwang SJE, Park JJW, Wakade D, et al. Cutaneous adverse events of anti-programmed death 1 antibodies combined with anti-cytotoxic T-lymphocyte-associated protein 4 therapy use in patients with metastatic melanoma. *Melanoma Res*. 2019;29(2):172-177.
19. Hartmann A, Bedenk C, Keikavoussi P, et al. Vitiligo and melanoma-associated hypopigmentation (MAH): shared and discriminative features. *J Dtsch Dermatol Ges*. 2008;6(12):1053-1059.
20. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* 2009;45(2):228-247.
21. Byrne KT, Turk MJ. New perspectives on the role of vitiligo in immune responses to melanoma. *Oncotarget*. 2011;2(9):684-694.
22. Hodi FS, Chiarion-Sileni V, Gonzalez R, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. *Lancet Oncol*. 2018;19(11):1480-1492.
23. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711-723.
24. Hamid O, Robert C, Daud A, et al. Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. *Ann Oncol*. 2019;30(4):582-588.
25. Bottlaender L, Amini-Adle M, Maucort-Boulch D, et al. Cutaneous adverse events: a predictor of tumour response under anti-PD-1 therapy for metastatic melanoma, a cohort analysis of 189 patients. *J Eur Acad Dermatol Venereol*. 2020;34(9):2096-2105.
26. Nakano E, Takahashi A, Namikawa K, et al. Correlation between cutaneous adverse events and prognosis in patients with melanoma treated with nivolumab: a single institutional retrospective study. *J Dermatol*. 2020;47(6):622-628.
27. Babai S, Voisin AL, Bertin C, et al. Occurrences and outcomes of immune checkpoint inhibitors-induced vitiligo in cancer patients: a retrospective cohort study. *Drug Saf.* 2020;43(2):111-117.
28. Conforti F, Pala L, Bagnardi V, et al. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol*. 2018;19(6):737-746.
29. Kannengiesser C, Spatz A, Michiels S, et al. EORTC Melanoma group. Gene expression signature associated with BRAF mutations in human primary cutaneous melanomas. *Mol Oncol*. 2008;1(4):425-430.
30. Frederick DT, Piris A, Cogdill AP, et al. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favourable tumor microenvironment in patients with metastatic melanoma. *Clin Cancer Res*. 2013;19(5):1225-1231.
31. Kelley MC. Immune responses to BRAF-targeted therapy in melanoma: is targeted therapy immunotherapy? *Crit Rev Oncog*. 2016;21(1-2):83-91.
32. Ferrucci PF, Ascierto PA, Pigozzo J, et al. Baseline neutrophils and derived neutrophil-to-lymphocyte ratio: prognostic relevance in metastatic melanoma patients receiving ipilimumab. *Ann Oncol*. 2018;29(2):524.
33. 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(1):74.
34. Delyon J, Mateus C, Lefeuvre D, et al. Experience in daily practice with ipilimumab for the treatment of patients with metastatic melanoma: an early increase in lymphocyte and eosinophil counts is associated with improved survival. *Ann Oncol*. 2013;24(6):1697-1703.
35. Guida M, Bartolomeo N, De Risi I, et al. The management of oligo-progression in the landscape of new therapies for metastatic melanoma. *Cancers (Basel)*. 2019;11(10):1559.
36. Auffray C, Sieweke MH, Geissmann F. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu Rev Immunol*. 2009;27:669-692.
37. Gouveia-Fernandes S. Monocytes and macrophages in cancer: unsuspected roles. *Adv Exp Med Biol*. 2020;1219:161-185.
38. Tcyganov E, Mastio J, Chen E, et al. Plasticity of myelopid-derived suppressor cells in cancer. *Curr Opin Immunol*. 2018;51:76-82.
39. Wu C, Wu X, Liu X, et al. Prognostic significance of monocytes and monocytic myeloid-derived suppressor cells in diffuse large B-cell lymphoma treated with R-CHOP. *Cell Physiol Biochem*. 2016;39(2):521-530.