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Vitiligo-like leukoderma as an indicator of clinical response to immune checkpoint inhibitors in late-stage melanoma patients

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Abstract

Purpose Although development of immune checkpoint inhibitors has revolutionized the treatment of metastatic melanoma, more than a half of treated patients experience disease progression during therapy. Cases of spontaneous vitiligo-like leukoderma have been described in melanoma patients and have been associated with a favorable outcome. This vitiligo-like leukoderma can also appear in melanoma patients undergoing immune therapies such as immune checkpoint inhibitors. However, no consensus exists about the relationship between vitiligo-like leukoderma onset and improved overall survival. Our study investigates the possible association between the onset of vitiligo-like leukoderma during immune checkpoint inhibitor treatment and a better prognosis.

Methods A non-concurrent cohort study was conducted by identifying retrospectively 280 patients who had inoperable or metastatic melanoma and had undergone immune therapy with checkpoint inhibitors in any line of treatment. Toxicities developed during therapy were evaluated.

Results Among the 280 study participants, 50% developed at least one type of toxicity, and vitiligo-like leukoderma was observed in 43 patients (15.4%). In the multivariate Cox model, a protective effect for mortality was observed for patients with vitiligo-like leukoderma development (HR : 0.23; 95% CI 0.11–0.44, $p < 0.0001$). In a sub-group analysis comprising only cutaneous melanoma in first line of treatment ($N = 153$), occurrence of vitiligo-like leukoderma was also an independent predictor factor for duration of clinical benefits measured by time to the next treatment (HR: 0.17; 95% CI 0.06–0.44).

Conclusion Our findings indicate that onset of vitiligo-like leukoderma during melanoma treatment could be a marker of favorable outcome in patients treated with immune checkpoint inhibitors.

Keywords Vitiligo · Leukoderma · Melanoma · Immune checkpoint inhibitors

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Abbreviations

MEK	Mitogen-activated protein kinase
ICI	Immune checkpoint inhibitors
CTLA-4	Cytotoxic T-lymphocyte antigen-4
PD-1	Programmed cell death protein 1
PFS	Progression free survival
OS	Overall survival
TMB	Tumor mutational burden
VLL	Vitiligo-like leukoderma
IL	Interleukin
irAE	Immune-related adverse events
LDH	Lactate dehydrogenase
TTNT	Time-to-next treatment
RECIST	Response Evaluation Criteria in Solid Tumors
CR	Complete response
PR	Partial response

SD	Stable disease
PD	Progressive disease
SD	Standard deviation
IQR	Interquartile range
HR	Hazard ratio
PD-L1	PD-ligand-1

Introduction

Treatment of metastatic melanoma has undergone a remarkable evolution in the last few years, leading to the development of innovative therapeutic strategies (Maverakis et al. 2015). Target inhibitors of the mitogen-activated protein kinase pathway, both inhibitors of mutated BRAF and of the mitogen-activated protein kinase (MEK) kinase, on one hand, and the immune checkpoint inhibitors (ICI), either anti-cytotoxic T-lymphocyte antigen (CTLA)-4 (ipilimumab) or anti-programmed cell death protein 1 (PD-1) (pembrolizumab or nivolumab), on the other hand, increased the median progression-free survival (PFS) and overall survival (OS) with a portion of long-survivors (Larkin et al. 2015; Dreno et al. 2018; Robert et al. 2019a, b). Despite these encouraging results, more than half of the patients experienced disease progression during treatment. Therefore, it is essential to understand the clinical and physio-pathological features of the patients who achieve the best outcome to define the most appropriate therapeutic strategies. The main risk factor for the development of cutaneous melanoma is exposure to solar or artificial ultraviolet rays which leads to the typical mutational signature with a $C > T$ nucleotide transition in dipyrimidine sites (León-Letelier et al. 2019). Then, other mutations occur during tumor progression, making melanoma the cancer type with the highest tumor mutational burden (TMB) (Büttner et al. 2019). Tumors with high TMB have a great probability of creating epitopes that can be presented by dendritic cells, leading to an efficient activation of immune cells (Chabanon et al. 2016). Despite this, the host immune system rarely succeeds in spontaneous melanoma clearance. However, presence of a high TMB confers a great advantage in the ICI treatment (Goodman et al. 2017), as it provides a range of antigens against which immune cells, once re-activated by ICI, can act.

