Association of Vitiligo With Tumor Response in Patients With Metastatic Melanoma Treated With Pembrolizumab

Camille Hua, MD; Lise Boussemart, MD, PhD; Christine Mateus, MD; Emilie Routier, MD; Céline Boutros, MD; Hugo Cazenave, MD; Roxane Viollet, MD; Marina Thomas, MD; Séverine Roy, CRA; Naima Benannoune, CRA; Gorana Tomasic, MD; Jean-Charles Soria, MD, PhD; Stéphane Champiat, MD; Matthieu Texier, MSc; Emilie Lanoy, PhD; Caroline Robert, MD, PhD

IMPORTANCE Vitiligo is an autoimmune skin disorder that reacts against melanocytes. The association of vitiligo with tumor response in patients with melanoma who undergo immunotherapy has been reported but is still controversial.

OBJECTIVE To prospectively evaluate the appearance of vitiligo in patients receiving pembrolizumab, a monoclonal antibody directed against the programmed death cell receptor.

DESIGN, SETTING, AND PARTICIPANTS This prospective observational study was conducted from January 1, 2012, through September 24, 2013, in a single tertiary care hospital with a unit dedicated to patients with melanoma. Sixty-seven patients with metastatic melanoma who received pembrolizumab treatment in the context of a phase 1 study were included and screened for the emergence of vitiligo. Data were collected from January 1, 2012, to February 28, 2014, and analyzed from February through December 2014.

MAIN OUTCOMES AND MEASURES Objective tumor response with regard to the occurrence of vitiligo in patients receiving pembrolizumab therapy. Correlation between vitiligo occurrence and overall survival was also estimated using the Kaplan-Meier product-limit method and compared with a log-rank test. To prevent guarantee- or lead-time bias, a landmark analysis approach after 12, 16, and 20 weeks of treatment was retained.

RESULTS Of the 67 patients included in the study, 17 (25%) developed vitiligo during pembrolizumab treatment and 50 (75%) did not. An objective (complete or partial) response to treatment was associated with a higher occurrence of vitiligo (12 of 17 [71%] vs 14 of 50 [28%]; \( P = .002 \)). The time to onset of vitiligo ranged from 52 to 453 (median, 126) days from the start of treatment. Of the 17 patients with vitiligo, 3 (18%) had a complete response, 9 (53%) had a partial response, 3 (18%) had stable disease, and 2 (12%) had progressive disease at the final follow-up. All the patients treated with pembrolizumab who developed vitiligo were alive at the time of analysis, with a median follow-up of 441 days.

CONCLUSIONS AND RELEVANCE Vitiligo, a clinically visible immune-related adverse event could be associated with clinical benefit in the context of pembrolizumab treatment.
Vitiligo is an autoimmune skin disorder characterized by hypopigmented skin lesions and originating from the loss of functional melanocytes from the epidermis. Several authors have described the occurrence of vitiligo in patients with melanoma during the past 40 years. The onset of vitiligo spontaneously or during treatment in patients with melanoma has been reported, and its incidence varies widely, with ranges from 2.8% to 4.1% and from 23% to 43%, which is much more frequent than in the normal population. Estimated risk for the development of vitiligo among patients with melanoma is thus as much as 10-fold higher than in the general population. The relationship between vitiligo and melanoma could result from an immune-mediated destruction through recognition of melanoma-associated antigens shared by normal melanocytes and melanoma cells and may reflect an immune response to melanoma.

A few previous studies have suggested that vitiligo could be associated with an improved prognosis for patients with melanoma. A more recent retrospective analysis performed on a large cohort included 2954 patients with melanoma and reported similar findings. In that study, vitiligo was present in 2.8% of patients with melanoma and was found to be an independent positive prognostic factor associated with overall survival in patients with stages III and IV melanoma and with distant metastasis-free survival in patients with stage III melanoma. Although their sample size was small and the trials were often underpowered to detect significant differences, several clinical trials evaluating immunotherapies in advanced metastatic melanoma also found a correlation between vitiligo and an improved clinical response, suggesting an immunomodulating effect against metastatic disease.

