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The Levels of Interferon-gamma Release as a Biomarker for Non-small-cell Lung Cancer Patients Receiving Immune Checkpoint Inhibitors

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Abstract. *Background/Aim: The present study aimed to prospectively examine the usefulness of interferon-gamma (IFN-γ) release (IGR) as a biomarker in non-small-cell lung cancer patients receiving immune checkpoint inhibitor treatment (ICI-Tx). Patients and Methods: IGR was measured using enzyme-linked immunosorbent assay at four time points: within 14 days before ICI-Tx (T1), and 8±3 (T2), 22±7 (T3), and 43±7 (T4) days after ICI-Tx. Results: Twenty-nine patients were divided into three groups based on IFN-γ levels in the IGR-positive control: Group-1 (n=8) with <10 IU/ml at T1, Group-2 (n=12) with a decrease in IFN-γ levels to <10 IU/ml at T3 and/or T4, and Group-3 (n=9) without changes in IFN-γ levels. Early progression and ICI-induced interstitial pneumonitis were frequently observed in Group-1 and Group-2, respectively. Group-3 exhibited more treatment cycles than the other groups. All three groups showed clear differences in clinical outcomes. Conclusion: IFN-γ levels could be a biomarker for ICI-Tx.*

After the *programmed cell death-1* (*PD-1*) gene was cloned (1), an anti-PD-1 antibody (2) was rapidly developed as an immune checkpoint inhibitor (ICI). ICIs have since become very important anticancer agents (3-5). However, there is currently no other biomarker for non-small-cell lung cancer (NSCLC) than programmed death-ligand 1 (PD-L1) (6).

Recently, we have reported on NSCLC patients who developed pulmonary *Mycobacterium tuberculosis* (MTB) infection while receiving nivolumab (7). We have shown that the development of this paradoxical response closely resembles that of pseudo progression after ICI treatment. Furthermore, several studies (8-10) have indicated that the PD-1/PD-L1 axis and interferon-gamma (IFN-γ) are very important for cellular immunity to *MTB*. The interferon-gamma release assay (IGRA) is widely used as a diagnostic method for latent *MTB* infection (11). The QuantiFERON®-TB Test is an IGRA that can measure IFN-γ released from T lymphocytes by whole blood-based enzyme-linked immunosorbent assay (ELISA). We hypothesized that changes in the PD-1/PD-L1 axis by ICI treatment affect IFN-γ release by T lymphocytes. Thus, the aim of the present prospective observational study was to verify our hypothesis and to examine the usefulness of monitoring IFN-γ as a biomarker in patients with NSCLC who are receiving ICI treatment.

Patients and Methods

Ethics. This study was approved by our institutional review board (approval no.: 884). All patients who participated in this study were enrolled after providing their written informed consent. Furthermore, this study was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000031881).

Patients. Patients with NSCLC were enrolled in this study between July 17, 2018 and February 25, 2019 at Osaka Habikino Medical Center. The main eligibility criteria were as follows: patients who were diagnosed with recurrent, unresectable stage III or IV NSCLC with measurable lesions, who received subsequent ICIs, such as nivolumab, pembrolizumab, and atezolizumab, without other concomitant anticancer therapies, and who had agreed to participate

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in this study by written informed consent. Other eligibility criteria included an age ≥20 years and Eastern Cooperative Oncology Group (ECOG) performance status of 0-2. The exclusion criteria were as follows: patients who had synchronous double tumors, had active infectious or hepatic disease, intended to become pregnant, or were deemed by a physician to be ineligible for the study.

Baseline patient characteristics and demographics. The baseline patient characteristics and demographics examined before ICI treatment were sex, age, smoking status, ECOG performance status, body weight, height, body mass index (BMI), medications, disease complications, past history, and history of cancer treatment.

