

Case Report

# Immune-Related Pancytopenia Induced by Anti-PD-1 Therapy – Interrupt or Continue Treatment – The Role of Immunohistochemical Examination

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## Keywords

Anti-PD-1 · Immunotherapy · Immune related adverse events · Melanoma · Pancytopenia · Pembrolizumab · Toxicity

## Abstract

Immune checkpoint inhibitors (ICIs), including anti-cytotoxic T-lymphocyte antigen 4 (anti-CTLA-4) and anti-programmed death receptor-1/ligand-1 (anti-PD-1/anti-PD-L1) caused a breakthrough in oncology and significantly improved therapeutic outcomes in cancer patients. ICIs generate a specific reaction in T cells, directed against antigens on cancer cells, leading to their damage and death. Through similar or the same antigens, activated lymphocytes may also have a cytotoxic effect on healthy cells, causing development of specific adverse effects – so-called immune-related adverse events (irAEs). We present the case report of a 56 year old patient with disseminated melanoma. During treatment with immunotherapy (anti PD-1), neutropenic fever and pancytopenia occurred. Trepanobiopsy of the bone marrow was performed to determine the cause of pancytopenia. Histopathological assessment of bone marrow combined with immunophenotype investigations may explain the cause of hematological disorders occurring in the course of treatment with ICIs, and support the choice of an appropriate treatment, directly translated into positive outcomes.

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## Introduction

Immune checkpoint inhibitors (ICIs), including anti-cytotoxic T-lymphocyte antigen 4 (anti-CTLA-4) and anti-programmed death receptor-1/ligand-1 (anti-PD-1/anti-PD-L1) caused a breakthrough in oncology and significantly improved therapeutic outcomes in cancer patients [1]. ICIs generate a specific reaction in T cells, directed against antigens on cancer cells, leading to their damage and death. Through similar or the same antigens, activated lymphocytes may also have a cytotoxic effect on healthy cells, causing development of specific adverse effects – so-called immune-related adverse events (irAEs) [2]. There are several hypotheses describing physio-pathological background of those toxic effects. Majority of them implies an association between immunological complications and ICIs-induced hyperactivation of T cells. Most commonly, ICIs-associated complications are result of activation of the immune system and lymphocytic infiltrations of healthy tissues. However, presentation of histological irAEs is not well understood. There are no unequivocal data that would allow foresee development of irAE based on a histopathological examination, and plan further treatment. From the histopathological point of view the best understood are irAEs associated with the skin and the gastrointestinal tract [3–9].

Bone marrow damage is one of rare immune complications associated with the use of ICIs. The complication may be clinically manifested by neutropenia, anemia, thrombocytopenia, and – in the most severe cases – pancytopenia [10–14].

We present a case of a patient who developed neutropenic fever with pancytopenia secondary to the treatment with pembrolizumab (antibody against the PD-1 receptor) for disseminated melanoma, and who was at the same time diagnosed with chronic lymphocytic leukemia. Histological diagnostics extended by immuno-phenotyping methods allowed making a correct diagnosis, detecting the second, independent tumor, and protecting the patient from unnecessary termination of the therapy and inferior outcome.

## Case Presentation

The male patient, 56-year-old, post removal in 2014 of skin melanoma localized in the left parietal area: histopathological diagnosis of melanoma malignum nodulare pT1b, present mutation in BRAF V600. In September 2017 imaging diagnostics demonstrated dissemination of the tumor to lungs, lymph nodes, the spleen and a single metastasis to the central nervous system (CNS). The patient was in overall very good condition, free from clinical symptoms of metastases to the CNS. Laboratory investigations (blood cell count, lactic dehydrogenase – LDH, hepatic tests, renal tests) demonstrated no departures from normal. Following a team consultation, the patient was qualified for immunotherapy with pembrolizumab (anti-PD-1 antibody) at the dose of 200 mg, intravenously (IV), every 3 weeks. The immunotherapy was started in November 2017.

Neutropenic fever with G4 (G-grade) leukopenia, G4 neutropenia, G2 thrombocytopenia and G2 anemia according to the Common Terminology Criteria for Adverse Events (CTCAE) [15] developed after two courses of pembrolizumab. The patient was admitted to a hospital. Additional investigations indicated no cause of fever. Empirical antibiotic therapy (amoxicillin/clavulanic acid), steroid therapy (intravenous dexamethasone) and subcutaneous filgrastim (G-CSF – granulocyte colony-stimulating factor) were introduced. The treatment resulted in disappearance of fever, improvement of the patient's general condition and improvement of blood count parameters (leukopenia G2, neutropenia G2, anemia G2). The treatment

with pembrolizumab was withheld. After subsequent 2 weeks laboratory investigations revealed maintained G1 leukopenia, G1 neutropenia and G2 anemia. Laboratory investigations demonstrated also an increase of the LDH level to approximately 1.5× ULN. A decision was made to perform trepanobiopsy in order to differentiate between infiltration of melanoma in bone marrow and irAE.

