Dear Editors,

Immune checkpoint inhibitors such as nivolumab, an antibody directed against the programmed death-1 (PD-1) protein, and ipilimumab, an antibody against cytotoxic T lymphocyte-associated protein-4 (CTLA-4), have revolutionized the therapy of metastatic melanoma [1]. The outstanding effectiveness of this treatment strategy is countered by therapy-induced side effects, which include immune-mediated thyroiditis, colitis, pneumonitis, hepatitis and pituitary gland inflammation [2–4]. Early detection and appropriate management of immune-mediated side effects are of central importance in ensuring the reliable use of this group of compounds.

In August 2017, a 74-year-old female presented at our clinic after an externally performed biopsy of a mass in the neck revealed histopathological findings of a lymph node metastasis of a malignant melanoma, with wild-type BRAF. The physical examination revealed no evidence of a primary tumor and the serum level of the S100 protein was elevated at 0.4 μg/l (normal value up to 0.1 μg/l). Examination by positron emission tomography/computer tomography (PET/CT) revealed left axillary and left clavicular lymph node metastases (Figure 1a). Magnetic resonance imaging (MRI) examination of the skull showed no evidence of metastases. After discussion of the findings at the interdisciplinary tumor conference, a combined immunotherapy with nivolumab (1 mg/kg bw) and ipilimumab (3 mg/kg bw) was initiated in October 2017. After the second therapy cycle, the patient developed immune-mediated colitis with aqueous diarrhea up to seven times a day. As part of the side effects management, the patient received systemic prednisolone at an initial dose of 100 mg once daily. Under this treatment the diarrhea subsided, allowing complete tapering of the glucocorticoid over a period of six weeks. Fortunately, the clinical staging conducted in June 2018 showed a complete remission of the lymph node metastases with no evidence of metastases in other organs (Figure 1a).

More than a year after the final round of immunotherapy, our patient was diagnosed with a new manifestation of pancytopenia in October 2019. In addition, the patient reported chills, dyspnea, night sweats and up to 39.7°C of fever that had been present for several days. Symptoms such as cough, head cold, diarrhea and vomiting were denied by the patient. On physical examination, neither the inspection of the skin nor the auscultation of the lungs gave any indication of the presence of an infectious disease. Laboratory diagnostics showed an increased inflammation marker value (CRP: 94.2 mg/l; normal value up to 3.0 mg/l) and pancytopenia (Table 1). Extensive infectiological investigations including urine analysis, chest x-rays, abdominal sonography and blood cultures did not reveal any infectious disease. Therefore, despite the time latency, immune-mediated pancytopenia as a side effect of melanoma therapy with ipilimumab and nivolumab was considered. Although hematological side effects of checkpoint inhibitors are generally rare, there are reports in the literature of thrombopenia, leukopenia, lymphopenia, neutropenia, agranulocytosis or pancytopenia as side effects of immunotherapy with checkpoint inhibitors [5, 6]. We administered

Figure 1 PET/CT scan. September 2017: Metastatic disease of lymph nodes left clavicular and left axillary (a). June 2018: Complete remission of the metastases (b).
immunosuppressive treatment with prednisolone (100 mg once daily) and antibiotics treatment with piperacillin/tazobactam (4/0.5 g three times daily) and meropenem (1 g twice daily) for a total of ten days. During this treatment, the fever did not subside and the blood count failed to normalize.

Because an abdominal ultrasound examination had detected splenomegaly, the bone marrow was biopsied to rule out lymphoma. Surprisingly, individual intracytoplasmic pathogens were found in CD1a expressing macrophages (Figure 2), leading to a diagnosis of visceral leishmaniasis. Our patient also reported a visit to Spain a few weeks previously.

Leishmania are protozoa that are transmitted to humans through the bite of a female sand fly and can lead to the clinical picture of leishmaniasis. A distinction is made between cutaneous, mucocutaneous and visceral leishmaniasis [6, 7]. Worldwide, especially in parts of North and East Africa, South America, Southern Europe and Asia, 500,000 new cases of visceral leishmaniasis occur every year with about 50,000 fatalities [8]. In Spain, between 150–250 new cases are reported each year and an average of 5.6 patients per million population are hospitalized [9]. Untreated, the disease is usually fatal [10]. Clinically, visceral leishmaniasis often manifests through hepatosplenomegaly as well as bone marrow suppression with the resulting pancytopenia. These changes result from an infection of the macrophages in the liver, spleen and bone marrow [11]. Treatment is carried out with various drugs effective against protozoa, such as amphotericin B or miltefosine [10].

**Table 1** Blood count with evidence of pancytopenia. Laboratory values from October 2019.

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Reference value</th>
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<tbody>
<tr>
<td>Leukocytes</td>
<td>2.06/μl</td>
<td>3.98–10.0/μl</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>3.62/μl</td>
<td>3.93–5.22/μl</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>8.8 g/dl</td>
<td>11.2–15.7 g/dl</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.94 × 10^3/μl</td>
<td>1.56–6.13 × 10^3/μl</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.12 × 10^3/μl</td>
<td>0.01–0.08 × 10^3/μl</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.02 × 10^3/μl</td>
<td>0.04–0.36 × 10^3/μl</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.31 × 10^3/μl</td>
<td>0.24–0.36 × 10^3/μl</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.67 × 10^3/μl</td>
<td>1.18–3.74 × 10^3/μl</td>
</tr>
<tr>
<td>Thrombocytes</td>
<td>68/μl</td>
<td>182–369/μl</td>
</tr>
</tbody>
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Figure 2: Histopathological staining of bone marrow: Bone marrow with macrophages containing Leishmania amastigotes (black arrows; Giemsa stain, x 400) (a, b). CD1a positive Leishmania amastigotes (black arrows, x 400; black bar: 50 μm) (c, d).
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After establishment of diagnosis our patient began treatment with amphotericin B 1 mg/kg bw, which was then gradually increased to 3 mg/kg bw. This resulted in the normalization of blood count values and resolution of all symptoms. The abdominal ultrasound examination also showed a decrease in splenomegaly.

The diversity of side effects mediated by checkpoint inhibitors is often a diagnostic and therapeutic challenge. In general, when an immune mediated side effect is suspected, other possible diseases such as infections must be ruled out. In particular, an unusual course of the disease and a lack of improvement of the symptoms through immunosuppressive therapy, as in our patient, should give reason to investigate other possible causes of the symptoms. This often requires interdisciplinary cooperation. Increasingly comprehensive registers of side effects are enabling registration and symptom monitoring even of rare side effects of checkpoint inhibitor therapy, expanding a wealth of experience and improving the application of these substances.

Conflict of interest
None.

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References