Pembrolizumab is a humanized IgG4 monoclonal antibody directed against the programmed death cell receptor 1 (PD-1), an inhibitory receptor expressed by T cells. As a consequence of the engagement between PD-1 and its main ligand, PD-L1, expressed on several types of cancer cells, infiltrating T cells are inhibited in their proliferating and antitumor cytolytic functions. Because pembrolizumab is a potent and selective inhibitor of PD-1, it was evaluated as an antitumor immunotherapy with the goal of reactivating an effective antitumor response. In patients with metastatic melanoma, pembrolizumab already has shown extremely promising clinical results, leading to an accelerated development and authorization. Pembrolizumab is now available in an expanded access program in Europe and was approved by the US Food and Drug Administration on September 4, 2014.

Treatment with pembrolizumab is well tolerated overall, with grade 3 or 4 adverse events in about 12% of the patients. The most frequent adverse events reported have been fatigue, diarrhea, pruritus, macular papular eruption, and arthralgia. The relationship between vitiligo and response to the anti–PD-1 antibody has, to our knowledge, never been reported. Vitiligo has been reported in 11% of the patients treated with pembrolizumab, but this adverse event could be underreported because a total body skin examination is not always performed in patients treated for melanoma. To evaluate the incidence of vitiligo in patients treated with pembrolizumab and to study the potential association between vitiligo and response to therapy, we conducted a prospective dermatology study in a population of patients treated with pembrolizumab for metastatic melanoma.

Methods

This prospective observational study systematically included patients treated with pembrolizumab in the context of the phase I study conducted at the Cancer Campus of Gustave Roussy Institute from January 1, 2012, to September 24, 2013. Final follow-up was completed on September 24, 2013, and data were collected from January 1, 2012, through February 28, 2014. The study was approved by the institutional review board of Gustave Roussy Institute and a national human subjects committee. Patients provided written informed consent, and patient data were deidentified.

Patients

Patients were eligible for inclusion if they had a confirmed diagnosis of unresectable stage III or IV melanoma according to the 2009 American Joint Committee on Cancer melanoma staging and classification. had received at least 3 infusions of pembrolizumab, and had undergone at least 1 radiologic evaluation 12 weeks after starting treatment. Vitiligo present before the initiation of pembrolizumab treatment was cause for exclusion.

Treatment

Patients were treated according to the phase 1 protocol consisting of pembrolizumab administered intravenously every 2 or 3 weeks at a dose ranging from 2 to 10 mg/kg of body weight. The treatment was continued until confirmation of disease progression or grade 3 or 4 toxic effects.

Evaluation

Patients underwent a complete dermatologic examination at each visit (every 2 or 3 weeks). Skin changes occurring during therapy were photographed and underwent biopsy for pathologic examination in willing patients. Skin reactions were graded according to the National Cancer Institute's common toxicity criteria, version 4.02 (2009; http://ctep.cancer.gov/protocolDevelopment/EVSMCLink/ctc.htm). Date of onset, detailed clinical features, and evolution of vitiligo were reported. Tumor responses to pembrolizumab were evaluated by computed tomography using Response Evaluation Criteria in Solid Tumours (RECIST), version 1.1, and defined as follows: disappearance of all measurable disease (complete response), at least a 30% decrease in the sum of target lesions (partial response), at least a 20% increase in the sum of target lesions or appearance of 1 or more new lesions (progressive disease), and insufficient shrinkage to qualify for a partial response and insufficient increase to qualify for progressive disease (stable disease).