Interferon-gamma release assay. The IGRA was performed in-house with the QuantiFERON®-TB Gold Plus (QFT-Plus; Qiagen, Germany) assay, according to the manufacturer's instructions (12). The QFT-Plus test was performed in two stages. First, whole blood was collected into QFT-Plus blood collection tubes, which included a Nil Control tube (NC: negative control tube), Tuberculosis 1 (TB1) antigen tube, TB2 antigen tube, and Mitogen tube (PC: positive control tube). The PC in QFT-Plus contained phytohemagglutinin (PHA), which is a mitogen used in the diagnosis of primary immunodeficiency syndrome (13). Each tube was strictly incubated at 37˚C for 20±0.5 h. Second, after incubation, the samples were returned to room temperature (17-27˚C) and centrifuged at 2000-3000 × *g* for 15 min. The plasma was collected, clarified by re-centrifugation, and subjected to an ELISA using the QFT-Plus kit. The absorbance was read at wavelengths of 450 and 650 nm.

Sample collection for IGRA. Sampling was performed during routine clinical procedures. Relative to treatment day (day 1), venous blood samples were collected for the IGRA at three time points, namely within 14 days before treatment (T1: day −14 to 1), on day 22±7 (T3: day 15 to 29), and on day 43±7 (T4: day 36 to 50) after treatment. After a protocol amendment, an additional time point for collection of the IGRA samples was added on day 8±3 (T2: day 5 to 11). However, the T2 IFN-γ levels were not used to categorize the enrolled patients.

Driver gene analysis and PD-L1 staining. The presence of epidermal growth factor receptor mutations was examined using the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method (LSI Medience Corporation Central Laboratory, Tokyo, Japan). Anaplastic lymphoma kinase (ALK) fusion was detected using Optiview ALK (D5F5) (Roche Diagnostics, K.K., Tokyo, Japan). ROS proto-oncogene 1, receptor tyrosine kinase (ROS1) fusion was detected using the AmoyDx ROS1 Fusion Test Kit (Riken Genesis Co LTD, Tokyo, Japan). PD-L1 expression was determined using the PD-L1 IHC 22C3 pharmDx Dako kit (Agilent Technologies, Santa Clara, CA, USA).

Laboratory tests. The following blood and biochemical tests were regularly performed on samples from T1, T2, T3, and T4: cell counts (white blood cells, lymphocytes, and neutrophils), serum albumin, blood urea nitrogen (BUN), creatinine, and C-reactive protein (CRP). The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the neutrophil count by the lymphocyte count.

Administration of immune checkpoint inhibitors. Nivolumab was intravenously administered at 3 mg/kg every 2 weeks between July 1 and August 31, 2018. After amendment of the approved dosage, 240 mg/person were administered intravenously every 2 weeks from September 1, 2018. Pembrolizumab and atezolizumab were intravenously administered at 1200 mg/person every 3 weeks and 200 mg/person every 3 weeks, respectively.

Assessment of response and adverse events. Regarding the assessment of response and adverse events, the observation period was 12 weeks after the first ICI dosage. Tumor responses were assessed by a Tumor Board after each treatment cycle to determine whether the patients could receive the next cycle of treatment. We assessed clinical responses according to the Response Evaluation Criteria in Solid Tumors (RECIST) guideline version 1.1 and the immune-related RECIST guideline. In this study, the responses were categorized as complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), and not evaluable (NE). The severities of adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 (14). The number of treatment cycles was counted until May 31, 2019.

Bronchoalveolar lavage, flow cytometry, and transbronchial lung biopsy. Bronchoalveolar lavage (BAL) was performed if the patient developed suspicious interstitial pneumonitis (IP). The tip of the bronchoscope was placed into the affected segment of the lung in patients with peripheral opacities. Fifty milliliters of sterile physiological saline were instilled through the bronchoscope, and BAL fluid (BALF) was retrieved by gentle hand suction applied to each syringe. The procedure was repeated three times and pooled BALF was centrifuged at 250 × *g* for 10 min to collect the cells. Subsequently, the cell preparation was examined by flow cytometry using the same method as described in a previous report (15). A transbronchial lung biopsy (TBLB) was performed after collecting BALF, as described previously (16).

