



Loss of nivolumab binding to T cell PD-1 predicts relapse of Hodgkin lymphoma

Reiki Ogasawara¹ · Daigo Hashimoto¹ · Junichi Sugita¹ · Fumihiko Yamawaki¹ · Tomoaki Naka² · Tomoko Mitsuhashi² · Shuichiro Takahashi¹ · Naohiro Miyashita¹ · Kohei Okada¹ · Masahiro Onozawa¹ · Yoshihiro Matsuno² · Takanori Teshima¹

Received: 11 June 2019 / Revised: 10 September 2019 / Accepted: 12 September 2019 / Published online: 19 September 2019
© Japanese Society of Hematology 2019

Abstract

Nivolumab is effective in the treatment of classical Hodgkin lymphoma that relapsed after allogeneic hematopoietic stem cell transplantation (SCT) with the risk of graft-versus-host disease; however, the optimal time and dose of nivolumab administration remain to be investigated. Nivolumab binding to PD-1 masks flowcytometric detection of PD-1 by the anti-PD-1 monoclonal antibody EH12.1. Using this method, we monitored nivolumab binding on T cells after nivolumab treatment in a patient with classical Hodgkin lymphoma relapsed after allogeneic SCT. Nivolumab was effective while prolonged nivolumab binding was evident, but restoration of PD-1 staining predicted tumor relapse. Flowcytometric monitoring of nivolumab binding on T cells could be a promising biomarker for predicting tumor relapse and determining the timing of nivolumab administration.

Keywords Checkpoint inhibitor · Allogeneic stem cell transplantation · Graft-versus-host disease · PD-1 · Hodgkin lymphoma

Introduction

PD-1 blockade after allogeneic hematopoietic stem cell transplantation (SCT) is highly effective against classical Hodgkin lymphoma (cHL) with the risk of graft-versus-host disease (GVHD) [1]. To reduce the risk of severe GVHD, it is recommended to start nivolumab from low dose and consider dose escalation in the selected patients [1]. However, an optimal dose and timing of nivolumab after allogeneic hematopoietic SCT remains to be determined, and there is an unmet medical need for development of biomarkers to estimate anti-tumor activity of nivolumab while avoiding harmful GVHD and immune-related adverse events. Nivolumab and anti-PD-1 monoclonal antibody EH12.1 competitively binds on PD-1 [2]. Thus, administration of nivolumab masks

flowcytometric detection of PD-1 by EH12.1. Here, we demonstrate results of flowcytometric monitoring of nivolumab binding on T cells after nivolumab treatment for nearly 1 year in a patient with cHL relapsing after allogeneic SCT.

Case report

A 24-year-old man was diagnosed with advanced cHL, involving the cervical, axillary, and mediastinal lymph nodes, and the left lung. Disease was refractory to ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) and ICE (ifosfamide, carboplatin, and etoposide) regimens, and residual mediastinal lesion was treated with involved-field radiotherapy. The patient then underwent high-dose chemotherapy MEAM (ranimustine, etoposide, cytarabine, and melphalan) followed by autologous peripheral blood stem cell transplantation (PBSCT) and achieved complete remission (CR). Two months later, the lung lesion recurred and was refractory to salvage chemotherapies including brentuximab vedotin and CHASE (cyclophosphamide, cytarabine, dexamethasone, and etoposide). Although the lesion

✉ Reiki Ogasawara
reiki_oga@yahoo.co.jp

¹ Department of Hematology, Faculty of Medicine, Graduate School of Medicine, Hokkaido University, N15 W7, Kita-ku, Sapporo 060-8638, Japan

² Department of Surgical Pathology, Hokkaido University Hospital, Sapporo, Japan

was regressed after local radiotherapy, the patient developed new pancreatic lesion.

Following GDP (gemcitabine, dexamethasone, and cisplatin) regimen, he underwent haploidentical allogeneic PBSCT (haplo-PBSCT) from his father with reduced intensity conditioning consisting of fludarabine, busulfan and 4 Gy total body irradiation. GVHD prophylaxis was post-transplant cyclophosphamide, followed by tacrolimus and mycophenolate mofetil. He developed grade II acute GVHD involving the skin, but successfully treated with 1 mg/kg of prednisolone (PDN). Although the patient once achieved second CR, the lymphoma relapsed at the left lung 6 months

after haplo-PBSCT. Profiles of T cells in the peripheral blood after haplo-PBSCT were monitored according to the protocol approved by the Institutional Review Board of Hokkaido University Hospital, in accordance with the Declaration of Helsinki.