In melanoma patients, spontaneous occurrence of cutaneous depigmentation or leukoderma has been reported (Failla et al. 2019). It is a clinical manifestation remarkably similar to vitiligo, an autoimmune disease characterized by the presence of autoimmune destruction of cutaneous melanocytes (Mason and Gawkrodger 2005). Antigens recognized by cytotoxic T lymphocytes nearby the leukoderma can be expressed both by normal melanocytes and by melanoma cells, thus explaining the coexistence of the neoplasm and the autoimmune response that determines skin depigmentation. Antigens recognized by T cells are generally characteristic of the melanogenesis process, such as gp100, MART-1, the tyrosinase enzyme, or the tyrosine-specific transport protein ½ (Teulings et al. 2014; Rodrigues et al. 2017). Recognition of these antigens by T lymphocytes seldom leads to regression of the cancerous lesions (Parmiani 2001).

Development of vitiligo-like leukoderma (VLL) has been described as a side-effect of immunomodulating treatments in cutaneous melanoma, starting with vaccine and interleukin (IL)-2 therapies up to modern treatments with ICI (Teulings et al. 2015). Incidence of VLL in patients treated with anti-CTLA-4 or anti-PD-1 was reported in a very variable range, from 3.4 and 28.0%, with an onset occurring on average at 30 weeks from inception of treatment (Belum et al. 2016; Hwang et al. 2019). Indeed, ICI therapy creates an imbalance in immune tolerance that may lead to uncontrolled immune reactions that manifest clinically as autoimmune events (Simeone et al. 2019). Besides VLL, other autoimmune reactions against normal melanocytes have been also observed in patients treated with ICI. These reactions target hair pigmentation (Dimitriou et al. 2020) or the retinal pigment epithelium, inducing a syndrome resembling the Vogt–Koyanagi–Harada disease (Gambichler et al. 2020). However, these reactions are rarer than VLL and only a few cases have been reported.

Recent systematic reviews focused on the evaluation of the possible link between immune-related adverse events (irAE) and clinical benefit in patients treated with ICI, analyzing retrospective studies and highlighting the association between autoimmune cutaneous toxicity and favorable outcome (Hua et al. 2016; Nakamura et al. 2017; Cortellini et al. 2019; Zitvogel et al. 2021). Regarding melanoma, a limited number of studies reported an association between development of irAE and better prognosis. This association was further confirmed only for autoimmune cutaneous effects such as VLL (Hua et al. 2016; Nakamura et al. 2017). However, the number of patients included in these studies was small, so that no conclusive evidence could be provided. The aim of our study was to investigate whether an association between the onset of VLL during immune checkpoint inhibitor treatment and a better prognosis exists.

Materials and methods

Patients

All patients at IDI-IRCCS, aged ≥ 18 years, who had inoperable or metastatic melanoma, according to the American Joint Committee on Cancer melanoma staging and classification (Gershenwald and Scolyer 2018) and who had undergone ICI therapy (anti-PD-1 or anti-CTLA-4) in any line

of treatment in the last 10 years, from September 2010 to December 2019, were identified and included in the study. The protocol was approved by the IDI-IRCCS Institutional Ethical Committee (n. 510/3, 2018). Demographic and clinical information was retrieved from medical records. Histological type, tumor thickness, ulceration, and regression were recorded, according to published guidelines (Clark et al. 1969, 1989; Breslow 1970; Busam and Barnhill 2004). The variables considered were: age at diagnosis of primary melanoma, gender, site of the primary melanoma, Breslow thickness, presence of ulceration, lymph-node status, presence of regression, presence of satellitosis, age at start of ICI therapy, melanoma staging at the start of treatment, number of sites affected by secondary lesions, serum lactate dehydrogenase (LDH), BRAF mutation, specific ICI drug used, line of treatment, use of adjuvant treatment (i.e., interferon-α, ICI), and type of toxicity developed during ICI therapy. Toxicities were evaluated through clinical examination, laboratory, and radiological assessments, using common toxicity grade criteria (Cancer Institute 2017). Patients affected by vitiligo before initiation of ICI therapy were excluded from the study.

Evaluation

The primary objective was to evaluate the association between VLL onset and the risk of death. Secondary objectives were to evaluate the association between VLL development and time to next treatment (TTNT) and the best overall response in ICI as first-line treatment. TTNT was used as an endpoint, since it reflects not only the duration of treatment efficacy, but it also incorporates the patient tolerance to the therapy. Tumor response was assessed using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (Eisenhauer et al. 2009). The Lazio Region Internet Portal of Health was accessed to obtain official, standardized information on the vital status of all patients. For the overall survival analysis, we calculated the duration of follow-up for each subject as the number of days from the start of the first ICI treatment to the date of death or to 31 December 2019, whichever came first. Time-to-next treatment was calculated as the number of days from the start of the first ICI treatment to the date of the decision to change treatment because of progression, exhaustion of the clinical benefit, and/or toxicity. For the best overall response, each patient was assigned to one of the following categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD).

