Global Health & Medicine. 2022; 4(6):301-308.
ORIGINAL ARTICLE
DOI: 10.35772/ghm.2022.01052

Association between increased peripheral blood CD86-positive plasmacytoid dendritic cells and immune-related adverse events in patients with non-small cell lung cancer

Masato Karayama[1,2,*], Yukihiro Mizoguchi[3], Yusuke Inoue[1], Hironao Hozumi[1], Yuzo Suzuki[1], Kazuki Furuhashi[1], Tomoyuki Fujisawa[1], Noriyuki Enomoto[1], Yutaro Nakamura[1], Naoki Inui[4], Takafumi Suda[1], Shigehisa Kitano[5], Kazunori Aoki[3], Yasuhide Yamada[2]

[1] Second Division, Department of Internal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan;
[2] Department of Medical Oncology, Hamamatsu University School of Medicine, Hamamatsu, Japan;
[3] Department of Immune Medicine, National Cancer Center Research Institute, Tokyo, Japan;
[4] Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, Hamamatsu, Japan;
[5] Division of Cancer Immunotherapy Development, Advanced Medical Development Center, The Cancer Institute Hospital of JFCR, Tokyo, Japan.

Abstract: The occurrence of immune-related adverse events (irAEs) after immune checkpoint inhibitors (ICIs) is unpredictable. Profiles of peripheral blood mononuclear cells (PBMCs) represent the host immune system and have the potential to predict irAEs. We analyzed PBMC subsets using multicolor flow cytometry before and at weeks 2 and 8 after the start of ICIs in patients with non-small cell lung cancer. Sixteen eligible patients were evaluated. The irAEs occurred in 6 patients (37.5%): diarrhea in 2, diarrhea and a rash in 1, pituitary dysfunction in 1, cholangitis in 1, and pneumonitis in 1. Patients experiencing irAEs had higher levels of $CD86^+$plasmacytoid dendritic cells (pDCs) at the baseline and weeks 2 and 8 after the ICIs than those not experiencing irAEs (p = 0.005, 0.038, and 0.050, respectively). In patients experiencing irAEs, the levels of $CD86^+$pDCs significantly decreased at weeks 2 and 8 compared to the baseline (p = 0 .034 and 0.025, respectively) but did not change in those not experiencing irAEs. The levels of other PBMC subsets were not significantly associated with irAEs. Higher levels of natural killer (NK) cells were significantly associated with an overall objective response (p = 0.024). In conclusion, higher levels of $CD86^+$pDCs at the baseline and a reduction in those levels 2 and 8 weeks after ICIs were associated with the occurrence of irAEs. Higher levels of NK cells were associated with an objective response to ICIs. Evaluation of PBMCs may help to predict the efficacy and safety of ICIs.

***Keywords*:** immunotherapy, immune-related adverse events (irAEs), immune checkpoint inhibitors (ICIs), peripheral blood mononuclear cells (PBMCs), $CD86^+$plasmacytoid dendritic cells (pDCs)

Introduction

Immune checkpoint inhibitors (ICIs) are an important part of cancer therapy. Conventional chemotherapy has direct toxicity to tumor cells, but ICIs activate anti-tumor immune responses that provide unprecedented clinical benefits. Treatment of patients with ICIs may result in immune-related adverse events (irAEs), which are defined as a spectrum of side effects that mimic autoimmune diseases (*1*). ICI treatment can cause irAEs in any organ at any time, hampering the prediction of their occurrence in clinical practice (*2*).

Assessment of host–tumor interactions and tumor characteristics is essential to understanding the modulatory effects of ICIs on the immune system of cancer patients. Research has increasingly revealed that the host immune status, assessed using peripheral blood mononuclear cell (PBMC) profiles, is associated with the clinical efficacy of ICIs. For example, higher levels of active natural killer (NK) cells and programmed death-1 (PD-1) positive $CD8^+$T cells in peripheral blood are associated with the clinical response to nivolumab, an anti-PD-1 antibody (*3*). Moreover, increased NK cells and decreased myeloid-derived suppressor cells are associated with the clinical response to nivolumab (*4*).

Little is known about the association between irAEs and PBMCs. The occurrence of irAEs after ICI treatment is likely caused by an activated immune system. A positive relationship exists between the efficacy of ICIs and the development of irAEs, which suggests common underlying mechanisms (*5,6*). Since irAEs may represent enhanced immune function, assessment

of PBMC profiles may provide useful information for understanding the mechanisms for development of irAEs. Using advanced multicolor flow cytometric analysis, this exploratory study comprehensively assessed PBMC profiles before and after ICI therapy and it analyzed the association between immune cell subsets and irAEs in patients with advanced non-small cell lung cancer (NSCLC).

Materials and Methods

Study design

This was an exploratory, prospective observational study conducted in accordance with the ethical standards of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of the Hamamatsu University School of Medicine (No. 16-080). Each patient provided written informed consent. The study was registered with the University Hospital Medical Information Network Clinical Trial Registry (000026140).