Nivolumab was administered at a dose of 3 mg/kg on day 200 posttransplant and every 2 weeks thereafter (Fig. 1). The patient was kept on tacrolimus and 5 mg of PDN to prevent GVHD relapse. Flowcytometric analysis performed prior to nivolumab injection showed that PD-1 expression was upregulated on CD4⁺ and CD8⁺ T cells in the peripheral blood (Fig. 1). After nivolumab administration, PD-1

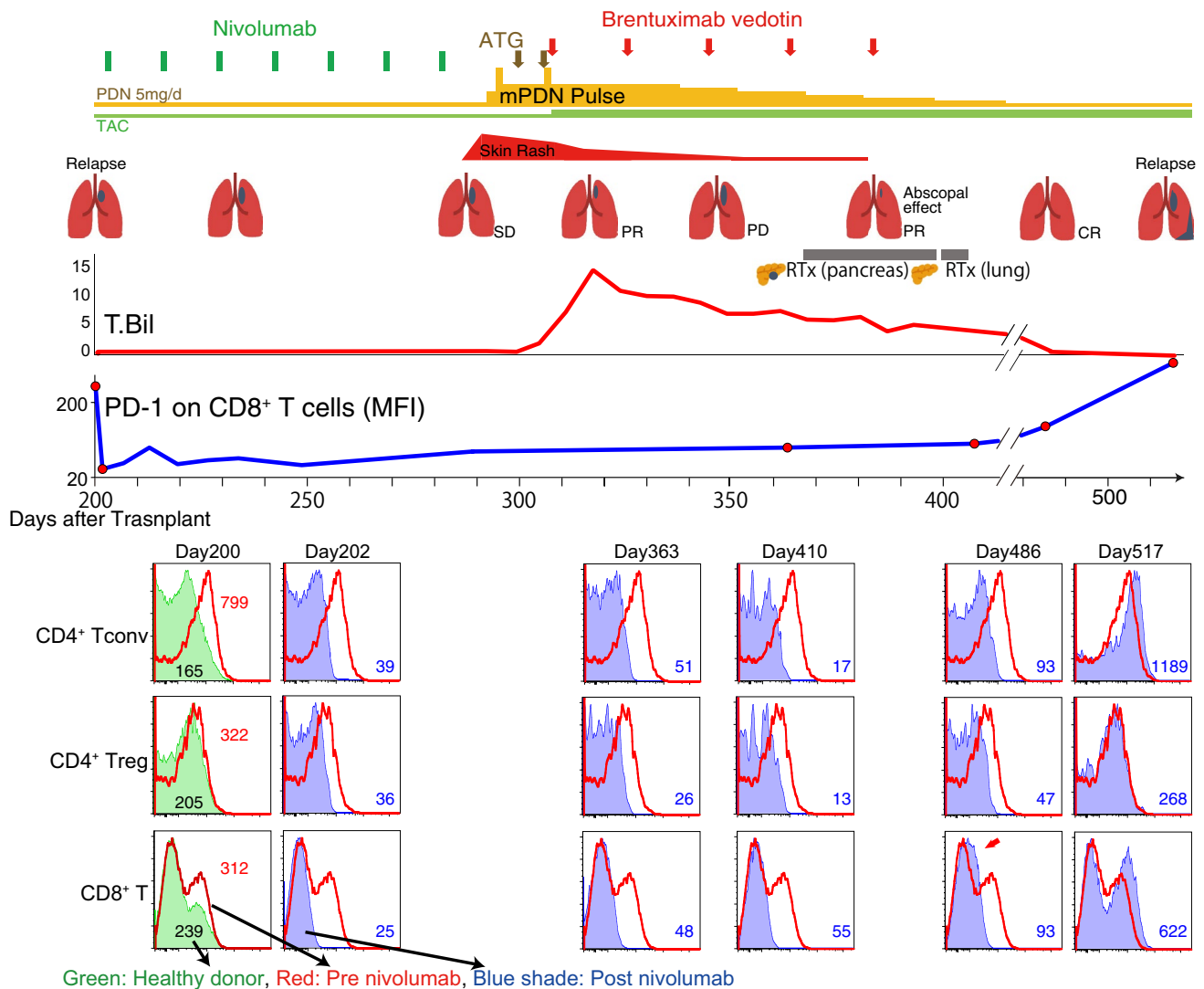


Fig. 1 Clinical course and flowcytometric studies of PD-1 on T cells. Peripheral blood mononuclear cells isolated from the patient prior to nivolumab treatment (red histograms) or at indicated time point after nivolumab treatment (blue shaded) were stained with fluorochrome-conjugated anti-PD-1 monoclonal antibodies (clone: EH12.1). Green-shaded histograms indicate PD-1 staining on T cells from the healthy donor. PD-1 staining on CD3⁺CD4⁺Foxp3⁻ conventional CD4⁺