Statistical analysis

To compare demographic, histological, clinical characteristics, and overall objective response between patients who

have developed VLL to those who have not developed this toxicity, the Fisher's exact test was used for the categorical variables, while the Mann–Whitney U test was used for the continuous variables. The Fisher's exact test was preferred, because for some variables, the expected counts were less than 5, and because of unbalanced data (group with leukoderma $n = 43$, group without leukoderma, $n = 237$) (Corcoran et al. 2005).

For the survival analysis, the Kaplan–Meier method and the log-rank test were used to compare the survival curves between different groups. Time zero corresponds to the start of the first ICI treatment. The Cox proportional-hazard model was used to investigate the association between VLL development and the risk of mortality, and it was also used to examine prognostic factors for TTNT.

For statistical analysis, the STATA software, release 15 (Stata Corp LLC, College Station, TX) was used.

Results

Two hundred and eighty patients were included in the study. The mean age of the patients at primary melanoma diagnosis was 62.9 years (standard deviation, SD = 14.9), with a median age of 65 years (interquartile range, IQR = 54–74). Most patients were males (60.7% versus 39.3%). Median follow-up time from melanoma diagnosis was 3.4 years (IQR = 1.8–6.2) for a total of 1286 person-years of follow-up. There were 144 deaths; 5- and 10-year overall survival were 56.2% and 30.8%, respectively (Table 1).

Regarding the primary melanoma, 77.9% was of skin onset (of which 44.0% of nodular type, 43.1% of superficial diffusion, and 12.8% of other histological type); 9.3% was mucosal; 3.2% was uveal and 4.3% was of unknown origin (occult melanoma). For 5.4%, no data on primary melanoma were available. The most frequent location of the primary melanoma was the trunk (35.7%), followed by lower extremities (21.1%), head and neck (18.6%), and the upper extremities (15.0%). Breslow thickness was 3.9 mm (SD = 3.2), with a median of 3 mm (IQR = 2–5). Ulceration was present in 59.6% of cases, absent in the 29.8%, unknown for a 10.6%. Regression was present only in 4.1% of cases and the presence of satellitosis in the 10.6% of cases. One hundred and two (102, 46.8%) patients had undergone lymphadenectomy, of which 59.8% were positive and 40.2% were negative for lymph-node metastasis (Table 1).

As shown in Table 2, patients at the beginning of treatment with ICI had a mean age of 66.1 years (SD = 14.1), with a median age of 68 years (IQR = 58–77). Out of the 280 patients, 84 (30.0%) were in stage M1, 51 (18.2%) in stage M1b, 97 (34.6%) in stage M1c, and 47 (16.8%) in stage M1d. Most of the patients showed the involvement of two metastatic organ sites (35.5%), followed by

Table 1 Characteristics of primary melanoma

Characteristics	N.	%
Age, years		
Mean (SD)	62.9 (14.9)	
Median (IQR)	65 (54–74)	
Sex		
Male	170	60.7
Female	110	39.3
Localization of primary		
Skin	218	77.9
Nodular	*96*	*44.0*
Superficial spreading	*94*	*43.1*
Other	*28*	*12.8*
Mucosal	26	9.3
Uveal	9	3.2
Occult	12	4.3
Unknown	15	5.4
Anatomic site		
Head/neck	52	18.6
Upper limbs	42	15.0
Trunk	100	35.7
Lower limbs	59	21.1
Unknown	27	9.6
Breslow thickness, mm[a]		
Mean (SD)	3.9 (3.2)	
Median (IQR)	3 (2–5)	
Ulceration[a]		
Present	130	59.6
Absent	65	29.8
Unknown	23	10.6
Regression[a]		
Yes	9	4.1
No	209	95.9
Presence of satellitosis[a]		
Yes	23	10.6
No	195	89.4
Lymphadenectomy[a]		
Performed	102	46.8
Positive	*61*	*59.8*
Negative	*41*	*40.2*
Not performed	116	53.2

SD standard deviation, *IQR* interquartile range

[a]Only for cutaneous melanoma

Italic in tables 1 has been used to better distinguish between sub-categories and categories.