Patient eligibility

Patients were included in this study if they presented with inoperable stage IIIB or IV NSCLC, had an Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0–2, and were scheduled for anti-PD-1 therapy. Patients who had a history of ICI therapy were excluded. The choice of anti-PD-1 therapy depended on the treating physician, and therapy was administered intravenously as follows: nivolumab at a dose of 3 mg/kg every 2 weeks or pembrolizumab at a dose of 200 mg every 3 weeks.

Treatment and evaluation schedule

Blood samples were collected before and at weeks 2 and 8 after the initiation of anti-PD-1 therapy. Chest computed tomography was performed before and 4 and 8 weeks after the initiation of anti-PD-1 therapy and repeated every 8 weeks until the cessation of ICI therapy. The expression of PD-L1 was assessed using the Dako 22C3 pharmDx (Agilent Technologies Santa Clara, CA, USA). Adverse events were graded using the Common Terminology Criteria for Adverse Events, version 5.0. Radiological response was evaluated according to the Response Evaluation Criteria in Solid Tumors, version 1.1.

Measurements of PBMCs

A total of 16 mL of peripheral blood samples was collected using two preparation tubes (8mL for each) containing sodium heparin (BD Vacutainer CPT; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The collection tubes were gently inverted 8 to 10 times to mix the anticoagulant with blood. After centrifugation at 1500 × g for 15 min at room temperature, PBMCs were present in a whitish layer just under the plasma layer. The PBMCs were collected with Pasteur pipettes, cryopreserved immediately in CellBanker 1® medium (Takara Bio Inc., Tokyo, Japan), and stored at −80°C. Aliquots of the frozen PBMCs were suspended in 50 μL phosphate buffered saline (PBS). Then, 50 μL of fluorescent labeled-surface marker antibody (Supplementary Table S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=57*) with Brilliant Stain Buffer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was combined with PBMCs to constitute 100 μL of cell solution, and the solution was incubated for 20 min at 4°C. PBMCs were immediately washed once with 2.0 mL of PBS, and after washing, samples were fixed using BD CellFIX (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). At least 50,000 live events were detected using an LSRFortessa™ X-20 flow cytometer with FACSDiva™ software (BD Biosciences, San Jose, CA, USA). For analysis of the immune cell profile, at the beginning of the gating strategy, the discrimination of single cells from doublets was performed using a forward scatter-area (FSC-A) versus forward scatter-height (FSC-H) bivariate plot, followed by use of a side scatter-area (SSC-A) versus side scatter-height (SSC-H) bivariate plot. Then, dead cells were removed using Fixable Viability Dye. All flow cytometric analyses were performed using FlowJo (BD Biosciences). Details of the FCM panel and gating strategy are described in Supplementary Figure S1 (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=57*).

Different immune cell populations were analyzed as follows: $CD4^+$T cells, $CD8^+$T cells, B cells, monocytes, proinflammatory monocytes, monocytic myeloid derived suppressor cells (mMDSCs), myeloid dendritic cells (mDCs), $CD141^+$ dendritic cells (DCs), plasmacytoid dendritic cells (pDC), basophils, natural killer (NK) cells, minor NK cells, and NK T cells (NKT). To evaluate the activation status, the expression of CD80, CD86, CD274 (PD-L1), and CD273 (PD-L2) was analyzed for each immune cell type. The definitions of immune cell types are described in Supplementary Tables S1 and S2 (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=57*). Immune cell populations were expressed as a percentage of PBMCs, and the activation status of each immune cell type was expressed as a percentage of the corresponding immune cell population.

Statistical analyses

The Wilcoxon rank sum test was used to compare $CD86^+$plasmacytoid dendritic cell (pDC) levels between the patients experiencing and not experiencing irAEs. A *p*

value < 0.05 (two-sided) was considered significant. All values were analyzed using the software JMP, version 13.0.0 (SAS Institute Japan, Tokyo, Japan).

Results

Patient characteristics

From September 2016 to December 2018, 23 patients were enrolled in this study. Of those, 7 patients were excluded from analysis because of rapid disease progression, which caused early withdrawal from ICI therapy (n = 3), and insufficient PBMC availability (n = 4). As a result, 16 patients had evaluable blood samples before ICI treatment and were available for analysis (Table 1). In addition to the baseline samples, 14 and 9 patients had evaluable blood samples at weeks 2 and 8, respectively, after the initiation of anti-PD-1 therapy. Only 1 patient (case 12) had a mutation in the epidermal growth factor receptor gene; the other patients had no oncogenic driver mutations. Eleven patients (68.8%) had positive PD-L1 expression with tumor proportion scores (TPS) ≥ 1%, 8 (50.0%) of whom had TPS ≥ 50%. No patients had a history of autoimmune disease or received systemic steroid therapy or immunosuppressive agents.