T cells (Tconv), CD3⁺CD4⁺Foxp3⁺ regulatory T cells (Treg), and CD3⁺CD8⁺ T cells are shown. Numbers in the histograms indicate mean fluorescence intensity (MFI) of PD-1 staining. Red circles indicate time points at which histograms were shown. *PDN* prednisolone, *mPDN* methylprednisolone, *TAC* tacrolimus, *ATG* anti-thymocyte globulin, *RTx* radiation therapy, *T.bil* total bilirubin

staining on CD4⁺ and CD8⁺ T cells was completely blocked due to competition of common binding epitope of nivolumab and staining antibody (clone: EH12.1), confirming complete blocking of PD-1 on T cells by nivolumab, as previously shown (Fig. 1) [2]. Nivolumab induced stable disease (SD). After being administrated with the seventh dose of nivolumab on day 284 posttransplant, the patient developed systemic skin rash, leading to cessation of nivolumab. The skin biopsy on day 287 showed interface dermatitis with lymphocyte infiltration, compatible with acute GVHD (Fig. 2a). Methylprednisolone (mPDN, 2 mg/kg) failed to ameliorate skin GVHD, and mPDN pulse therapy and subsequent anti-thymocyte globulin improved skin GVHD. Nonetheless, serum levels of bilirubin and transaminase increased. Liver biopsy on day 315 showed portal infiltration of CD3⁺ T cells, cholestasis, and spotty necrosis, suggestive of coexistence of acute GVHD and drug-induced lobular hepatitis, which are often seen in patients who received nivolumab after SCT (Fig. 2b, c) [3–5]. GVHD progressed to grade III with stage 3 liver involvement and was successfully treated with brentuximab vedotin targeting CD30⁺ activated T cells, as previously shown [6]. Development of GVHD induced a graft-versus-lymphoma (GVL) effect, and the patient achieved partial response (PR) on day 318. However, CT scan on day 356 showed a new pancreatic lesion with regrowth of the lung lesion (Fig. 2d). Involved-field radiation therapy (40 Gy/ 20 fr) administrated from day 367 to day 395 to the pancreatic lesion was effective but

surprisingly the lung lesion also diminished, indicating that pancreatic irradiation evoked the abscopal effect (Fig. 2e) [7]. The patient achieved CR after subsequent 16 Gy radiotherapy to the lung lesion. Flowcytometric analysis on day 410 demonstrated that PD-1 staining on T cells remained to be completely blocked. However, PD-1 staining began to be restored on day 486 and completely restored on day 517 after allogeneic SCT; it is 233 days from the last dose of nivolumab. The lung tumor relapsed on day 528 but chemoradiotherapies were not effective at all, and subsequently he died of lymphoma.

Discussion

This case represents highly refractory cHL against chemotherapies, brentuximab vedotin, autologous PBSCT, and even HLA haploidentical PBSCT. In this patient, nivolumab was a drug of last resort. However, treatment with PD-1 blockade after allogeneic SCT is often complicated with severe, and steroid-refractory acute GVHD with frequent liver involvement [1, 8, 9]. It has been shown that a history of prior GVHD and shorter time from SCT to administration of nivolumab are risks of GVHD development [1, 9]. Our patient had these risk factors and developed severe GVHD often seen in this setting. GVHD rapidly occurs usually after 1–2 cycles of PD-1 blockade [9]; however, GVHD develops after seventh cycles of