Table 2 Patient characteristics at Immune Checkpoint Inhibitors (ICI) therapy baseline

Characteristics	N.	%
Age, years		
Mean (SD)	66.1 (14.1)	
Median (IQR)	68 (58–77)	
Metastatic stage		
M1a	84	30.0
M1b	51	18.2
M1c	97	34.6
M1d	47	16.8
Unknown	1	0.4
Number of organs involved		
1	97	34.8
2	99	35.5
≥ 3	83	29.7
Serum LDH		
Normal	126	45.0
High[a]	75	26.8
Unknown	79	28.2
BRAF status		
Mutation	83	29.6
V600E	*72*	*86.7*
V600K	*10*	*12.0*
K601E	*1*	*1.2*
No mutation	186	66.4
Unknown	11	3.9

SD standard deviation, *IQR* interquartile range, *LDH* lactate dehydrogenase

[a]> upper limit of normal (ULN)

Italic in tables 2 has been used to better distinguish between sub-categories and categories.

in 66.4% of patients; for 3.9% of patients, these data were not available.

As shown in Table 3, two hundred patients (71.4%) received ICI therapy as a first-line treatment, 76 (27.1%) as a second-line treatment, and only 4 patients (1.4%) as third- or fourth-line treatment. Among the patients who received ICI as a first-line therapy, 103 patients (51.5%) received nivolumab, 47 pembrolizumab (23.5%), and 50 ipilimumab (25.0%). Out of 280 patients, 248 (88.6%) did not receive any adjuvant therapy before ICI treatment. Among the 280 patients studied, 140 (50.0%) developed at least one type of toxicity. The following toxicities were observed: gastrointestinal (19.3%); endocrinological (15.4%); VLL (15.4%); skin toxicity excluding VLL (11.4%); pulmonary (4.3%); skeletal muscle (2.5%); neurological (1.4%); cardiovascular (0.7%); hematological (0.7%); ocular (0.7%) and renal toxicity (0.4%). It is important to note that, among the 43 patients who developed VLL, 25 patients (58.1%) did not

one (34.8%), and by three or more (29.7%). Serum LDH value was normal in 45.0% of patients and high in 26.8%. A BRAF mutation was present in 29.6% of patients (with V600E mutation in 86.7% of cases, V600K in 12.0% of cases, one case with K601E mutation), and was not present

Table 3 Type of therapies and toxicity

	N.	%
ICI line of treatment		
First-line	200	71.4
Second-line	76	27.1
Third- or fourth-line	4	1.4
First-line drug		
Ipilimumab	50	25.0
Pembrolizumab	47	23.5
Nivolumab	103	51.5
Adjuvant therapy		
No	248	88.6
Yes	30	10.7
Unknown	2	0.7
Toxicity		
No	140	50.0
Yes	140	50.0
Type of toxicity		
Leukoderma	43	15.4
Only leukoderma	25	8.9
Gastro-intestinal	54	19.3
Endocrine	43	15.4
Cutaneous (excluding leukoderma)	32	11.4
Pulmonary	12	4.3
Skeletal muscle	7	2.5
Neurological	4	1.4
Cardiovascular	2	0.7
Hematological	2	0.7
Ocular	2	0.7
Renal	1	0.4

ICI immune checkpoint inhibitors

have any other toxicity. The median time of VLL onset after the start of ipilimumab, pembrolizumab, and nivolumab as a first-line treatment was 7 months (IQR = 3–14), 6 months (IQR = 4–8), and 10 months (4–17) respectively. No difference was found between ICI treatment types and onset of VLL ($p = 0.713$). From the start of ICI therapy, the median follow-up time was 9.9 months (ranging from 6 days to 81 months) for a total of 4651 months of follow-up.

A significant survival advantage in patients who developed VLL during ICI treatment compared to those who did not develop such a toxicity was observed (log-rank test, $p < 0.0001$; Fig. 1A). Five-year overall survival for persons who developed VLL was 75.1% versus 24.2% for those who did not develop it. This effect was only observed for VLL and not for other toxicities, regardless of the toxicity grade. A significant poorer survival was found for patients with high LDH compared to those with normal serum LDH levels (log-rank test, $p < 0.0001$; Fig. 1B); with metastatic disease M1c and M1d compared to M1a–M1b (log-rank test, $p < 0.0001$; Fig. 2A); and in patients who did not undergo previous adjuvant treatment compared to those who did (log-rank test, $p = 0.047$; Fig. 2B).