Vitiligo Definition and Assessment

Vitiligo, defined as the appearance of hypopigmented skin areas, was classified as localized or generalized according to
the distribution of the lesions.5,18 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 perimeta-

Vitiligo and Melanoma Response

Statistical Analysis
Data analysis was performed from February through December 2014. Associations between the presence of vitiligo (yes vs no) and response (yes vs no) or the occurrence of an immune-related adverse event (IRAE) (yes vs no) were systematically assessed using the Fisher exact test. Because vitiligo is a time-dependent factor that could induce lead-time bias, we retained a landmark analysis approach to assess the effect of the occurrence of vitiligo during anti–PD-1 therapy on overall survival. We considered time points at 12, 16, and 20 weeks after treatment initiation. Only patients alive at each of these 3 points were included in the respective analysis and were divided into groups that presented or did not present with vitiligo before the considered time point. Overall survival, defined as times from 12, 16, and 20 weeks after diagnosis to death from any cause, was estimated using the Kaplan-Meier product-limit method in each stratum and was defined by the occurrence of vitiligo from treatment initiation to 12, 16, and 20 weeks after diagnosis. Strata were compared using the log-rank test. All analyses were performed using SAS software (version 9.3; SAS Institute Inc).

Results
Patient Characteristics
From January 1, 2012, through September 30, 2013, 74 patients were treated with pembrolizumab in the context of the phase 1 study. Seven patients were excluded from our analysis. Five of them received only 1 infusion of pembrolizumab owing to rapid disease progression (3 patients) or severe toxic effects (2 patients). The other 2 patients were excluded because they had vitiligo before receiving the first pembrolizumab infusion. Of the 67 enrolled patients, 29 (43%) were women and 38 (57%) were men. Their ages ranged from 20 to 74 years, with a mean age of 54 years. Three patients (4%) had stage III melanoma and 64 patients (96%) had stage IV melanoma (eTable in the Supplement). Vitiligo occurred in 17 of the 67 patients with melanoma for a cumulative incidence of 25% (Table 1 and Figure 1). The mean age at vitiligo occurrence was 57 (range, 28-72) years, with a male prevalence. Of these 17 patients, 6 (35%) had been treated previously with immunotherapy; 9 (53%), with cytotoxic chemotherapy; and 1 (6%), with targeted agents (eTable in the Supplement). Baseline characteristics were not statistically different, except that fewer patients received targeted agents in the group with vitiligo (1 of 17 [6%] vs 20 of 50 [40%]; P = .01) and the time since the first diagnosis was shorter in patients with vitiligo (25.0 vs 47.5 months; P = .04) (eTable in the Supplement). We found no relationship between vitiligo occurrence and previous immunotherapy (eTable in the Supplement; Fisher exact test, P = .55). No association between pembrolizumab dose and vitiligo occurrence was found. The time to onset of vitiligo ranged from 52 to 453 (median, 126) days from the start of pembrolizumab treatment. The median number of infusions of pembrolizumab before the onset of vitiligo was 7 (range, 4-31). Of the 17 patients, vitiligo was localized in 2 (12%), generalized in 14 (82%), and mixed in 1 (6%) (Table 1). Hypomelanotic lesions developed around cutaneous metastases in 3 patients. Two patients had grade 2 vitiligo with hypopigmentation covering more than 10% of their body surface area and reported a psychosocial impact of the skin lesions. One of them developed universal vitiligo after 6 pembrolizumab infusions with a rapid extension of the skin lesions, which preceded a complete tumor response, and the other had stable disease at the final follow-up. Of the 2 patients with localized vitiligo, one had progressive disease and one had a partial response at the final follow-up.

Tumor Responses
The median time from the initiation of pembrolizumab treatment to the last dose of pembrolizumab was 168 (range, 41-702) days. Among the 17 patients who developed vitiligo during the treatment, 12 (71%) had an objective response. Thus, we observed a complete response for 3 patients (18%) and a partial response for 9 patients (53%). Three patients (18%) who developed vitiligo had stable disease, and 2 patients (12%) had progressive disease at the final follow-up. Of the 50 patients who did not develop vitiligo, 14 (28%) had an objective (complete or partial) tumor response, 1 (2%) had stable disease, and 35 (70%) had progressive disease (Table 2). The difference