Trepanobiopsy of January 2018: Increased bone marrow cellularity (of approx. 70%) with maintained cell lines. Scattered megakaryocytes are present in the tissue, various sizes, majority normotypical, with presence of few atypical forms. In the tissue there are intraparenchymal and peritrabecular clusters of lymphocytes. Immunohistochemical staining reveals they are mostly composed of B cells, with a rather abundant admixture of T cells – CD4 and CD8 positive. Proliferative activity in Ki-67 staining is minor in the above-mentioned clusters. Moreover, there are also rather numerous CD8-positive lymphocytes scattered in bone marrow. Presentation of bone marrow may correspond to changes caused by the applied immune therapy. No melanoma infiltrations are found in examined sections. Cytometric tests demonstrated no signs of proliferation of NHL (Fig. 1, 2).

Laboratory investigations carried out two weeks later demonstrated a further improvement of blood cell parameters – only G1 anemia persisted. A complete diagnostics for anemia was carried out, and iron deficiency was found. Iron in therapeutic doses was recommended. Imaging diagnostics (10 weeks after introduction of the treatment) demonstrated a partial remission. A decision was made to continue the immunotherapy with pembrolizumab (February 2018).

In July 2018, considering persistent G1 anemia (acc. to CTCAE [15]), a control examination of bone marrow was carried out. 2 osseous cylinders, total length of approx. 14mm, with partially increased cellularity up to approx. 50% and maintained cell lines were observed in examined sections. On the background of a physiological pattern of the tissue there are some focal, nodular, intertrabecular (in case of a single focus – peritrabecular) infiltrations composed of small CD20 and bcl2 positive B cells. CD5 reaction doubtful in B cells, and CD23, CD10, bcl6, MUM1 negative. Lymphocytes are accompanied by a significant admixture of T cells, both CD4 and CD8-positive. Proliferative activity in Ki-67 staining is minor in the above-mentioned infiltrations. Results of cytometric analyses were inconclusive. A minor population of abnormal lymphocytes was found, demonstrating the CLL/SLL immunophenotype (Fig. 3, 4, 5, 6). Considering inconclusive presentation, immunophenotyping of peripheral blood was carried out. The investigation indicated: lymphocytes 28 %, monocytes 6%, granulocytes 66%. B cells prevailed among lymphocytes (slightly over 50%). All of them demonstrated a classic phenotype of CLL/SLL cells: they possessed the CD19 antigen, reduced expression of CD20 and CD22 antigens, co-expression of CD23 and CD5, and absence of light chains on the surface.

Considering reports suggesting efficacy of PD-1 inhibitors in treatment of CLL [16], and a good outcome observed in the discussed patient, a decision was made to continue the treatment with pembrolizumab. At present the patient continues the treatment with pembrolizumab (36th administration in June 2019) with the effect of partial remission.

## Discussion

Hematological complications associated with the use of ICIs are rare, but may be violent in nature [10, 11, 17–20]. Few cases of hematological complications due to the use of anti PD-1 have been reported and only in individual cases shown histology [21]. In the presented case symptoms of irAE were also rather violent, but were quickly controlled by introduction of

steroid therapy and G-CSF. This was a recommended course of action [10, 11]. Considering persistent neutropenia, a decision was made to carry trephine biopsy out, for a more precise differentiation of possible causes. Histopathological examination excluded invasion of bone marrow by melanoma cells, and allowed identification of lymphocytic infiltrations composed of B and T cells, with numerous cytotoxic CD8+ cells. That confirmed the association of parameters of the blood cell count with cellular stimulation of bone marrow secondary to irAE. As for continued anti-PD-1 therapy, there were no precise recommendations regarding the further course of actions. No guideline indicated if the therapy should be terminated or it could be continued [10, 11]. A fact should be noted that development of grade 3 or 4 irAE acc. to CTCAE [15] is usually considered to be an indication for discontinuation of immune therapy, considering possible occurrence of hard to manage complications in case of continued treatment. However, some authors allow continuation of immune therapy in selected cases [10, 11]. Considering withdrawal of symptoms, the patient's very good general condition and a partial remission confirmed by imaging diagnostics, a decision was made to continue the anti-PD-1 therapy. Considering absence of straightforward guidelines, and to determine if lesions in the bone marrow become intensified after re-introduction of pembrolizumab, a control trephine biopsy was carried out after subsequent 4 months of treatment. Re-assessment of bone marrow demonstrated regression of proliferative bone marrow changes, but with presence of numerous infiltrations of small B and T cells. Immunohistochemical staining demonstrated no immunophenotype specific for cancerous proliferation of lymphocytes, but cytometry revealed a minor population of abnormal B cells demonstrating the immunophenotype of CLL/SLL cells. The immunophenotypic assessment of peripheral blood using the flow cytometry method indicated that all B cells had abnormal immunophenotype of CLL/SLL cells, despite physiological parameters of blood cell count. The obtained result was surprising – chronic lymphocytic leukemia/ monoclonal B-cell lymphocytosis, and was confirmed by additional determinations. Co-existence of chronic lymphocytic leukemia with melanoma is reported in literature [22]. According to recent data, melanoma co-exists in approx. 4% of CLL cases [23]. The diagnosis of a second, independent tumor rose a question of further treatment of the patient. Some reports indicate efficacy of pembrolizumab and nivolumab in treatment of CLL undergoing Richter's transformation [24]. In the discussed case the continued immune therapy seemed advisable, which was confirmed by observation of maintained response to the therapy, as evidenced by imaging techniques, and a physiological parameters of blood cell count.