Table 4 shows the results of the multivariate analysis for mortality. After controlling for sex, age, staging, LDH levels, use of adjuvant therapy, and first-line use of ICIs, VLL development was associated with reduced mortality (hazard ratio, HR: 0.32; 95% CI 0.22–0.48, $p < 0.0001$). The protective effect was even greater if the analysis was restricted to patients with cutaneous melanoma who underwent treatment with ICI in first line (HR: 0.24; 95% CI 0.12–0.45, $p < 0.0001$). For patients in which ICI was the first-line therapy and VLL was the only toxicity observed, mortality risk was 6 times lower than in patients with no toxicity (HR: 0.15; 95% CI 0.05–0.43, $p < 0.0001$). High serum LDH levels (HR: 2.33; 95% CI 1.51–3.60, $p < 0.0001$), staging (M1d
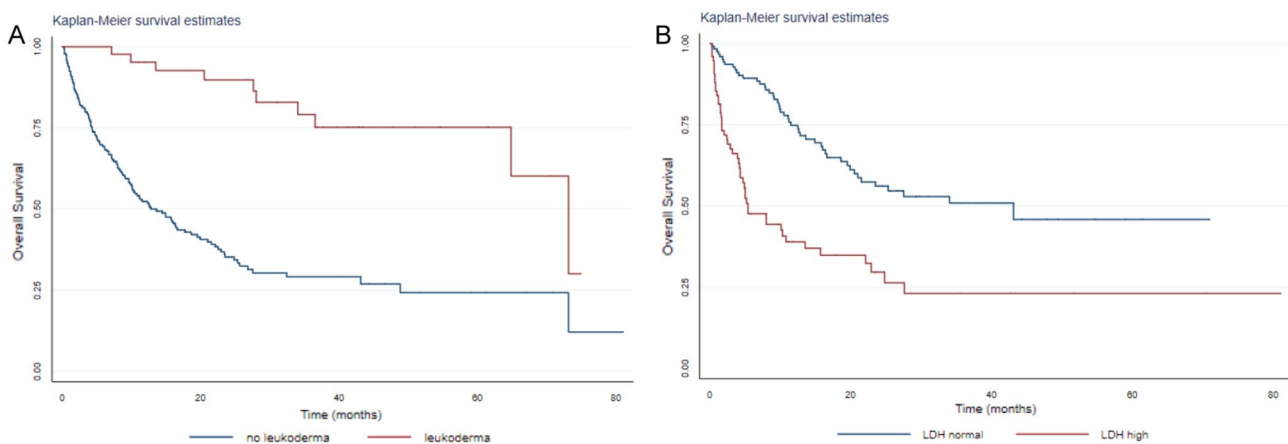

Fig. 1 Overall survival analysis. Kaplan–Meier survival curves for analysis in patients who had developed or not VLL during ICI treatment (**A**), with normal or elevated LDH serum levels (**B**)
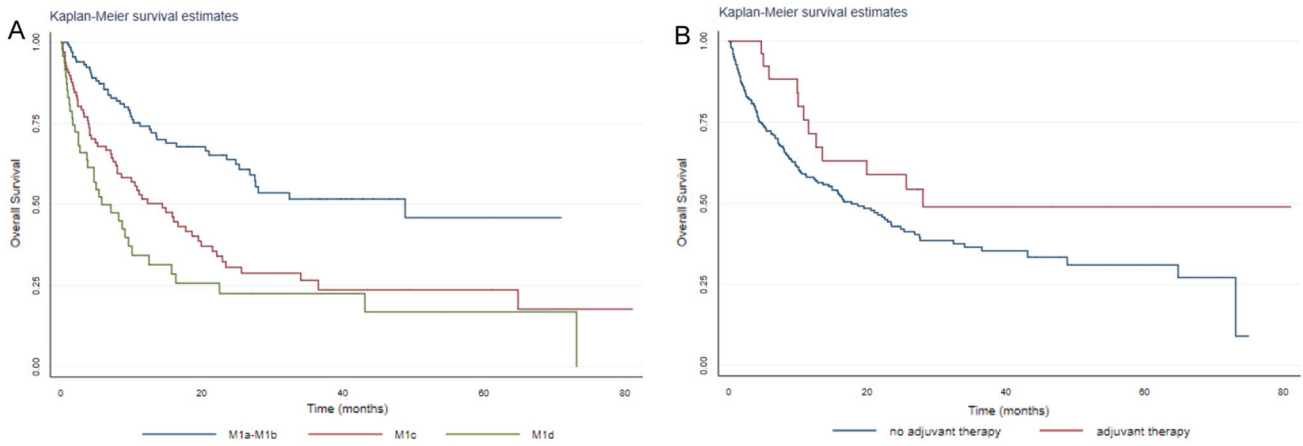
Fig. 2 Overall survival analysis. Kaplan–Meier survival curves for analysis in patients with M1a/b or M1c or M1d metastatic stage disease (**A**), who performed or not adjuvant treatment (**B**)