Table 1. Clinical Features of Melanoma-Associated Vitiligo

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Data*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitiligo incidence</td>
<td>17/67 (25)</td>
</tr>
<tr>
<td>Time to onset of vitiligo, median (range), d</td>
<td>126 (52-453)</td>
</tr>
<tr>
<td>No. of injections before onset of vitiligo, median (range)</td>
<td>7 (4-31)</td>
</tr>
<tr>
<td>Onset of inflammatory skin lesions before vitiligo</td>
<td>10/17 (59)</td>
</tr>
<tr>
<td>Vitiligo lesion classification</td>
<td></td>
</tr>
<tr>
<td>Generalized</td>
<td>14/17 (82)</td>
</tr>
<tr>
<td>Acrofacial</td>
<td>5/17 (29)</td>
</tr>
<tr>
<td>Vulgaris</td>
<td>8/17 (47)</td>
</tr>
<tr>
<td>Universal</td>
<td>1/17 (6)</td>
</tr>
<tr>
<td>Localized</td>
<td>2/17 (12)</td>
</tr>
<tr>
<td>Focal</td>
<td>0</td>
</tr>
<tr>
<td>Segmental</td>
<td>0</td>
</tr>
<tr>
<td>Around cutaneous metastases or halo nevi</td>
<td>2/17 (12)</td>
</tr>
<tr>
<td>Mixed (acrofacial and perimetastatic)</td>
<td>1/17 (6)</td>
</tr>
</tbody>
</table>

* Unless otherwise indicated, data are expressed as number/total number (percentage) of patients.
between the vitiligo and nonvitiligo groups in objective response (71% vs 28%) was significant (Fisher exact test, \(P = .002\)). Of the 2 patients excluded from the analysis because they had preexisting vitiligo, one had stable disease and one had progressive disease at the final follow-up.

**Effect of Vitiligo During Therapy on Overall Survival**

All 17 patients treated with pembrolizumab who developed vitiligo are still alive, with a median follow-up of 441 days. Figure 2 shows that no death was observed among the patients who developed vitiligo after 12, 16, and 20 weeks of treatment, whereas the 1-year conditional survival rates were 70% (95% CI, 55%-81%), 75% (95% CI, 58%-86%), and 81% (95% CI, 61%-91%), respectively, among patients without vitiligo. However, overall survival of patients presenting with vitiligo during the first 12, 16, and 20 weeks was not significantly improved compared with patients free of vitiligo (\(P = .35\), \(P = .17\), and \(P = .17\), respectively).

**Association of Vitiligo With IRAEs**

Overall, 35 patients (52%) had at least 1 IRAE, including the 17 patients with vitiligo. The most common IRAEs were skin related (34 patients [51%]), endocrine dysfunctions (11 patients [16%]), and arthralgia (11 patients [16%]) (Table 3). A significantly higher percentage of IRAEs was found in patients with vitiligo (13 of 17 patients [76%]), especially for dermatologic events (12 patients [71%]), compared with patients without vitiligo (22 of 50 [44%]) (Fisher exact test, \(P = .01\)) (Table 3). Seven patients with vitiligo (41%) had a cutaneous reaction compared with 11 patients without vitiligo (22%). Three of 67 patients (5%) had grade 3 or 4 IRAEs that led to discontinuation of treatment. All grades 3 and 4 IRAEs occurred

**Table 2. Response to Treatment at Final Follow-up**

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Response, No. (%) of Patients</th>
<th>Disease, No. (%) of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete</td>
<td>Partial</td>
</tr>
<tr>
<td>Vitiligo (n = 17)</td>
<td>3 (18)</td>
<td>9 (53)</td>
</tr>
<tr>
<td>No vitiligo (n = 50)</td>
<td>4 (8)</td>
<td>10 (20)</td>
</tr>
<tr>
<td>All (N = 67)</td>
<td>7 (10)</td>
<td>19 (28)</td>
</tr>
</tbody>
</table>

\(a\) The difference between the vitiligo and nonvitiligo groups in objective (complete and partial) response (12 of 17 [71%] vs 14 of 50 [28%]) was significant (Fisher exact test, \(P = .002\)).
in patients with melanoma who developed vitiligo during treatment. Colitis and autoimmune hemolytic anemia resolved with high-dose corticosteroid treatment. In 10 patients with vitiligo (59%), lesions were preceded by erythematous inflammatory lesions on the same body areas (Table 1).