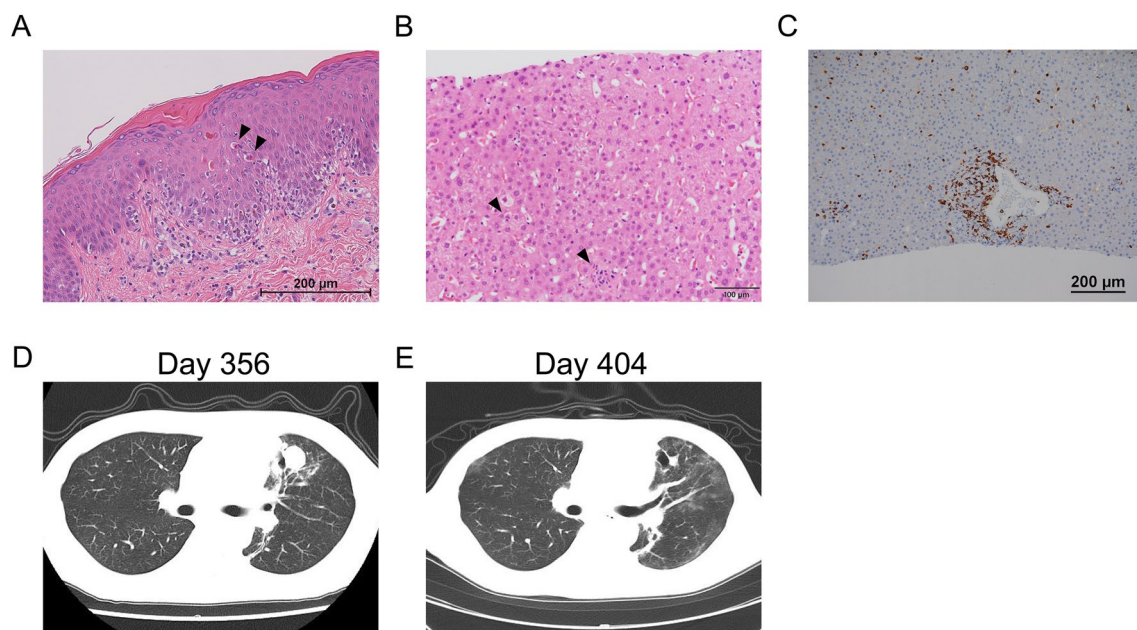


Fig. 2 Pathological and radiological images. **a** H&E images of skin biopsy obtained on day 287 posttransplant demonstrated lymphocyte infiltration along the dermal–epidermal junction and vacuolar interface dermatitis. Arrow heads indicate keratinocyte apoptosis. **b**, **c**

Liver biopsy was performed on day 315. H&E image (**b**) and immunohistochemical image targeting CD3 (**c**) demonstrated spotty necrosis and T cell infiltration. Arrow heads indicate spotty necrosis

nivolumab in our patient. This delay appears to be due to concomitant administration of tacrolimus and low-dose PDN, suggesting that PD-1 blockade can be safely and effectively administered in high-risk patients for GVHD under low-dose immunosuppression. It is of note that the lymphoma remained to be SD for 3 months until the onset of GVHD, regressed after the onset of GVHD, and progressed after effective GVHD treatment, suggesting a link between nivolumab-related GVHD and GVL effects.

Although ATG is effective as GVHD prophylaxis, its effect as the salvage therapy for steroid-refractory GVHD is limited and more effective against skin GVHD compared to liver or gut GVHD, as observed in the current case [10, 11]. Brentuximab vedotin administered after ATG was effective against liver GVHD. Since mPDN pulse, ATG, and brentuximab vedotin were administered for GVHD treatment, it is difficult to specify which agent induced tumor regrowth. It is worthy of note that abscopal effect was observed during brentuximab vedotin treatment, even though the patient's lymphoma was primarily refractory against brentuximab vedotin, suggesting that brentuximab vedotin might ameliorate GVHD with preserving GVL effects. The impact of brentuximab vedotin on GVL effects should be tested in the future studies.

Tumor radiation could stimulate anti-tumor immunity by spreading tumor-associated antigens and danger signals, leading to enhancement of activation of anti-tumor T cells by antigen-presenting cells [7]. PD-1 blockade enhances this process and induced so-called abscopal effect. To our knowledge, this is the first demonstration of abscopal effect by PD-1 blockade given after allogeneic SCT. Abscopal effect may be more potent after allogeneic SCT due to the presence of more antigens to be presented, including alloantigens in addition to tumor-associated antigens, and alloantigens are major targets of GVL effects [12].

Given the risks of devastating GVHD after PD-1 inhibitors, lower doses and longer intervals of PD-1 inhibitor administration could be considered, but the optimal dose and schedule of PD-1 inhibitors after allogeneic SCT remain to be clarified [1]. While flowcytometric detection of nivolumab or pembrolizumab bound to T cells using anti-IgG4 antibodies is useful to study immunological sequences after PD-1 blockade [13, 14], a recent study showed that monitoring of PD-1 staining blockade is more sensitive way to detect restoration of binding between PD-L1 and PD-1 on cell surface [2]. This study suggested an association between restoration of PD-1 staining and relapse of lung cancer [2]. In our case, nivolumab was effective when prolonged nivolumab binding was evident, but restoration of PD-1 staining predicted tumor relapse. Furthermore, radiotherapy was only effective while persistent binding of nivolumab to T cells, suggesting that nivolumab enhanced radiation effect and abscopal effect. Administration of nivolumab

immediately after flowcytometric detection of PD-1 staining may restore anti-tumor immunity.