Table 4 Prognostic factors for mortality: uni- and multi-variate Cox model

	all Melanoma (N=280)				Only cutaneous melanoma (N=218)		Only cutaneous melanoma and first-line ICI (N=153)	
	HRcrude (95% CI)	P	HRadj[a] (95% CI)	P	HRadj[a] (95% CI)	P	HRadj[a] (95% CI)	P
Sex								
Male	1		1		1		1	
Female	0.78 (0.56–1.10)	0.162	0.74 (0.51–1.07)	0.110	0.75 (0.49–1.16)	0.198	0.82 (0.45–1.48)	0.505
Age at start of ICI	1.00 (0.99–1.02)	0.658	1.01 (1.00–1.03)	0.076	1.01 (0.99–1.03)	0.190	1.04 (1.01–1.06)	0.002
Toxicity								
None	1		1		1		1	
Leukoderma and other	0.26 (0.18–0.38)	<0.0001	0.32 (0.22–0.48)	<0.0001	0.28 (0.17–0.45)	<0.0001	0.24 (0.12–0.45)	<0.0001
Only leukoderma	0.23 (0.12–0.44)	<0.0001	0.23 (0.11–0.44)	<0.0001	0.22 (0.11–0.46)	<0.0001	0.15 (0.05–0.43)	<0.0001
Serum LDH								
Normal	1		1		1			
High[a]	2.75 (1.84–4.10)	<0.0001	2.33 (1.51–3.60)	<0.0001	2.76 (1.66–4.58)	<0.0001	2.75 (1.46–5.16)	<0.0001
Metastatic stage								
1a–1b	1		1		1		1	
1c	2.21 (1.51–3.23)	<0.0001	1.44 (0.96–2.16)	0.075	1.52 (0.95–2.43)	0.084	1.92 (1.01–3.64)	0.047
1d	3.22 (2.08–4.99)	<0.0001	2.25 (1.39–3.63)	0.001	1.76 (1.00–3.10)	0.051	2.80 (1.36–5.78)	0.005
Adjuvant therapy								
Yes vs. no	0.55 (0.31–1.00)	0.051	0.54 (0.29–0.99)	0.047	0.47 (0.25–0.91)	0.024	1.22 (0.57–2.61)	0.605
ICI line of treatment								
First line	1		1		1		-	
Second line and beyond	1.94 (1.39–2.72)	<0.0001	1.47 (1.02–2.13)	0.040	1.53 (1.00–2.33)	0.047	-	

HR hazard ratio, *adj* adjusted, *CI* confidence intervals, *LDH* lactate dehydrogenase, *ICI* immune checkpoint inhibitors

[a]HR adjusted for all items in table

[b]> Upper limit of normal (ULN)

versus M1a-1b) (HR: 2.25; 95% CI 1.39–3.63, $p=0.001$), and use of ICIs not as a first-line treatment (HR: 1.47; 95% CI 1.02–2.13, $p=0.040$), were all independently associated with an increased risk of mortality in the multivariate model, while the use of adjuvant therapy was associated with a protective effect (HR: 0.54; 95% CI 0.29–0.99, $p=0.047$).

A sub-group analysis was conducted only for patients with ICIs as a first-line therapy ($N=153$). The median TTNT was 4.9 months. The occurrence of VLL was also an independent predictor factor for duration of clinical benefits measured by TTNT (only VLL), HR: 0.17; 95% CI 0.06–0.44, $p<0.0001$; VLL and other toxicities HR: 0.35; 95% CI 0.21–0.59, $p<0.0001$. Both high LDH serum levels and advance staging were associated with shorter TTNT (Table 5).

All other factors considered in the study, such as Breslow thickness, ulceration, site of primary melanoma, presence of regression, presence of satellitosis, lymphadenectomy, BRAF status, and types of ICI used in first line (ipilimumab, pembrolizumab, or nivolumab), were not associated with mortality.

Demographic and clinical characteristics of patients who developed VLL and of the ones who do not developed it were examined (Table 6). No difference between groups were found for age, types of melanoma, anatomic site of the primary melanoma, BRAF status, use of adjuvant therapy, and type of ICI. However, statistically significant differences were observed for sex ($p=0.043$) and LDH levels

($p=0.021$). Most patients who developed VLL were females (53.5% versus 36.7%) and had normal serum LDH levels (80.0% versus 59.0%). For patients with ICI as first-line treatment, the best objective response was also analyzed. The rates of clinical complete responses (CR) (35.3% versus 9.1%) and partial responses (PR) (38.2% versus 28.0%) were higher in patients who developed VLL than in patients who did not have such a toxicity, while progressive disease (PD) was significantly lower in patients with VLL (5.9% versus 40.9%, $p<0.0001$).