Delivery of ICI therapy

Nivolumab was administered to 10 patients (62.5%) as second line or later therapy, and pembrolizumab was administered to 6 patients (37.5%) as first line therapy. An objective response was observed in 9 patients (56.3%); 3 (18.8%) had stable disease and 4 (25.0%) had progressive disease. The median duration of anti-PD-1 treatment was 3.9 months (range: 1.0–18.5 months), and the median observation time was 12.3 months (range: 3.1–24.6 months).

Occurrence of irAEs

The occurrence of irAEs was observed in 6 patients (37.5%) (Table 2). The median time to an irAE was 44 days (range: 7–133 days). Systemic steroid therapies were administered to 3 patients, and the patient with pituitary dysfunction received a low-dose mineralocorticoid supplementation. Patient 1

Table 1. Characteristics of the patients in this study who presented with non-small cell lung cancer

Case	Group	Age, years	Sex	ECOG-PS	Stage	Pathology	PD-L1: TPS, %	Treatment line of ICI	ICI therapy	Best response
1	irAE	79	Female	1	IV	Ad	Unknown	2nd	Nivolumab	PD
2	irAE	73	Male	1	IV	Ad	≥ 50	1st	Pembrolizumab	PR
3	irAE	77	Female	1	IV	Ad	≥ 50	1st	Pembrolizumab	SD
4	irAE	81	Male	0	IV	Ad	≥ 50	1st	Pembrolizumab	PR
5	irAE	70	Male	1	IV	Sq	0	4th	Nivolumab	CR
6	irAE	56	Male	0	IV	Ad	≥ 50	3rd	Nivolumab	CR
7	Non-irAE	69	Male	1	IIIB	Ad	≥ 50	2nd	Nivolumab	PR
8	Non-irAE	67	Male	1	IV	Ad	0	2nd	Nivolumab	PD
9	Non-irAE	48	Male	0	IV	Others	1–49	2nd	Nivolumab	PD
10	Non-irAE	66	Male	0	IV	Ad	1–49	3rd	Nivolumab	PD
11	Non-irAE	41	Male	1	IV	Others	1–49	2nd	Nivolumab	PR
12	Non-irAE	73	Male	1	IV	Ad	0	7th	Nivolumab	SD
13	Non-irAE	69	Male	1	IV	Ad	≥ 50	1st	Pembrolizumab	PR
14	Non-irAE	77	Male	0	IV	Sq	≥ 50	1st	Pembrolizumab	CR
15	Non-irAE	65	Male	0	IV	Ad	≥ 50	1st	Pembrolizumab	CR
16	Non-irAE	58	Female	0	IV	Ad	0	3rd	Nivolumab	SD

Ad, adenocarcinoma; CR, complete response; ECOG-PS, Eastern Cooperative Oncology Group-performance status; ICI, immune checkpoint inhibitor; irAE, immune-related adverse event; PD, progressive disease; PD-L1, programmed death-ligand 1; PR, partial response; SD, stable disease; Sq, squamous cell carcinoma; TPS, tumor proportion score.

Table 2. The clinical courses of six patients with non-small cell lung cancer who developed immune-related adverse events

Case	irAE	Grade	Time to an irAE, days	ICI therapy	Treatment for irAE	Outcomes of irAE
1	Diarrhea	1	7	Continued	Antidiarrheal	Not alleviated
	Rash	1	28		Steroid ointment	
2	Diarrhea	2	84	Discontinued	Antidiarrheal	Alleviated
3	Diarrhea	3	23	Discontinued	Antidiarrheal and oral steroid	Alleviated
4	Pneumonitis	2	119	Discontinued	Oral steroid	Alleviated
5	Pituitary dysfunction	3	133	Discontinued	Mineralocorticoid supplementation	Not alleviated
6	Cholangitis	5	57	Discontinued	Intravenous steroid and immunosuppressant	Not alleviated/died

ICI, immune checkpoint inhibitor; irAE, immune-related adverse event.

developed grade 1 diarrhea and a rash at 7 and 28 days, respectively, which was alleviated by antidiarrheal and steroid ointment without systemic steroid therapy. Patient 3 developed grade 3 diarrhea 23 days after the start of ICI therapy; however, an antidiarrheal was administered first and then oral steroids were initiated 63 days after the start of ICI therapy (40 days after the development of diarrhea). Thus, no patient received systemic steroids or immunosuppressive agents during the 8 weeks of the period studied. The occurrence of irAEs was not significantly associated with age, sex, or ECOG-PS.

