ORIGINAL ARTICLE

Vitiligo-like lesions occurring in patients receiving anti-programmed cell death−1 therapies are clinically and biologically distinct from vitiligo

Maiana Larsabal, MD,[a] Aurélie Marti, MD,[a] Clément Jacquemin, PhD,[b] Jérôme Rambert, PhD,[b] Denis Thiolat, BS,[b] Léa Dousset, MD,[a] Alain Taieb, MD, PhD,[a,b] Caroline Dutriaux, PhD, MD,[a] Sorilla Prey, MD, PhD,[a] Katia Boniface, PhD,[a,b] and Julien Seneschal, MD, PhD[a,b]
Bordeaux, France

Background: The use of anti-programmed cell death (PD)-1 therapies in metastatic tumors is associated with cutaneous side effects including vitiligo-like lesions.

Objective: We sought to characterize clinically and biologically vitiligo-like lesions occurring in patients receiving anti-PD-1 therapies by studying a case series of 8 patients with metastatic tumors and 30 control subjects with vitiligo.

Methods: Eight patients receiving anti-PD-1 therapies with features of vitiligo-like lesions seen in our department were recruited. Clinical features and photographs were analyzed. For some patients, skin and blood samples were obtained. Results were compared with the vitiligo group.

Results: All patients developed lesions localized on photoexposed areas with a specific depigmentation pattern consisting of multiple flecked lesions without Koebner phenomenon. In contrast to vitiligo, patients receiving anti-PD-1 therapies who developed vitiligo-like lesions did not report any personal or family histories of vitiligo, thyroiditis, or other autoimmune disorders. Analysis of blood and skin samples revealed increased C-X-C motif ligand 10 levels in serum of patients developing vitiligo-like lesions, associated with skin infiltration of CD8 T-cells expressing C-X-C motif receptor 3 and producing elevated levels of interferon-γ and tumor necrosis factor-alfa.

Limitations: This cross-sectional study concerned a single center.

Conclusions: Clinical and biological patterns of vitiligo-like lesions occurring in patients receiving anti-PD-1 therapies differ from vitiligo, suggesting a different mechanism. (J Am Acad Dermatol http://dx.doi.org/10.1016/j.jaad.2016.10.044.)

Key words: anti-programmed cell death−1 therapy; nivolumab; pembrolizumab; vitiligo; vitiligo-like lesions.

Immunotherapy is poised to play a more central role in the treatment of cancers, as shown in metastatic melanoma. Indeed, antibodies against the protein programmed cell death (PD)-1, a major checkpoint in the effector phase of cytotoxic T cells, have shown promising clinical results in cancer management immunotherapy. The selective PD-1 inhibitors pembrolizumab and nivolumab have shown impressive clinical results in the treatment of metastatic melanoma, leading to accelerated

From the Department of Dermatology and Pediatric Dermatology, National Reference Center for Rare Skin Disorders, Hôpital Saint-André,[a] and Institut National de la Santé Et de la Recherche Médicale (INSERM) U1035, Biothérapies de Maladies Génétiques, Inflammatoires et Cancers (BMGIC), Immuno-dermatology ATIP-AVENIR, University of Bordeaux.[b]

Drs Larsabal and Marti are co-first authors. Drs Boniface and Seneschal are co-last authors.

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Conflicts of interest: None declared.

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Reprint requests: Julien Seneschal, MD, PhD, Department of Dermatology and Pediatric Dermatology, National Reference Center for Rare Skin Disorders, Hôpital Saint-André, 1 rue Jean Burguet, 33075 Bordeaux, Cedex, France. E-mail: julien.seneschal@chu-bordeaux.fr.