Discussion

In recent years, treatment of metastatic melanoma has undergone a sudden and encouraging evolution. Search for effective therapies to improve the prognosis of patients with inoperable melanoma has led to the development of innovative drugs, including CTLA-4 and PD-1/PD-ligand-1 (PD-L1) inhibitors, used alone or in combination. Despite these reassuring results, many patients have primary resistance to such treatments, obtaining limited benefit from these therapeutic strategies. Understanding clinical aspects related to treatment response would allow the selection of patients who would truly benefit from these therapies. The possible link between the onset of adverse events and the response to ICI treatment is one of the subjects of greatest interest in

Table 5 Prognostic factors for time to the next treatment (TTNT): uni- and multi-variate Cox model

	Only cutaneous melanoma and first-line ICI (N = 153)			
	HRcrude (95% CI)	P	HRadj[a] (95% CI)	P
Sex				
Male	1		1	
Female	0.96 (0.61–1.49)	0.846	1.02 (0.63–1.66)	0.934
Age at start of ICI	1.00 (0.99–1.02)	0.564	1.01 (0.99–1.02)	0.513
Toxicity				
None	1		1	
Leukoderma and other	0.32 (0.20–0.51)	<0.0001	0.35 (0.21–0.59)	<0.0001
Only leukoderma	0.19 (0.07–0.48)	<0.0001	0.17 (0.06–0.44)	<0.0001
Serum LDH				
Normal	1		1	
High[b]	1.95 (1.19–3.20)	0.008	2.06 (1.19–3.59)	0.010
Metastatic stage				
M1a-1b	1		1	
M1c	1.93 (1.15–3.24)	0.013	1.53 (0.88–2.65)	0.130
M1d	2.84 (1.66–4.88)	<0.0001	2.08 (1.14–3.79)	0.017
Adjuvant therapy				
Yes vs. no	1.20 (0.65–2.22)	0.553	1.35 (0.70–2.60)	0.364

HR hazard ratio, *adj* adjusted, *CI* confidence intervals, *LDH* lactate dehydrogenase

[a]HR adjusted for all items in table

[b]> Upper limit of normal (ULN)

Table 6 Demographic and clinical characteristics of the patients and onset of leukoderma

Characteristics	Leukoderma (N=43)		No leukoderma (N=237)		
	N.*	%	N.*	%	P^a
Age at start of ICI					
Mean (SD)	66.2 (12.7)		66.1 (14.4)		0.831[b]
Sex					
Male	20	46.5	150	63.3	
Female	23	53.5	87	36.7	0.043
Localization of primary					
Cutaneous	35	83.3	183	82.1	
Mucosal	4	9.5	22	9.9	
Uveal	0	–	9	4.0	
Occult	3	7.1	9	4.0	0.537
Anatomic site					
Head/neck	5	13.2	47	21.9	
Upper limbs	3	7.9	39	18.1	
Trunk	16	42.1	84	39.1	
Lower limbs	14	36.8	45	20.9	0.089
Metastatic stage					
M1a-1b	28	65.1	107	45.3	
M1c	10	23.3	87	36.9	
M1d	5	11.6	42	17.8	0.071
Serum LDH					
Normal	28	80.0	98	59.0	
High[a]	7	20.0	68	41.0	0.021
BRAF status					
Mutation	29	69.0	157	69.2	
No mutation	13	31.0	70	30.8	1.00
Adjuvant therapy					
No	35	81.4	213	90.6	
Yes	8	18.6	22	9.4	0.104
First-line drug					
Ipilimumab	7	20.6	43	25.9	
Pembrolizumab	9	26.5	38	22.9	
Nivolumab	18	52.9	85	51.2	0.801
Best objective response[d]					
Complete response	12	35.3	15	9.1	
Partial response	13	38.2	46	28.0	
Stable disease	7	20.6	36	22.0	
Progressive disease	2	5.9	67	40.9	<0.0001

[a]Fisher's exact test

[b]Mann–Whitney U test

[c] > upper limit of normal (ULN)

[d]To first-line therapy

oncology, due to the hypothesis that greater toxicity would also indicate greater anti-neoplastic effect.

One of the side-effect signs that appears to be related to a better response to ICI treatment in cutaneous melanoma is VLL development. Indeed, some studies suggest the existence of a link between development of VLL and a better outcome in patients treated with ICI (Hua et al. 2016; Nakamura et al. 2017). However, these studies were conducted in a small number of patients and no multi-variate analysis was performed to control for other known prognostic factors of progression (e.g., LDH and metastatic stage). Thus, no firm conclusions could be drawn (Hua et al. 2016; Nakamura et al. 2017).