Association between CD86$^+$pDCs and the occurrence of irAEs

Compared to 10 patients not experiencing irAEs, the 6 patients who experienced irAEs had significantly higher CD86$^+$pDC levels at the baseline (a median of 3.15% *vs.* 15.0%, p = 0.005) (Figure 1A). CD86$^+$myeloid dendritic cells (mDCs) and the other analyzed PBMC subsets were not significantly associated with the occurrence of irAEs (Figure 1B, Supplementary Table S3, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=57*). CD86$^+$pDCs levels in patients experiencing irAEs decreased significantly weeks 2 and 8 after ICI therapy compared to the baseline values for those patients (p = 0.034 and 0.025, respectively); however, the levels did not change from the baseline in patients not experiencing irAEs (Figure

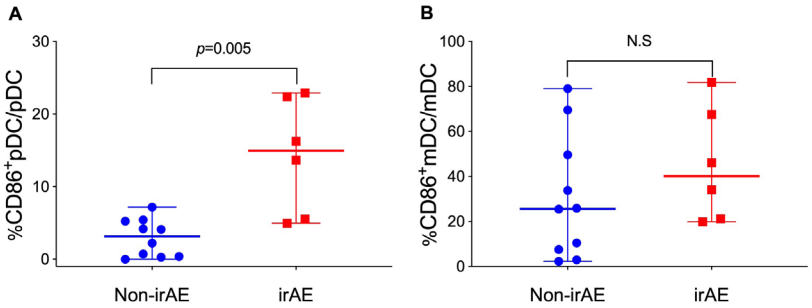

Figure 1. Association between circulating dendritic cells at the baseline and the occurrence of immune-related adverse events. Levels of circulating **(A)** CD86$^+$plasmacytoid dendritic cells (pDCs) and **(B)** CD86$^+$myeloid dendritic cells (mDCs) at the baseline. Patients experiencing and not experiencing immune-related adverse events (irAEs) are indicated in red and blue, respectively. Horizontal lines and error bars represent the median and the minimum and maximum, respectively. Activation status is expressed as a percentage of each corresponding immune cell type.

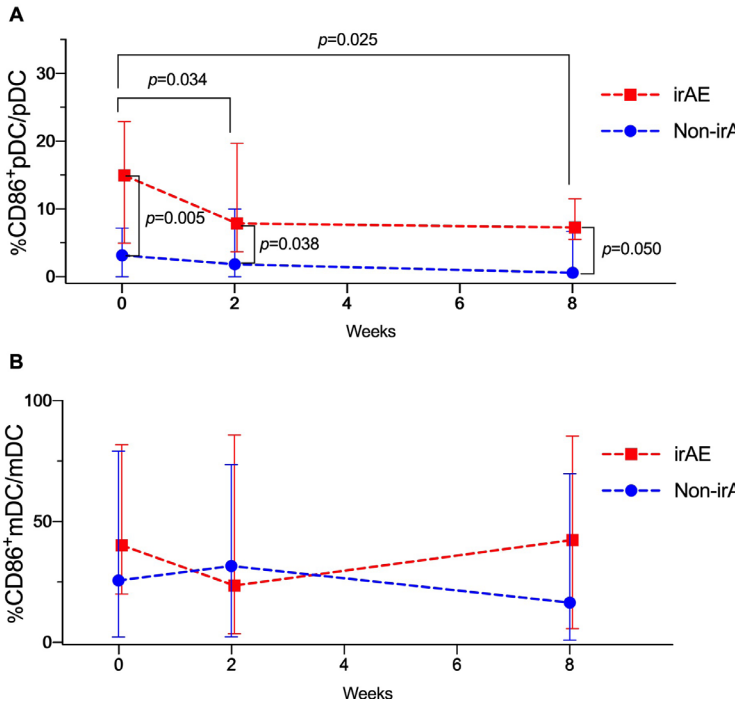

Figure 2. Changes in circulating dendritic cells after treatment with immune checkpoint inhibitors in patients experiencing and not experiencing immune-related adverse events. Changes in circulating **(A)** CD86$^+$plasmacytoid dendritic cells (pDCs) and **(B)** CD86$^+$myeloid dendritic cells (mDCs) at weeks 2 and 8 during treatment with immune checkpoint inhibitors (ICIs). Patients experiencing and not experiencing immune-related adverse events (irAEs) are displayed in red and blue, respectively. Horizontal lines and error bars represent the median and the minimum and maximum, respectively. Activation status is expressed as a percentage of each corresponding immune cell type.

2A). Even with decreased levels after ICI treatment, the patients experiencing irAEs still had significantly higher CD86$^+$pDC levels at 2 weeks (p = 0.038) and tended to have higher levels at 8 weeks (p = 0.050) compared to those not experiencing irAEs (Figure 2A). A decreased proportion of CD86$^+$pDC after ICI therapy was observed in all patients experiencing irAEs (except Patient 3, who did not have evaluable samples 2 and 8 weeks after ICI therapy). The time course of CD86$^+$pDC in individual patients is shown in Supplementary Figure S2 (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=57*). The representative flow cytometric data for CD86$^+$pDCs in patients experiencing and not experiencing irAEs are shown in Figure 3. The levels of CD86$^+$mDCs and other PBMC subsets did not change 2 and 8 weeks after the initiation of ICI therapy (Figure 2B). CD86$^+$pDC levels were not closely associated with the grade of or time to an irAE (Supplementary Figure S3-S4, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=57*). An increased proportion of NK cells in PBMCs was significantly associated with the objective response rate (ORR, p = 0.024), whereas the other PBMC subsets and activation status, including that of CD4$^+$T cells, CD8$^+$T cells, B cells, CD86$^+$pDCs, and CD86$^+$mDCs, were not associated with the ORR (Figure 4A–F). CD86$^+$pDC levels were not significantly associated with age, sex, or ECOG-PS.

