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Transition of the PD-1 occupancy of nivolumab on T cells after discontinuation and response of nivolumab re-challenge

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Abstract. Although nivolumab is administered every two or four weeks, high programmed cell death-1 (PD-1) binding of nivolumab on T cells lasting for several months has been reported. A relationship between the PD-1 occupancy rate on T-cells and the efficacy of nivolumab is not yet fully understood. The present study used flow cytometric analyses to determine the time-dependence of PD-1 occupancy in five patients who discontinued nivolumab. The relationship between PD-1 occupancy at relapse and the efficacy of re-challenge was also studied. Occupancies after discontinuation were measured at a total of 32 points. The data indicated that it took 32.4 and 48.9 weeks to decrease occupancy by 50 and 70%, respectively. Subsequently, two patients had recurrence and were re-challenged with nivolumab. At that time, one patient had 70.8% occupancy while the other had 6.6%. Treatment was effective only for the patient with lower occupancy. Overall, the present study suggests that re-challenge with nivolumab may be efficacious in patients with low occupancy at recurrence.

Introduction

Immune checkpoint inhibitors (ICI) targeting programmed cell death-1 (PD-1) have been developed as cancer immunotherapy agents (1,2). Given that PD-1 is an inhibitory immunogenic molecule, the ability of ICI to enhance the immune system allows it to eliminate cancer cells (1,2). Patients treated with nivolumab often expect longer-term survival than would be obtained with conventional chemotherapies, and overall survival curves have been seen to flatten in some clinical trials (3-5). However, extended administration of nivolumab raises concerns of immune-related adverse events (irAEs) and can create financial issues (6,7). Therefore, in some patients, nivolumab may be discontinued for reasons other than disease progression.

In some reports, long-lasting responses were observed in patients even after discontinuation of nivolumab treatment and no further anti-cancer treatment (8-10). Although the mechanisms of long-lasting response are not fully understood, prolonged binding of nivolumab on T cells is one possible explanation (11,12). Some groups have indeed reported prolonged nivolumab binding on T cells after the last nivolumab infusion, which has stimulated study of the kinetics of nivolumab binding after discontinuation of treatment (11,12). However, a relationship between nivolumab binding at relapse and the efficacy of nivolumab re-challenge has not been reported.

Here, we monitored PD-1 occupancy on T cells of five patients who discontinued nivolumab treatment for reasons other than tumor progression and determined the relationship between the level of nivolumab binding at relapse and the efficacy of additional nivolumab therapy.

Materials and methods

Patients and sample collections. Between May 2019 and June 2020, peripheral blood samples were collected from patients at Kobe University Hospital who had discontinued nivolumab administration after long-term use. Samples were collected in heparinized tubes. Peripheral blood mononuclear cells (PBMCs) then were isolated by density gradient centrifugation using Ficoll-Paque Plus (GE Healthcare, UK) and SepMate-50 tubes (STEMCELL Technologies, Canada), according to the manufacturer's instructions.

Flow cytometric analysis for PD-1 receptor occupancy. To detect nivolumab (a human IgG4 monoclonal antibody) binding to PD-1 molecules on circulating CD3+ T cells, PBMCs were incubated (4°C, 20 min) with a saturating concentration (20 μg/ml) of either unlabeled human IgG4 (isotype control) or nivolumab, washed extensively, and then co-stained with anti-human CD3-APC (Biolegend, USA) and murine anti-human IgG4 biotin (Invitrogen, USA) plus BV421 streptavidin (BD Biosciences, USA). PD-1 binding was determined as the ratio of the percent of CD3+ T cells stained

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with anti-human IgG4 after *in vitro* saturation with isotype control antibody (indicating *in vivo* binding) to that observed after nivolumab saturation (showing total available binding sites) (11). The flow cytometry gating strategy for analysis of occupancy rate is shown in Figs. S1 and S2.

Results

Patient characteristics. Five patients who discontinued nivolumab treatment for reasons other than tumor progression were investigated (Table I). Patient characteristics are described in Table I. One patient had completed a year of adjuvant nivolumab following surgery. The other four patients with recurrent disease had been treated with nivolumab for 52-104 weeks, but decided to discontinue treatment due to the stable state of their disease and concern over the development of irAEs. All patients underwent computed tomography scans every 3-4 months to monitor tumor growth, during which time no further cancer treatments were done.