To further verify the existence of this association, we performed a retrospective analysis on 280 metastatic melanoma patients who had undergone at least one ICI treatment. Our findings confirm, after controlling for confounding factors, a significant association between VLL development and a better prognosis in terms of overall survival and TTNT that agrees with other studies published elsewhere (Hua et al. 2016; Nakamura et al. 2017). The effect was even stronger for patients in which no other toxicity, other than VLL. The latter finding could be related to a probable greater use of high-dose corticosteroids in patients who developed other autoimmune toxicities with consequent partial anti-inflammatory and immunosuppressive effects, as well as to the higher probability of discontinuing treatment in the presence of other toxicities. Indeed, VLL development itself is devoid of clinical significance, except for its psychological impact that could alter patient quality of life.

In our study, 43 out of 280 patients (15.4%), developed VLL which is lower in comparison with other studies (Hua et al. 2016; Nakamura et al. 2017). In our study, the median time of VLL onset after the start of ipilimumab, pembrolizumab, and nivolumab as a first-line treatment was 7 months (IQR = 3–14), 6 months (IQR = 4–8), and 10 months (4–17), respectively, which is in agreement with a previous study (Babai et al. 2020).

Our data show that complete objective response was reached by 35.3% of the patients who developed VLL, while in the study by Nakamura et al. (2017), conducted on 35 unresectable stage III or IV melanoma patients treated with nivolumab, 9 patients (25.7%) developed VLL and objective response was 22.2% among patients with VLL. Hua et al. (2016) conducted a study on 67 unresectable stage III or IV melanoma patients treated with pembrolizumab. During the treatment with pembrolizumab, VLL. appeared in 17 (25%) patients and complete objective response was 18% among patients who developed VLL. The median overall survival was 18.2 months in the group of patients that developed

VLL compared to 8.5 months in the group of non-VLL. In our study, median overall survival in the group of patients that developed VLL. was 34.0 months vs 8 months in the group of non-VLL.

It is interesting to note that VLL, or at least nevus lightening, was reported in melanoma patients treated with BRAF/MEK inhibitors (Zhao et al. 2018). Since a role for BRAF/MEK inhibition in the activation of the immune system was hypothesized (Smalley 2020), there is the possibility that a higher infiltration of activated T cells in the melanoma lesion would lead, in genetically predisposed patients treated with this target therapy, to the development of VLL. In either case, VLL development could be considered as a sign of active immune responses in the treated patients. Nevertheless, the number of reported cases is too small to associate VLL development with a positive response to treatment with BRAF/MEK inhibitors or with a better overall survival.

LDH within normal limits, M1a and/or M1b disease, and a previous adjuvant treatment, remained independent factors associated with a better prognosis in terms of survival, in line with the literature data (Keung et al. 2020). The analysis of patients' clinical characteristics showed an association with gender, disease burden, metastatic sites, and the likelihood of VLL onset. Patients of female sex, with metastatic disease involving only skin, lymph nodes or lungs, and/or with serum LDH within the normal limits, were more likely to develop VLL. This evidence could be explained by the fact that a greater aggressiveness of tumor disease, and the presence of visceral and/or encephalic metastases, could underlie a lower ability to develop an effective immune reaction. It is known that some metastatic niches, such as those in the liver and brain, have a greater ability to evade the immune response due to a more immunosuppressive environment (Tumeh et al. 2017; Di Giacomo et al. 2019). Regarding gender, female sex is known to be associated with the ability to develop a more efficient immune response (Klein and Flanagan 2016). A higher probability of longer progression-free survival in female patients was also reported in a recent multicenter study that analyzed clinical and biological features of melanoma patients who developed VLL after ICI treatment (Guida et al. 2021). Differently from our present study, the recent paper from Guida et al. (2021) did not match the VLL patient subset with one without VLL and could not obtain conclusive data about significance of VLL development in the entire population.

It is important to note that this is the largest observational study conducted so far that shows an independent association between VLL development and better prognosis measured by objective overall survival, overall response, and TTNT.

In conclusion, our study confirms, on a large patient cohort of advanced cutaneous melanoma, that VLL development as an ICI toxicity is an independent marker of a more favorable prognosis. Therefore, VLL onset should be considered as a sign of positive response to ICI treatment. Future studies of the biological mechanisms and of the actual causal chain leading to VLL development would clarify the different degrees of response to ICI treatment, possibly leading to an optimization of the therapeutic strategies.

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Availability of data and materials All relevant data are within the papers.

Declarations

Conflicts of interest No competing interests.

Ethics approval and consent to participate The protocol was approved by the IDI-IRCCS Institutional Ethical Committee (n. 510/3, 2018).

Consent for publication Not applicable.

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