Discussion

This prospective exploratory study comprehensively assessed multiple PBMC subsets in patients with NSCLC who were receiving ICI therapy. The patients who experienced irAEs had increased CD86$^+$pDC levels at the baseline compared to patients who did not experience irAEs. Moreover, CD86$^+$pDC levels in patients experiencing irAEs decreased significantly weeks 2 and 8 after initiation of anti-PD-1 therapy. The CD86$^+$mDC levels were not associated with irAEs. In addition, an increased proportion of NK cells in PBMCs was associated with the efficacy of ICI. Results indicate that the assessment of PBMC subsets may aid in predicting the efficacy and safety of ICI therapy.

DCs are a group of antigen-presenting cells that stimulate T cells *via* co-stimulatory factors, including CD86 (also known as B7-2) (*7,8*). Among several subsets of DCs, pDCs produce type I interferons (IFN-I) in response to viruses, unlike mDCs, which mainly act as conventional antigen-presenting cells (*9*). Although pDCs constitute only 0.2%–0.8% of PBMCs, emerging evidence suggests that pDCs contribute to the pathogenesis of autoimmune diseases including systemic lupus erythematosus (SLE), systemic sclerosis, psoriasis, autoimmune thyroid diseases, type I diabetes, autoimmune pancreatitis, and inflammatory bowel diseases (IBDs), as well as having a role in immune responses to infectious diseases (*10-16*). For example, antibody-mediated depletion of pDCs reduced type I interferon responses and disease activity in patients with cutaneous lupus (*10*). In patients with IBDs, increased numbers of pDCs were observed in inflamed mucosa, and increased levels of peripheral CD86$^+$pDCs were associated with disease activity (*14*). Moreover, patients with type I diabetes had significantly higher pDC levels in their peripheral blood than healthy individuals (*17*). Interestingly, autoimmune thyroid disease, IBDs, and

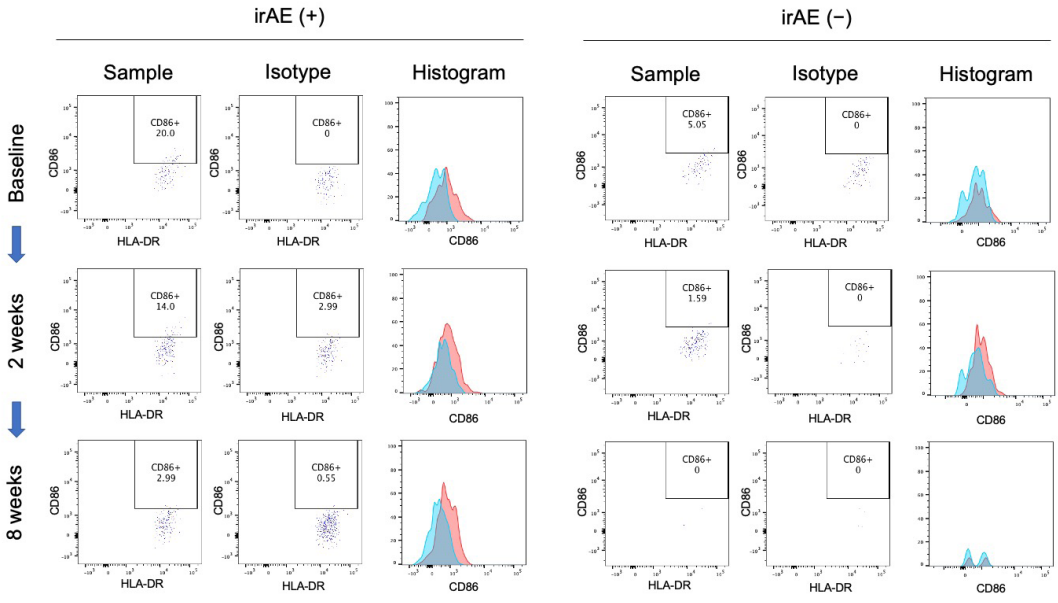

Figure 3. Representative flow cytometric data for CD86$^+$pDCs. Levels of circulating CD86$^+$ plasmacytoid dendritic cells (pDCs) in patients experiencing and not experiencing immune-related adverse events (irAEs) at the baseline and 2 and 8 weeks after immune checkpoint inhibitor therapy.