Monitoring occupancy of nivolumab on PD-1 of CD3 T cells after discontinuation. We monitored the binding of nivolumab to T cells by flow cytometric analysis at 32 different times (6.4 points/patient). Linear regression analysis indicated that it took 32.4 and 48.9 weeks to decrease occupancies by 50 and 70%, respectively (Fig. 1). The individual ratios for each patient are shown in Fig. 2.

Relationship between PD-1 occupancy at relapse and efficacy of retreatment. Patients 4 and 5 had disease recurrence 28 and 45 weeks after their last nivolumab infusion, respectively, at which time treatment was re-initiated. Nivolumab binding at recurrence was 70.8 and 6.6%, respectively (Fig. 2). The flow cytometry plots for patients 4 and 5 are shown in Supplemental Fig. 2. Only treatment of the patient with 6.6% binding was efficacious (Fig. 3). Occupancy in Patient 5 at 4 weeks after re-administration was 68.3% (Fig. 2).

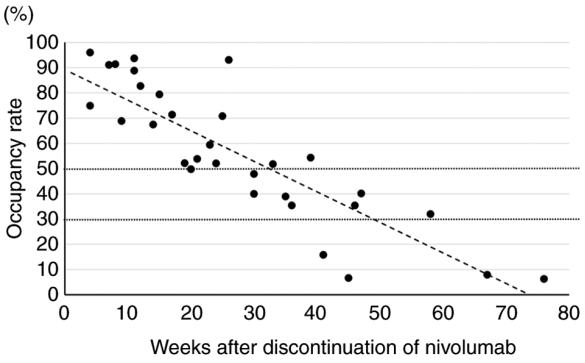

Figure 1. PD-1 binding vs. time. Binding of nivolumab to T cells was determined at 32 different time-points after discontinuation of treatment. The broken line was derived by linear regression of the data.

Discussion

The optimal duration of treatment with nivolumab remains unknown. In a largely community-based phase IIIb/IV study, CheckMate 153 (13), the effects of either one-year or continuous treatment were evaluated. An important result was that continuing nivolumab beyond one year improved outcomes (13). On the other hand, the financial burden and irAE occurrence on long-term use of nivolumab should be considered. We note that therapy with another anti-PD-1 antibody, pembrolizumab, was ended after 2 years in most clinical trials (14,15). Nivolumab discontinuation is one strategy that should be considered for patients with prolonged stable disease due to long-term use of nivolumab. Further studies about discontinuation of ICI are ongoing (16).

A few previous studies have examined the binding of nivolumab to PD-1 on T cells after nivolumab discontinuation (11,12). As in our study, Osa *et al* investigated nivolumab binding on T cells in lung cancer patients and reported that prolonged binding (more than 20 weeks) was maintained (12). Consistent with these reports, several studies have demonstrated prolonged remission in patients after the discontinuation of nivolumab therapy (8,17,18). Accordingly, a correlation may be present between long-term PD-1 occupancy and a prolonged treatment effect (9). In our study, two patients experienced disease recurrence after discontinuation of nivolumab. Patient 4 relapsed 28 weeks after discontinuation, at which point occupancy was 70.8%. This case might therefore suggest that relapse is not associated with a lack of nivolumab. It may instead be due to tumor cells developing resistance to the drug or to changes in the relationship between the cancer and its microenvironment, including immune cells and alterations in genes encoding components of the antigen processing and/or presentation apparatus (e.g., class I MHC, β2-microglobulin) (19,20). In this case, re-administration of nivolumab was not effective. In contrast, patient 5 relapsed 45 weeks after the last dose, displaying only 6.6% occupancy. In such cases, re-administration of nivolumab to increase the level of binding may be a useful strategy. In fact, occupancy quickly increased up to 68.3% at 4 weeks after re-administration.