In summary, flowcytometric monitoring of nivolumab binding on T cells could be a potential biomarker to predict nivolumab effect and help determining dose and schedule of PD-1 blockade in high-risk patients for GVHD and immune-related adverse events, but this should be tested in the future clinical studies.

Acknowledgements This study was supported by the Japan Agency for Medical Research and Development (AMED, 19ek0510025h0002 to TT). We thank Ms. Chiaki Yokoyama for her technical assistance.

Author contribution DH and TT developed the conceptual framework of the study, designed and conducted the studies, analyzed the data and wrote the paper. RO, JS, FY, ST, NM, KO, and MO conducted the studies, analyzed the data and wrote the paper.

Funding This study was supported by the Japan Agency for Medical Research and Development (AMED, 19ek0510025h0002 to TT).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Herbaux C, Merryman R, Devine S, Armand P, Houot R, Morschhauser F, et al. Recommendations for managing PD-1 blockade in the context of allogeneic HCT in Hodgkin lymphoma: taming a necessary evil. *Blood*. 2018;132:9–16.
- Osa A, Uenami T, Koyama S, Fujimoto K, Okuzaki D, Takimoto T, et al. Clinical implications of monitoring nivolumab immunokinetics in non-small cell lung cancer patients. *JCI Insight*. 2018;3:e59125.
- Michot JM, Bigenwald C, Champiat S, Collins M, Carbone F, Postel-Vinay S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur J Cancer*. 2016;54:139–48.
- Merryman RW, Kim HT, Zinzani PL, Carlo-Stella C, Ansell SM, Perales MA, et al. Safety and efficacy of allogeneic hematopoietic stem cell transplant after PD-1 blockade in relapsed/refractory lymphoma. *Blood*. 2017;129:1380–8.
- Alessandrino F, Tirumani SH, Krajewski KM, Shinagare AB, Jagannathan JP, Ramaiya NH, et al. Imaging of hepatic toxicity of systemic therapy in a tertiary cancer centre: chemotherapy, haematopoietic stem cell transplantation, molecular targeted therapies, and immune checkpoint inhibitors. *Clin Radiol*. 2017;72:521–33.
- Chen YB, Perales MA, Li S, Kempner M, Reynolds C, Brown J, et al. Phase I multicenter trial of brentuximab vedotin for steroid-refractory acute graft-versus-host disease. *Blood*. 2017;129:3256–61.
- Buchwald ZS, Wynne J, Nasti TH, Zhu S, Mourad WF, Yan W, et al. Radiation, immune checkpoint blockade and the abscopal effect: a critical review on timing dose and fractionation. *Front Oncol*. 2018;8:612.
- Haverkos BM, Abbott D, Hamadani M, Armand P, Flowers ME, Merryman R, et al. PD-1 blockade for relapsed lymphoma

- post-allogeneic hematopoietic cell transplant: high response rate but frequent GVHD. *Blood*. 2017;130:221–8.
9. Herbaux C, Gauthier J, Brice P, Drumez E, Ysebaert L, Doyen H, et al. Efficacy and tolerability of nivolumab after allogeneic transplantation for relapsed Hodgkin lymphoma. *Blood*. 2017;129:2471–8.
 10. McCaul KG, Nevill TJ, Barnett MJ, Toze CL, Currie CJ, Sutherland HJ, et al. Treatment of steroid-resistant acute graft-versus-host disease with rabbit anti-thymocyte globulin. *J Hematother Stem Cell Res*. 2000;9:367–74.
 11. Van Lint MT, Milone G, Leotta S, Uderzo C, Scime R, Dallorso S, et al. Treatment of acute graft-versus-host disease with prednisolone: significant survival advantage for day +5 responders and no advantage for nonresponders receiving anti-thymocyte globulin. *Blood*. 2006;107:4177–81.
 12. Reddy P, Maeda Y, Liu C, Krijanovski OI, Korngold R, Ferrara JL. A crucial role for antigen-presenting cells and alloantigen expression in graft-versus-leukemia responses. *Nat Med*. 2005;11:1244–9.
 13. Kamphorst AO, Pillai RN, Yang S, Nasti TH, Akondy RS, Wientland A, et al. Proliferation of PD-1+ CD8 T cells in peripheral blood after PD-1-targeted therapy in lung cancer patients. *Proc Natl Acad Sci USA*. 2017;114:4993–8.
 14. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, et al. T cell invigoration to tumour burden ratio associated with anti-PD-1 response. *Nature*. 2017;545:60–5.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.