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development and authorization.[1-3] Nonetheless, anti-PD-1 therapies are often associated with an increased burden of toxicities in cancers (eg, melanoma) and some of the side effects are described as immune-related adverse effects.[4] Among them, the occurrence of vitiligo-like lesions is of particular interest in the context of melanoma, because it is hypothesized that this side effect is associated with increased survival. The incidence of vitiligo-like lesions in patients receiving pembrolizumab was recently estimated to be 25% of patients treated,[5] which is much more frequent than spontaneously occurring vitiligo (prevalence between 0.5%-1% in the general population).[6] Although the occurrence of vitiligo-like lesions has received considerable attention, a clear description of the affected sites and pattern of depigmentation is lacking, as are clinical and biological comparisons with vitiligo. Therefore, we sought to better characterize clinically and biologically the vitiligo-like lesions occurring in patients receiving anti-PD-1 therapies, and compared our results with those of a cohort of patients with vitiligo followed up in our center.

CAPSULE SUMMARY

- Vitiligo-like lesions are a classic side effect occurring in patients receiving anti-programmed cell death-1 therapies.
- Vitiligo-like lesions are characterized by flecked depigmented macules occurring on photoexposed areas without Koebner phenomenon.
- Vitiligo-like lesions display a clinical and biological pattern different from vitiligo.

METHODS

Patients

This prospective single-center study included systematically all patients who developed vitiligo-like lesions after receiving pembrolizumab or nivolumab, which were used according to the French national recommendations between January 2015 and December 2015. Patients with vitiligo who attended our vitiligo clinic in the Department of Dermatology at Bordeaux Hospital between March and April 2016 were included as a control group. In addition to clinical notes, the Vitiligo European Task Force questionnaire was completed and ultraviolet photographs were taken for each patient.

For some patients, blood samples, a 4-mm punch biopsy specimen, or both were taken from perilesional skin. Healthy skin was obtained as discarded human tissue from cutaneous plastic surgery.

All the studies involving human tissues were approved by the local institutional ethics committee and the Commission Nationale de l'Informatique et des Libertés.

Skin T-cell extraction

T cells were extracted from the skin using a previously published method that isolates T cells from the skin.[7] T cells were isolated from 3-week explant cultures maintained in interleukin (IL)-2 and IL-15 (R&D Systems, Minneapolis, MN). Analysis of cytokine expression from extracted T cells was then performed as described below.

Flow cytometry

For the analysis of cytokine production, peripheral blood mononuclear cells or skin isolated T cells were stimulated for 4 hours with phorbol myristate acetate (50 ng/mL) (Sigma, St Louis, MO) and ionomycin (500 ng/mL) (Sigma) in the presence of GolgiStop protein transport inhibitor containing monensin (BD Biosciences, San Jose, CA). Cells were then surface-stained with anti-CD8 (clone: SK1) and anti-CD3 (clone: SK7) antibodies (Abs), fixed, permeabilized, and stained with anti-tumor necrosis factor (TNF)-alfa (clone: monoclonal antibody 11) and anti-interferon (IFN)-γ (clone: B27) Abs (Biolegend, San Diego, CA) using the Cytofix/Cytoperm plus kit (BD Biosciences, San Jose, CA). Data were acquired on a Canto II cytometer (BD Biosciences) and analyzed with DIVA (BD Biosciences) or FlowJo (Ashland, OR) software.

Enzyme-linked immunosorbent assay

Serum C-X-C motif ligand (CXCL) 10 levels were measured by using human CXCL10 duoset, according to the manufacturer's instructions (R&D Systems).

Immunofluorescence

We prepared 3-μm sections from formalin-fixed, paraffin-embedded skin biopsy specimens. Sections were deparaffinized and subjected to a heat-induced epitope retrieval step. Slides were rinsed in cool running water and washed in Tris-buffered saline (pH 7.4) before incubation with relevant primary antibodies. Monoclonal Abs specific for C-X-C motif receptor (CXCR) 3 (R&D System) and CD8 (Abcam, Cambridge, UK) were used as primary Abs, according to the manufacturer's protocols. Then the slides were incubated with appropriate secondary Abs: Alexa Fluor 488-conjugated antimouse (IgG2a) and Alexa Fluor 555-conjugated antimouse (IgG1) (Thermo Fisher Scientific Inc, St Louis, MO). After subsequent washing, the sections were mounted with Prolong Gold antifade reagent with 4′,6-diamidino-2-phenylindole (Thermo