Quantitative Analysis of Immune-Reactive Cells among Leukocytes Is Useful for the Diagnosis of Drug Eruptions Caused by Bexarotene

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Abstract
Bexarotene is a third-generation retinoid X receptor-selective retinoid that has been approved for use in the treatment of cutaneous T-cell lymphomas (CTCLs). Since the objective response rate of bexarotene is relatively high, with no racial differences, bexarotene can be administered to patients with phototherapy-resistant early CTCL as one of the first-line therapies in real-world clinical practice. Although various adverse events caused by bexarotene have been reported, there have been no reports of drug eruptions caused by bexarotene. One of the possible reasons is that it is difficult to distinguish a drug eruption from recurrence of CTCL, histologically. In this report, drug eruptions in 2 patients with CTCL treated with bexarotene diagnosed by quantitative analysis of immunohistochemical staining by digital microscopy are described.
Introduction

Cutaneous T-cell lymphomas (CTCLs), such as mycosis fungoides (MF), generally progress slowly from early to advanced stages [1]. Recently, several immunomodulatory drugs have become available for the treatment of CTCLs that are resistant to local skin therapies [2]. Among them, bexarotene is used as one of the most promising anti-CTCL drugs in first-line systemic therapy [2]. Since CTCL is a hematopoietic malignancy of lymphocyte origin [1], unlike solid tumors, it is difficult to distinguish tumor cells from tumor-infiltrating lymphocytes (TILs). In this report, drug eruptions occurring in 2 patients with CTCL treated with bexarotene are described.

Case 1

A 70-year-old Japanese man visited our outpatient clinic with a 9-year history of erythema on the trunk and extremities. He had been treated with topical steroid and narrow-band ultraviolet B for early-stage MF (T1bN0M0B0 stage I). On his visit, he had developed an infiltrated erythematous plaque on the trunk and extremities (Fig. 1a), though his phototherapy and topical therapy were continued. A biopsy specimen from the left side of the abdomen showed band-like infiltration of atypical lymphocytes with epidermotropism in the superficial dermis (Fig. 1b). Immunohistochemical staining showed that these atypical lymphocytes were positive for CD2, CD3, CD4, CD45, and CCR4 and negative for CD5, CD7, CD8, and CD30. Positron emission tomography-CT at the initial visit showed no evidence of lymph node or visceral involvement. The diagnosis was MF (T2N0M0B0 stage IIA), and he was administered oral bexarotene (300 mg/m²). Eight days after the administration of bexarotene, he had developed symmetrical, infiltrated, and disseminated erythema on the trunk and extremities with severe pruritus (Fig. 1c). A biopsy specimen from the newly arising eruption showed superficial perivascular infiltration of lymphocytes with prominent eosinophils (Fig. 1d). The diagnosis was a drug eruption caused by bexarotene. With oral prednisolone 30 mg/day, his eruption disappeared rapidly.

Case 2

A 66-year-old Japanese woman visited our outpatient clinic with erythematous nodules on the face, trunk, and extremities (Fig. 2a). A biopsy specimen showed dense infiltration of atypical lymphocytes without epidermotropism throughout the dermis (Fig. 2b). Immunohistochemical staining showed that these atypical lymphocytes were positive for CD2, CD3, CD4, CD7, and CCR4 and negative for CD5, CD8, and CD30. Positron emission tomography-CT at the initial visit showed no evidence of lymph node or visceral involvement. The diagnosis was primary cutaneous peripheral T-cell lymphoma not otherwise specified (T1N0M0B0 stage I), and she was treated with oral bexarotene (300 mg/m²). Nine days after the administration of bexarotene, she developed symmetrical, infiltrated, and disseminated erythema on the trunk and extremities with severe pruritus (Fig. 2c). A biopsy specimen from a newly arising eruption showed superficial perivascular infiltration of lymphocytes with prominent eosinophils (Fig. 2d). The diagnosis was a drug eruption caused by bexarotene. With oral prednisolone 30 mg/day, her eruption disappeared rapidly.
Quantitative Analysis of Leukocytes

To make the differential diagnosis between drug eruption and recurrence of MF, immunohistochemical staining of CD3, CD4, CD8, Foxp3, as well as CD163, was performed, and the percentage of IHC-positive cells per all tumor-infiltrating cells was quantitatively analyzed by BZ-X800 (KEYENCE, Tokyo, Japan), as we previously reported [3, 4]. The
proportion of CD8+ cells and CD163+ cells among the leukocytes in the dermis was increased at the onset of the drug eruption in both cases; whereas, there were no increases in CD3+ cells, CD4+ cells, and Foxp3+ cells among the leukocytes in the dermis (Fig. 3).

**Discussion**

Bexarotene is a third-generation retinoid X receptor-selective retinoid that has been approved for use in the treatment of CTCLs [2, 3, 5–8]. Since the objective response rate of bexarotene is relatively high, with no racial differences, bexarotene can be administered to patients with phototherapy-resistant early CTCL as one of the first-line therapies in real-world clinical practice [2, 3]. In addition, several clinical trials and multicenter, retrospective studies suggested that bexarotene causes characteristic adverse events such as hypothyroidism, hyperlipidemia, and leukopenia [2, 3, 5–8]. Although various adverse events have been reported, there have been no reports of drug eruptions caused by bexarotene, even in real-world clinical experience data from post-marketing surveillance [4]. One of the possible reasons is that it is difficult to distinguish a drug eruption from recurrence of CTCL, histologically.

Although flow cytometers are widely used for diagnosing CTCL subtypes, they are difficult to use if the number of infiltrating cells is limited, such as the early stage of MF and drug eruptions. Digital microscopic analysis is useful for quantitative analysis of immunohistochemical staining, especially to identify the profiles of lymphocytes such as TILs [9, 10]. For example, we previously evaluated TILs in metastatic melanoma, the profile of which is dramatically changed by the administration of nivolumab plus ipilimumab combined therapy [10]. These reports suggested that digital microscopic analysis is even useful for the analysis of profiles of CTCL instead of flow cytometry. Indeed, in the present cases, digital microscopic analysis showed increased ratios of CD8+ and CD163+ cells, suggesting that the developed eruption was a drug eruption caused by bexarotene rather than recurrence of CTCL. Since the present cases were both CD4-dominant CTCLs, digital microscopic analysis was useful to make the differential diagnosis of drug eruption, which should show CD8-dominant lymphocyte infiltration.
Conclusion

This report presents only 2 cases, but further cases may provide fundamental insights into the usefulness of digital microscopic analysis for the diagnosis of drug eruptions in CTCL patients.

Statement of Ethics

Written, informed consent was obtained from the patients for publication of this case report and any accompanying images. The protocol for this human study was approved by the Ethics Committee of Tohoku University Graduate School of Medicine, Sendai, Japan (permit no. 2019-1-308).

Conflict of Interest Statement

The authors have no conflicting interests to declare.

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Author Contributions

T. Fujimura designed the research study. H. Chiba, Y. Kambayashi, E. Tamabuchi, K. Ohuchi, R. Amagai, A. Hasimoto, and T. Fujimura treated the patient and acquired the clinical data. K. Ohuchi performed immunohistochemical staining and its analysis. H. Chiba and T. Fujimura wrote the manuscript. T. Fujimura supervised the study.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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