In addition to occupancy, a number of other factors have been identified as being associated with the effectiveness of nivolumab. One group reported a relationship between metastatic sites and their numbers and the efficacy of ICI in patients with advanced non-small cell lung cancer (21). In their report, patients with brain metastases had shorter overall survival than those without, and a greater number of involved metastatic organs was related with a poorer response to ICIs. In our study, the two patients with recurrence both had a single site of recurrence (Patient 4, adrenal grand; and Patient 5, lymph node), and the relationship between the number of metastases and the effect is not clear. Further studies are needed to reveal other factors.

Some studies have investigated ICI re-challenge in cancer patients. One group reported an objective response rate on nivolumab re-challenge of 2.9% and disease control rate of 42.9% (20). Regarding pembrolizumab, another PD-1 antibody, a second group reported outcomes in patients who completed 2 years of pembrolizumab treatment: among 21 patients who underwent re-challenge after recurrence, 11 (52.4%) had an objective response (22). Furthermore, another group reported the results of pembrolizumab re-treatment in patients with melanoma who relapsed 6 months after completing 1 year of adjuvant pembrolizumab therapy. Although only 9 patients were evaluable, one achieved a complete response and three

Table I. Patient characteristics.

Patient	Age, years	Sex	Cancer type	Prior treatment	Reason for nivoluma[b] treatment (stage)	Dosing duration of nivoluma[b] (times)	Liver function[c] (AST/ALT U/ml)	Creatinine clearance[c] (Cockcroft-Gault, ml/min)	irAE[d] (Grade)	Recurrence[e] (number, site, size)
Patient 1	71	Male	Melanoma	Surgery	Adjuvant setting (Stage II)	52 weeks (22)	25/23	63.3	None	-
Patient 2	75	Male	Laryngeal cancer	Chemoradiotherapy[a] after surgery	Recurrence (Stage IV)	100 weeks (49)	23/16	25.2	Dermatitis (G1)	-
Patient 3	72	Female	Cancer of unknown primary in the head and neck	Chemoradiotherapy[a] after surgery	Recurrence (Stage IV)	98 weeks (47)	23/9	24.5	None	-
Patient 4	70	Male	Oropharyngeal cancer	Chemoradiotherapy[a] after surgery	Recurrence (Stage IV)	58 weeks (28)	20/16	51.5	Dermatitis (G1) Thyroiditis (G2)	1, Left adrenal grant, 15.6-mm
Patient 5	68	Male	Cancer of unknown primary in the mediastinal lymph node	Chemotherapy[b]	Metastasis (Stage IV)	104 weeks (50)	26/30	66.1	Thyroiditis (G2)	1, Right hilar lymph node, 25.1-mm

[a]Cisplatin and radiotherapy; [b]Carboplatin + Paclitaxel + Bevacizumab; [c]laboratory data at base line; [d]no patients required steroid treatment; [e]recurrence after nivolumab treatment. irAE, immune-related adverse event; AST/ALT, aspartate aminotransferase/alanine transaminase.