## Conclusion

Histopathological assessment of bone marrow combined with immunophenotype investigations may explain the cause of hematological disorders occurring in course of the treatment with ICIs, and support the choice of an appropriate treatment, directly translated into positive outcomes.

In the presented case, treatment with immunotherapy was very effective. However, treatment should always be used individually and the patient should be closely monitored.

## Statement of Ethics

Informed consent was obtained from patient.

### Disclosure Statement

The authors declare that they have no competing interests.

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Not applicable.

### Author Contributions

BCS interpreted the patient data and was a major contributor in writing the manuscript. AG and MN obtained, analyzed and interpreted the patient data, and were contributors in editing the manuscript. All authors read and approved the final manuscript.

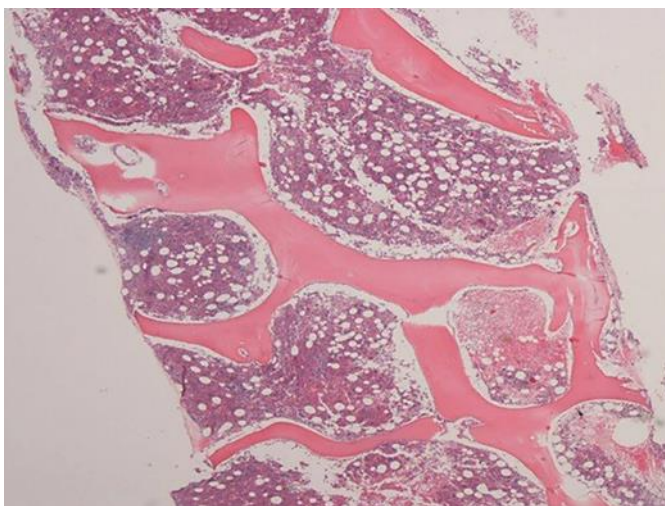
### Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