Discussion

The results of this prospective cohort study suggest that the onset of vitiligo in patients treated with pembrolizumab for metastatic melanoma could be a clinically visible IRAE associated with clinical benefit. Indeed, vitiligo did not develop in any of the 22 patients who died at final follow-up, and 12 of the 17 patients who developed vitiligo (71%) had an objective tumor response (complete or partial) vs 14 of 50 patients (28%) who did not develop vitiligo ($P = .002$).

The conclusion that the occurrence of vitiligo might be an early marker of tumor response is difficult to make because the median time to the onset of vitiligo was 126 days and the first radiologic evaluation occurred earlier, after 84 days. However, for 5 patients (including the patient who presented with universal vitiligo), vitiligo clearly preceded the partial response that was observed at the first radiologic evaluation.

Vitiligo that occurs during pembrolizumab treatment represents a time-dependent factor. When we evaluate the association between the occurrence of vitiligo and survival after starting pembrolizumab administration, we must consider a selection bias: patients who presented with vitiligo received treatment long enough and lived long enough to present with this event. This selection bias of patients with vitiligo who live longer than patients without vitiligo is termed lead-time or guarantee-time bias.\textsuperscript{19,20} To control for this bias, a landmark analysis approach was considered. The effect of vitiligo occurring before the 3 chosen time points (12, 16, and 20 weeks) was estimated; the factor evaluated at the time point was no longer time dependent and the selection bias was avoided. However, for a given time point, patients who died before that point are excluded from analysis. Therefore, the sample size for each time point is reduced, which leads to a decrease in power. Thus, after correction for lead-time bias, associations between the appearance of vitiligo and overall survival did not reach statistical significance.

Vitiligo is known to be associated often with other autoimmune diseases, such as autoimmune thyroiditis, type 1 diabetes mellitus, and organ-specific autoantibodies.\textsuperscript{21,22} In our study, we found that patients with vitiligo were characterized by a higher frequency and severity of IRAEs than those without vitiligo. These results are supported by a report from Quaglino et al,\textsuperscript{5} who found that patients with melanoma-associated vitiligo have more immune-mediated diseases. Association between IRAEs and the response to anti–cytotoxic T-lymphocyte antigen 4 therapy has been demonstrated\textsuperscript{20} but not with anti–PD-1 therapy, and our prospective design that focuses mainly on skin reactions does not allow us to study this potential association.

Our study found a 25% incidence of vitiligo in patients treated with pembrolizumab. This ratio is difficult to compare with those of previous studies performed in patients with melanoma because patients with preexisting vitiligo were not always excluded and prospective studies are rare ($n = 7$).\textsuperscript{5,6,8,23-26} This incidence of one-quarter of the patients is much higher than what has been reported in the first safety and efficacy report of pembrolizumab ($9\%-11\%$).\textsuperscript{13} This difference probably results from an underestimation of the frequency of vitiligo in clinical trial safety reports because pa-
We found that vitiligo occurring during pembrolizumab treatment of patients with melanoma was more often symmetrically and bilaterally distributed (14 of 17 patients [82%]) than localized. These results are concordant with those reported by Hartmann et al, who showed that 75% of melanoma-associated vitiligo had a rather generalized distribution.

Development of melanoma-associated vitiligo after immunotherapy has already been correlated with improved survival, as has the appearance of other autoimmune manifestations. Richards et al described first a positive correlation (P < .005) of vitiligo with tumor regression in 47% of 36 patients treated with sequences of chemotherapy (carmustine, dacarbazine, and cisplatin) and immunotherapy (interleukin 2 after the induction of chemotherapy. The median overall survival was 18.2 months in the group of patients who developed vitiligo compared with 8.5 months for the nonvitiligo group. However, when time to occurrence of vitiligo was taken into account as a time-dependent covariate, vitiligo was not a significant predictor of survival (hazard ratio, 0.55; P = .09). Gogas et al reported an incidence of vitiligo of 43% in 49 patients with metastatic melanoma who were treated with injections of interleukin 2 after the induction of chemotherapy. The median overall survival was 18.2 months in the group of patients who developed vitiligo compared with 8.5 months for the nonvitiligo group. However, when time to occurrence of vitiligo was taken into account as a time-dependent covariate, vitiligo was not a significant predictor of survival (hazard ratio, 0.55; P = .09).