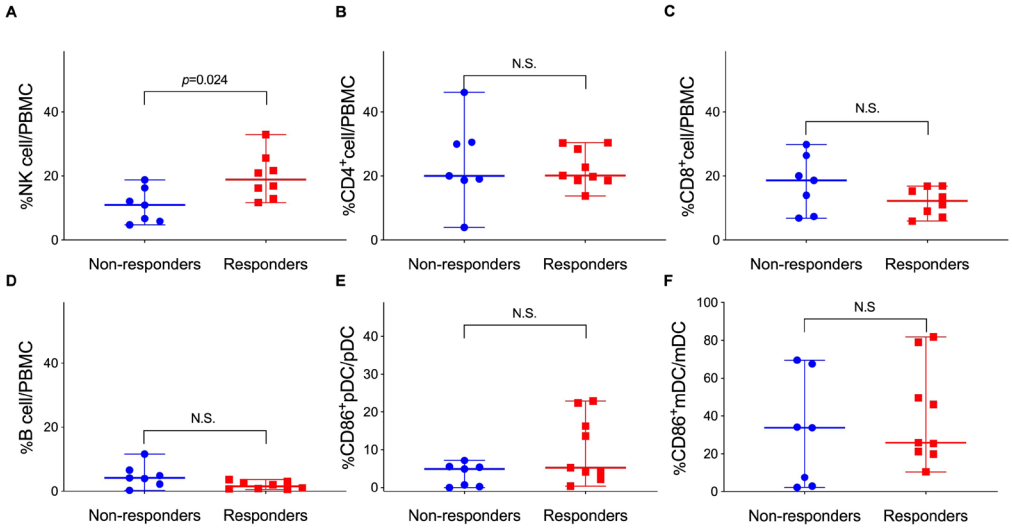

Figure 4. Association between circulating immune cells and the efficacy of immune checkpoint inhibitor therapy. Circulating **(A)** natural killer cells (NK cells), **(B)** $CD4^+$T cells, **(C)** $CD8^+$T cells, **(D)** B cells, **(E)** $CD86^+$plasmacytoid dendritic cells (pDCs), and **(F)** $CD86^+$myeloid dendritic cells (mDCs) at the baseline. Patients in whom an objective response was achieved (responders) and those in whom such a response was not achieved (non-responders) are displayed in red and blue, respectively. Horizontal lines and error bars represent the median and the minimum and the maximum, respectively. Data are expressed as a percentage of peripheral blood mononuclear cells (PBMCs) (A–D) or each immune cell type (E, F).

type I diabetes are known to develop after ICI therapy. Given the autoimmune nature of irAEs, one can reasonably deduce that an increase in activated pDCs at the baseline may affect the occurrence of irAEs.

The decrease in $CD86^+$pDC levels after ICI therapy in patients experiencing irAEs displayed another interesting similarity to autoimmune diseases. In patients with type I diabetes, the increased number of peripheral blood pDCs at diagnosis tended to decrease after 2 years whereas the number of peripheral pDCs was stable over 2 years in controls (*17*). In patients with SLE, psoriasis, and autoimmune thyroid diseases, pDC levels decreased in peripheral blood instead of increasing (*13,15,18*). This seemingly paradoxical behavior is thought to be due to an accumulation of pDCs in tissue lesions. In fact, increased pDCs have been observed in involved organs (such as the skin, lymph nodes, kidneys, or thyroid) in patients with SLE, psoriasis, or autoimmune thyroid diseases (*13,15,19*). The decrease in circulating $CD86^+$pDCs after ICI therapy may reflect the recruitment of these cells from the peripheral blood to specific organs.

The accumulation of pDCs in lesional tissues is a common characteristic in several autoimmune diseases; however, controversy exists regarding the levels of circulating pDCs. Those levels increased in type I diabetes and IBDs and decreased in SLE, psoriasis, or autoimmune thyroid diseases (*13-15,17,19*). Although the precise mechanisms involved are unknown, the timing of the evaluation during the course of disease (at diagnosis or after progression) or the specific organs involved may be associated with differences in circulating pDCs among autoimmune diseases.

The pathogenesis of irAEs and differences in that pathogenesis among different irAEs are largely unknown. Shared antigens between tumors and organs, inflammation generated by cytokines and activated immune cells, or pre-existing organ inflammation (*i.e.* autoimmune diseases) might be potential mechanisms (*5*). irAEs occur in a wide variety of organs, and therefore, organ-specific mechanisms may exist for each irAE. However, some common mechanisms might also be involved because all of the irAEs were caused by autoimmunity triggered by ICIs. As well as having a role in innate immunity by producing IFN-I, pDCs promote the differentiation and maintenance of autoreactive B cells (*20,21*). Given their multiple roles in innate and adaptive immunity, pDCs might be involved in various irAEs. However, there were no obvious differences in the baseline levels of $CD86^+$pDCs among the different types of irAEs. Moreover, a decreased proportion of $CD86^+$pDCs was observed after ICI therapy regardless of the type of irAE. These results indicate that pDCs were associated with a common mechanism across irAEs. However, the current study evaluated a limited number and type of irAEs. The organ-specific roles of pDCs should be investigated further.

The association between increased peripheral blood NK cell levels and ICI efficacy has previously been reported and was also observed in the current study (*3,22*). In animal models, depletion of NK cells attenuated the anti-tumor effects of a PD-1/PD-L1 blockade. In addition, PD-1/PD-L1 signaling resulted in reduced anti-tumor activity of NK cells, which was restored after a PD-1/PD-L1 blockade (*23*). The underlying mechanisms are largely unknown; however, these results indicate the considerable importance of NK cells in ICI therapy as a predictor of efficacy or delivery to the therapeutic target.