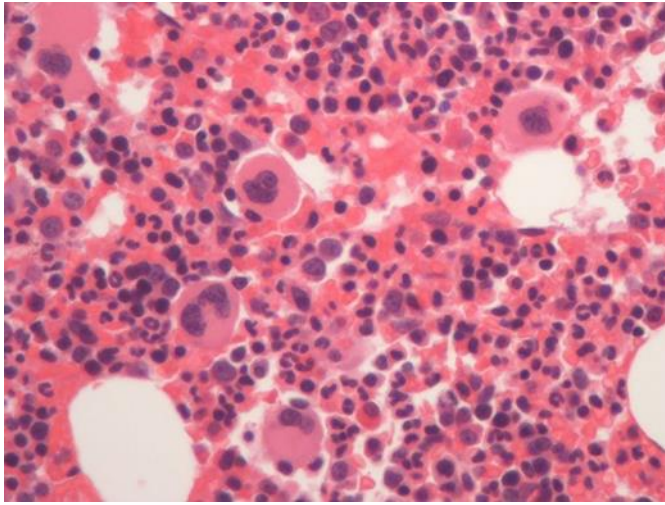
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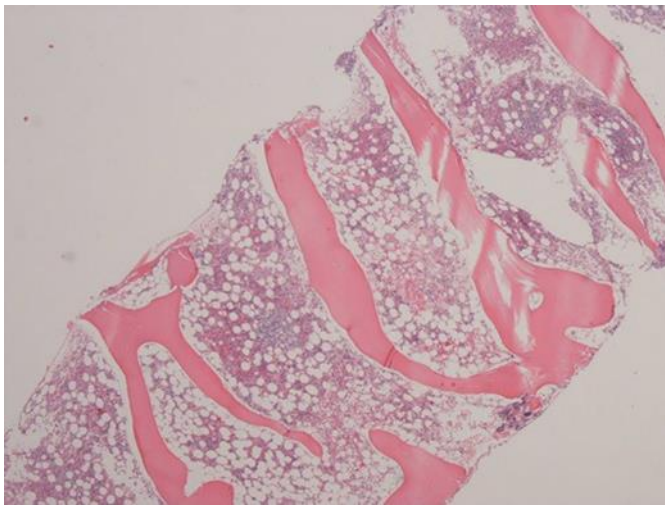
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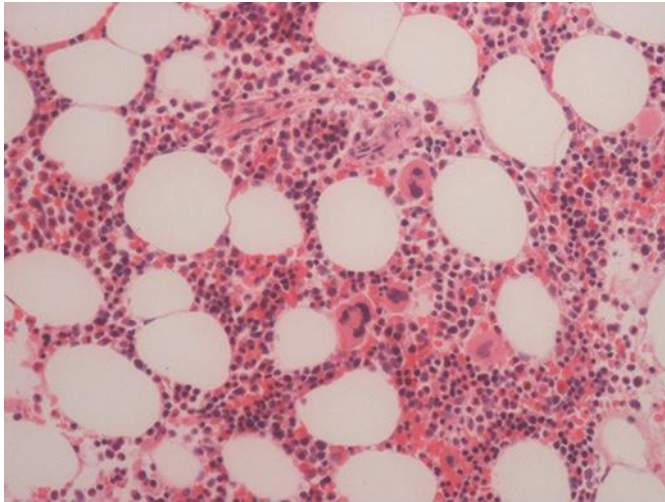
**Fig. 1.** H&E (hematoxylin-eosin staining), 20× magnification. The bone marrow is significantly cellular. Visible 2 empty spaces after aspirate collection.



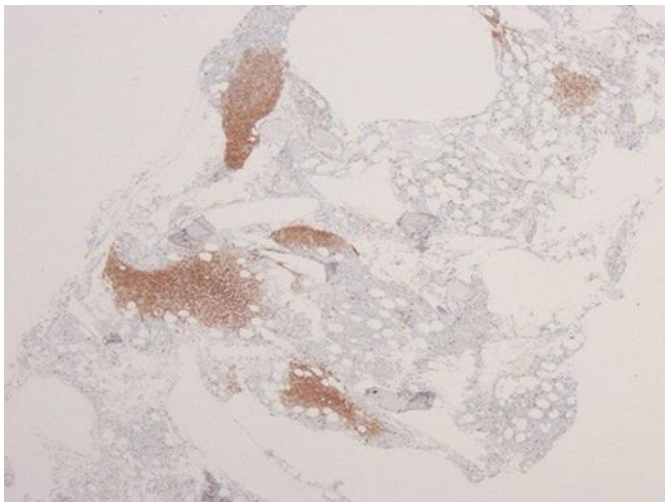
**Fig. 2.** H&E (hematoxylin-eosin staining), 400× magnification. Marrow cell, the current cell lines are mixed, numerous megakaryocytes.



**Fig. 3.** H&E (hematoxylin-eosin staining), 20× magnification. Control bone marrow biopsy, visible cellular normalization, compare with Figure 1.

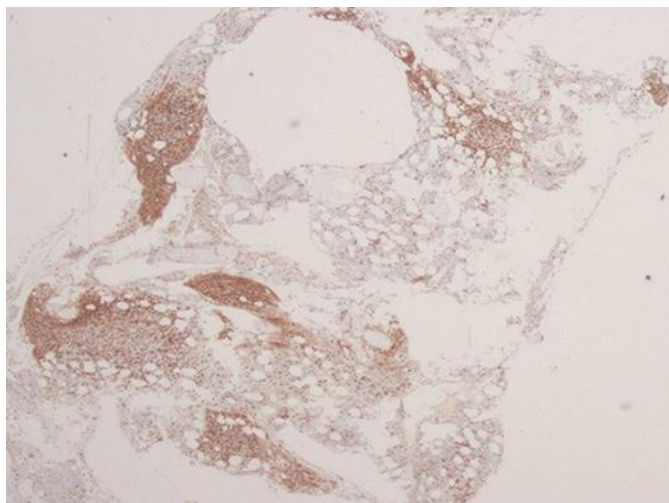


**Fig. 4.** H&E (hematoxylin-eosin staining), 200× magnification. Normalization of the bone despite continued treatment pembrolizumab.



**Fig. 5.** Immunohistochemical staining for CD 20. 20× magnification. Concentration of B cells in the bone marrow.





**Fig. 6.** Immunohistochemical staining for CD 3. 20× magnification. Numerous T lymphocytes in the bone marrow. Figure should be compared with Figure 5.