Conclusions

Our study is the first, to our knowledge, to suggest an association between vitiligo and responses to anti–PD-1 therapy. The cause of melanoma-associated vitiligo is not well known, but the infiltration of the same clone of CD8 T cells in the tumor and in vitiligo lesions and the circulation of antibodies against melanoma-associated antigens shared by melanoma cells and normal melanocytes (MART-1, gp100, and tyrosinase-related proteins 1 and 2), each implicated in melanin synthesis, suggest an autoimmune mechanism. Thus, the overexpression of melanocytic antigens in tumor cells and the release of these antigens by therapy could explain the breakdown of immune tolerance to these self-antigens. In pembrolizumab treatment, PD-1 antibodies activate the immune response by preventing an inhibitory signal and probably induce tumor responses against antigens shared by melanomas and normal melanocytes. This hypothesis is supported by the fact that vitiligo has not been reported in patients with cancers distinct from melanoma, such as renal cell cancer. However, further prospective dermatologic examination of patients with other cancers treated with anti–PD-1 will be needed to validate this hypothesis.

### Table 3. Immune-Related Adverse Events

<table>
<thead>
<tr>
<th>Event</th>
<th>No. (%) of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal tract</td>
<td>All (N = 67)</td>
</tr>
<tr>
<td></td>
<td>With Vitiligo (n = 17)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6 (9)</td>
</tr>
<tr>
<td>Colitis</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Endocrine system</td>
<td>11 (16)</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>10 (15)</td>
</tr>
<tr>
<td>Type 1 diabetes mellitus</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Dermatologic</td>
<td>34 (51)</td>
</tr>
<tr>
<td>Dermatologicb</td>
<td>16 (24)</td>
</tr>
<tr>
<td>Eczematiform, lichenoid, or psoriasiform skin irritation</td>
<td>18 (27)</td>
</tr>
<tr>
<td>Other</td>
<td>35 (52)</td>
</tr>
</tbody>
</table>

*Graded according to the National Cancer Institute’s common toxicity criteria, version 4.02 (2009; http://evs.nci.nih.gov/ftp1/CTCAE/About.html).

Indicates the number of patients with at least 1 dermatologic adverse event.

Patients could have more than 1 immune-related adverse event.
Vitiligo and Melanoma Response

Original Investigation Research

Institut National de la Santé et de la Recherche Médicale U 981, Cancer Campus, Gustave Roussy Institute, Grand Paris, France (Champiat, Robert); Biostatistics and Epidemiology Unit, Gustave-Roussy Institute, Villejuif, France (Texier, Lanoy). Centre de recherche en épidémiologie et Santé des populations, Institut National de la Santé et de la recherche médicale, Université Paris-Sud, Université de Versailles Saint Quentin en Yvelines, Université Paris-Saclay, Villejuif, France (Texier, Lanoy).

Author Contributions: Drs Hua and Robert had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Hua, Boussermain, Roy, Benannoune, Soria, Champiat, Texier, Lanoy, Robert. Acquisition, analysis, or interpretation of data: Hua, Boussermain, Mateus, Routier, Boutrous, Cazenave, Viollet, Thomas, Benannoune, Tomasic, Champiat, Texier, Lanoy, Robert. Drafting of the manuscript: Hua, Boussermain, Roy, Benannoune, Tomasic. Critical revision of the manuscript for important intellectual content: Hua, Mateus, Routier, Boutrous, Cazenave, Viollet, Thomas, Soria, Champiat, Texier, Lanoy, Robert. Administrative, technical, or material support: Boussemart, Mateus, Routier, Boutros, Cazenave, Viollet, Thomas, Soria, Champiat, Texier, Lanoy, Robert. Study supervision: Viollet, Thomas, Lanoy, Robert.

Conflict of Interest Disclosures: None reported. Additional Contributions: We thank the patient for granting permission to publish this information.

REFERENCES


Copyright 2016 American Medical Association. All rights reserved.