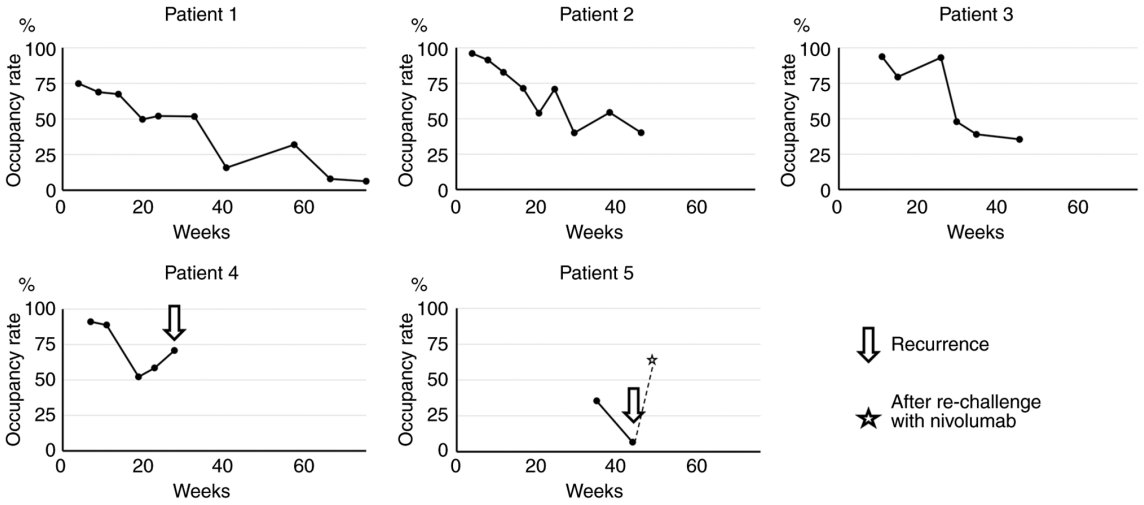

Figure 2. Temporal changes in nivolumab binding to T cell PD-1. The x-axis indicates weeks after the final dose of nivolumab. After discontinuation of treatment, binding was determined for each patient periodically. Arrows indicate when disease recurrence was observed in patients 4 and 5. The star indicates the occupancy rate after re-challenged with nivolumab in Patient 5.

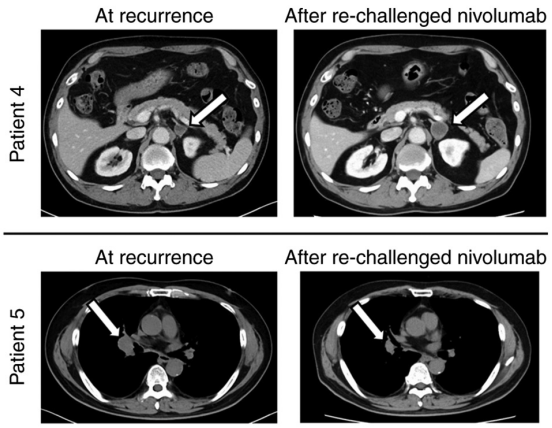

Figure 3. Axial computed tomography of patients 4 and 5 at the time of recurrence and after resumption of nivolumab treatment. Arrows indicate each tumor.

patients were categorized with stable disease by re-challenge (23). These reports suggest that re-challenge might even benefit those patients with a history of disease progression after ICI therapy. Nevertheless, it remains unclear which patients will benefit from re-challenge. Research into the relationship between occupancy and efficacy may aid the identification of candidates for re-administration.

Our study has some limitations. First, it was a small case series and only two patients received re-administration of nivolumab. Clarifying the relationship between efficacy of re-administration and occupancy rate will require prospective confirmation in larger studies. Second, compared with the serum half-life of nivolumab, occupancy is maintained for an extended period. However, the mechanism underlying the long-term residual binding of nivolumab on T cells is still unclear. Furthermore, factors influencing the fluctuation of binding rate are also unclear. Although two of five patients had reduced renal function in our study, there was no clear difference in the trend in occupancy between these patients and those with normal renal function. Further research into the pharmacokinetics of this agent is required. Third, in patients 4 and 5, because the period between the date of registration and recurrence was short, only five and two measurements were made, respectively. These numbers may be insufficient to clarify the precise trends. We also do not have data on occupancy during nivolumab treatment in all patients. To improve the accuracy of the trend, measurement at additional points would be necessary. Finally, although we believe this measurement method for occupancy is sufficient to show a trend, degree of measurement error will be present, owing to the complicated and multi-process nature of FACS.

In conclusion, nivolumab occupied PD-1 on T cells for an extended period. Re-challenge with nivolumab may provide a good response in patients with lowered occupancy.

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Availability of data and materials

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

Authors' contributions

TN and YF conceived and designed the study, collected, analyzed and interpreted the data and drafted and wrote the article. HS and YN collected and interpreted the data. YI, MT and NK collected and interpreted the data, and were involved in patient management. HM supervised the project, interpretated the data and reviewed and revised the manuscript. All authors read and approved the final version of the article. TN and YF confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present investigation was approved by the Kobe University Hospital Ethics Committee (approval no. 180152) and was conducted in accordance with the Declaration of Helsinki.

Patient consent for publication

All patients provided written informed consent for this investigation and publication of their data.

Competing interests

Hironobu Minami has received research grants and honoraria from Bristol-Myers Squibb and Chugai Pharmaceutical. Naomi Kiyota has received research grants from Bristol-Myers Squibb. The other authors declare that they have no competing interests.

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