The current study had four main limitations. First,

this was an exploratory study with a limited number of subjects. Multiple surface markers were evaluated on immune cells, and this may have led to false positives. Moreover, PBMCs contained a small fraction of pDCs, and therefore, the results of this study should be interpreted with caution. To validate the utility of $CD86^+$pDC levels in predicting irAEs, further studies need to be conducted with larger cohorts. Second, the association between $CD86^+$pDC levels and the severity of or time to onset of irAEs was not evaluated because of the limited number of the patients. Moreover, irAEs are considered to be the result of complex immune responses. Although the current findings suggest that $CD86^+$pDCs play an important role in the occurrence of irAEs, other unknown factors may be associated with the severity of or time to onset of irAEs. The differences in pDC function among the organs involved in the irAEs are also unknown. ICI-induced irAEs are a heterogeneous group of conditions that occur in various organs, and the roles of pDCs may differ depending on the irAE phenotype. Third, differences in the distribution of immune cells between peripheral blood and organs were not determined. Although circulating $CD86^+$pDC levels were not associated with the efficacy of ICI treatment, tumor-infiltrating pDCs likely play an essential role in cancer immunity. In animal models, tumor-infiltrating pDCs induced antitumor immunity by activating NK cells, conventional DCs, and $CD8^+$T cells (*24*). In the tumor microenvironment, anti-tumor activity by tumor-infiltrating pDCs is attenuated by tumor-derived soluble factors, including transforming growth factor-β, which results in immune tolerance to the tumor (*25*). Fourth, the current study only evaluated anti-PD-1 therapy. Several single or combinatorial ICI therapeutic strategies exist, including anti-PD-ligand(L)1 or anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4) antibody therapy, combinations of ICI with chemotherapy, and combinations of ICI antibody therapies. Immune status may differ depending on the type of immunotherapy used. In fact, the combination of anti-PD-1 and anti-CTLA-4 therapeutics results in frequent irAEs (*26,27*). CTLA-4 suppresses T cell activity by binding to CD86; therefore, $CD86^+$pDC behavior during anti-CTLA-4 therapy is of interest. Immunotherapies will become more diversified in the near future as new immunomodulating agents emerge. The association between host immune status and immunotherapy efficacy and safety should be investigated further.

In conclusion, NSCLC patients who experienced irAEs had increased $CD86^+$pDC levels in their peripheral blood at the baseline. These $CD86^+$pDC levels decreased after ICI treatment. Assessment of host immune status by profiling PBMCs may help to predict the efficacy and safety of ICI treatment.

Funding: None.

Conflict of Interest: Dr. Aoki received grants from Ono Pharmaceutical Co., Eisai Co., Daiichi Sankyo Company, Chugai Pharmaceutical Co., Otsuka Pharmaceutical Co., and Chiome Bioscience Inc. and honoraria for lectures from Bayer Yakuhin outside the submitted work. Dr. Yamada received honoraria for lectures from Nippon Boehringer Ingelheim, Ono Pharmaceutical Co. and Taiho Pharmaceutical Co. outside the submitted work. No other disclosures were reported.

References

1. Chen TW, Razak AR, Bedard PL, Siu LL, Hansen AR. A systematic review of immune-related adverse event reporting in clinical trials of immune checkpoint inhibitors. Ann Oncol. 2015; 26:1824-1829.
2. Brahmer JR, Lacchetti C, Schneider BJ, *et al.* Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol. 2018; 36:1714-1768.
3. Mazzaschi G, Facchinetti F, Missale G, Canetti D, Madeddu D, Zecca A, Veneziani M, Gelsomino F, Goldoni M, Buti S, Bordi P, Aversa F, Ardizzoni A, Quaini F, Tiseo M. The circulating pool of functionally competent NK and CD8+ cells predicts the outcome of anti-PD1 treatment in advanced NSCLC. Lung Cancer. 2019; 127:153-163.
4. Youn JI, Park SM, Park S, *et al.* Peripheral natural killer cells and myeloid-derived suppressor cells correlate with anti-PD-1 responses in non-small cell lung cancer. Sci Rep. 2020; 10:9050.
5. Das S, Johnson DB. Immune-related adverse events and anti-tumor efficacy of immune checkpoint inhibitors. J Immunother Cancer. 2019; 7:306.
6. Maher VE, Fernandes LL, Weinstock C, *et al.* Analysis of the association between adverse events and outcome in patients receiving a programmed death protein 1 or programmed death ligand 1 antibody. J Clin Oncol. 2019; 37:2730-2737.
7. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 2013; 13:227-242.
8. Bourque J, Hawiger D. Immunomodulatory bonds of the partnership between dendritic cells and T cells. Crit. Rev. Immunol. 2018; 38:379-401.
9. Reizis B. Plasmacytoid dendritic cells: Development, regulation, and function. Immunity. 2019; 50:37-50.
10. Karnell JL, Wu Y, Mittereder N, *et al.* Depleting plasmacytoid dendritic cells reduces local type i interferon responses and disease activity in patients with cutaneous lupus. Sci Transl Med. 2021; 13:eabf8442
11. Caielli S, Athale S, Domic B, *et al.* Oxidized mitochondrial nucleoids released by neutrophils drive type I interferon production in human lupus. J Exp Med. 2016; 213:697-713.
12. van Bon L, Affandi AJ, Broen J, *et al.* Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis. N Engl J Med. 2014; 370:433-443.
13. Nestle FO, Conrad C, Tun-Kyi A, Homey B, Gombert M, Boyman O, Burg G, Liu YJ, Gilliet M. Plasmacytoid predendritic cells initiate psoriasis through interferon-α production. J Exp Med. 2005; 202:135-143.
14. Baumgart DC, Metzke D, Guckelberger O, Pascher A, Grötzinger C, Przesdzing I, Dörffel Y, Schmitz J, Thomas

S. Aberrant plasmacytoid dendritic cell distribution and function in patients with Crohn's disease and ulcerative colitis. Clin Exp Immunol. 2011; 166:46-54.
15. Leskela S, Rodríguez-Muñoz A, De La Fuente H, Figueroa-Vega N, Bonay P, Martín P, Serrano A, Sánchez-Madrid F, González-Amaro R, Marazuela M. Plasmacytoid dendritic cells in patients with autoimmune thyroid disease. J Clin Endocrinol Metab. 2013; 98:2822-2833.
16. Minaga K, Watanabe T, Hara A, Yoshikawa T, Kamata K, Kudo M. Plasmacytoid dendritic cells as a new therapeutic target for autoimmune pancreatitis and IgG4-related disease. Front Immunol. 2021; 12: 713779.
17. Allen JS, Pang K, Skowera A, Ellis R, Rackham C, Lozanoska-Ochser B, Tree T, Leslie RD, Tremble JM, Dayan CM, Peakman M. Plasmacytoid dendritic cells are proportionally expanded at diagnosis of type 1 diabetes and enhance islet autoantigen presentation to T-cells through immune complex capture. Diabetes. 2009; 58:138-145.
18. Cederblad B, Blomberg S, Vallin H, Perers A, Alm GV, Rönnblom L. Patients with systemic lupus erythematosus have reduced numbers of circulating natural interferon-α-producing cells. J Autoimmun. 1998; 11:465-470.
19. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon-α/β-producing cells) accumulate in cutaneous lupus erythematosus lesions. Am J Pathol. 2001; 159:237-243.
20. Jego G, Palucka AK, Blanck JP, Chalouni C, Pascual V, Banchereau J. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. Immunity. 2003; 19:225-234.
21. Menon M, Blair PA, Isenberg DA, Mauri C. A regulatory feedback between plasmacytoid dendritic cells and regulatory B cells is aberrant in systemic lupus erythematosus. Immunity. 2016; 44:683-697.
22. Youn JI, Park SM, Park S, *et al.* Peripheral natural killer cells and myeloid-derived suppressor cells correlate with anti-PD-1 responses in non-small cell lung cancer. Sci Rep. 2020; 10:9050.
23. Hsu J, Hodgins JJ, Marathe M, *et al.* Contribution of NK cells to immunotherapy mediated by PD-1 / PD-L1 blockade. J Clin Invest. 2018; 128:4654-4668.
24. Liu C, Lou Y, Lizée G, Qin H, Liu S, Rabinovich B, Kim GJ, Wang YH, Ye Y, Sikora AG, Overwijk WW, Liu YJ, Wang G, Hwu P. Plasmacytoid dendritic cells induce NK cell-dependent, tumor antigen-specific T cell cross-priming and tumor regression in mice. J Clin Invest. 2008; 118:1165-1175.
25. Terra M, Oberkampf M, Fayolle C, Rosenbaum P, Guillerey C, Dadaglio G, Leclerc C. Tumor-derived TGFb alters the ability of plasmacytoid dendritic cells to respond to innate immune signaling. Cancer Res. 2018; 78:3014-3026.
26. Paz-Ares L, Ciuleanu TE, Cobo M, *et al.* First-line nivolumab plus ipilimumab combined with two cycles of chemotherapy in patients with non-small-cell lung cancer (CheckMate 9LA): An international, randomised, open-label, phase 3 trial. Lancet Oncol. 2021; 22:198-211.
27. Hellmann MD, Paz-Ares L, Bernabe Caro R, *et al.* Nivolumab plus ipilimumab in advanced non–small-cell lung cancer. N Engl J Med. 2019; 381:2020-2031.

Received July 20, 2022; Revised November 5, 2022; Accepted November 15, 2022.

Released online in J-STAGE as advance publication November 20, 2022.

**Address correspondence to*:
Masato Karayama, Second Division, Department of Internal Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, Japan 431-3192.
E-mail: karayama@hama-med.ac.jp