Statistical analysis. The primary goal of this study was to examine the correlation between the levels of IFN-γ and clinical outcomes in NSCLC patients treated with ICI. In this study, we hypothesized that patients with CR, PR, or hyper-progressive disease (HPD) would be immunologically activated by ICI (RG: Reaction group) and those with PD, but not HPD or SD, would not (NRG: non-Reaction group). Based on the study by Ferrara *et al*. (17), the RG and NRG would be expected to comprise 33% and 67% of the enrolled patients, respectively. We assumed that 60% of the RG and 10% of the NRG would show some type of immunological reaction as assayed by the IGRA. Subsequently, at least 28 patients were required to be able to reject the null hypothesis at $p<0.05$ and a power of 85%. Under the assumption that approximately two patients would withdraw/drop out of the study, a minimum of 30 patients needed to be enrolled. All analyses were conducted using the statistical software package R (18). The patient background data were compared using the chi-square test and Fisher's exact test for categorical variables. A difference was considered statistically significant if the *p*-value was below 0.05.

Results

Patient demographics. Thirty-one patients were enrolled in this study. Two patients were excluded because one did not receive ICI treatment due to disease progression and the other withdrew study consent. The first patient received ICI

Table I. *Patient demographics and laboratory data before immune checkpoint inhibitor treatment grouped according to interferon-gamma status in positive control*.

Variables	Total (n=29)	Group-1 (n=8)	Group-2 (n=12)	Group-3 (n=9)
Gender				
Male	24	8	9	7
Female	5	0	3	2
Age				
Median (range)	73 (44-83)	69.5 (55-83)	74.5 (57-84)	73 (44-79)
Performance status				
0	2	0	1	1
1	17	3	7	7
2	10	5	4	1
Histology				
Adenocarcinoma	24	6	10	8
Squamous cell carcinoma	3	1	1	1
Others	2	1	1	0
Stage				
IIIB-C	3	1	1	1
IV	18	4	9	5
Recurrence	8	3	2	3
Previous corticosteroid treatment				
Yes	3	0	0	3
No	26	8	12	6
PD-L1 staining				
<1%	6	2	3	1
>1%, <50%	6	1	1	4
>50%	15	5	7	3
Unknown	2	0	1	1
Driver mutation				
Yes[a]	1	1	0	0
No	28	7	12	9
Treatment Line				
First	13	5	5	3
Second	10	0	5	5
Third or more	6	3	2	1
Immune checkpoint inhibitor				
Pembrolizumab	17	5	7	5
Atezolizumab	5	1	4	2
Nivolumab	5	2	1	2
Body mass index (kg/m^2)				
Mean±S/D	22.7±4.0	20.5±1.1	22.5±4.0	24.5±4.5
Laboratory data (Mean±S/D)				
NLR	4.9±3.3	7.7±5.0	3.9±1.3	3.8±1.7
Lymphocyte (/μl)	1220±456	919±523	1293±355	1390±427
CRP (mg/dl)	2.6±3.6	5.8±5.2[†]	2.2±2.1	0.5±0.8
Serum albumin (g/dl)	3.8±0.6	3.3±0.6[‡]	3.8±0.5	4.2±0.3

[a]epidermal growth factor receptor exon 19 deletion; NLR: neutrophil-to-lymphocyte ratio; CRP: C-reactive protein; S/D: standard deviation. [†]Group 1 *vs*. Group 3: p=0.0268; [‡]Group 1 *vs*. Group 3: p=0.0169.

treatment on July 23, 2018 and the last patient received treatment on February 26, 2019. The patient demographics are shown in Table I.

IGRA results. As shown in Figure 1, IFN-γ levels in TB1, TB2, and the NC changed did not differ significantly between the sampling points except for five patients (one patient was an overlap case). Figure 1A and B shows that two of these patients had changes in IFN-γ levels at the TB1 and TB2 samples or only at the TB1 sample. One patient was diagnosed as having squamous NSCLC with active pulmonary *MTB*. Since *MTB* patients with severe complications such as lung cancer often require longer courses of *MTB* treatment than those without, he received long-term anti-*MTB* chemotherapy

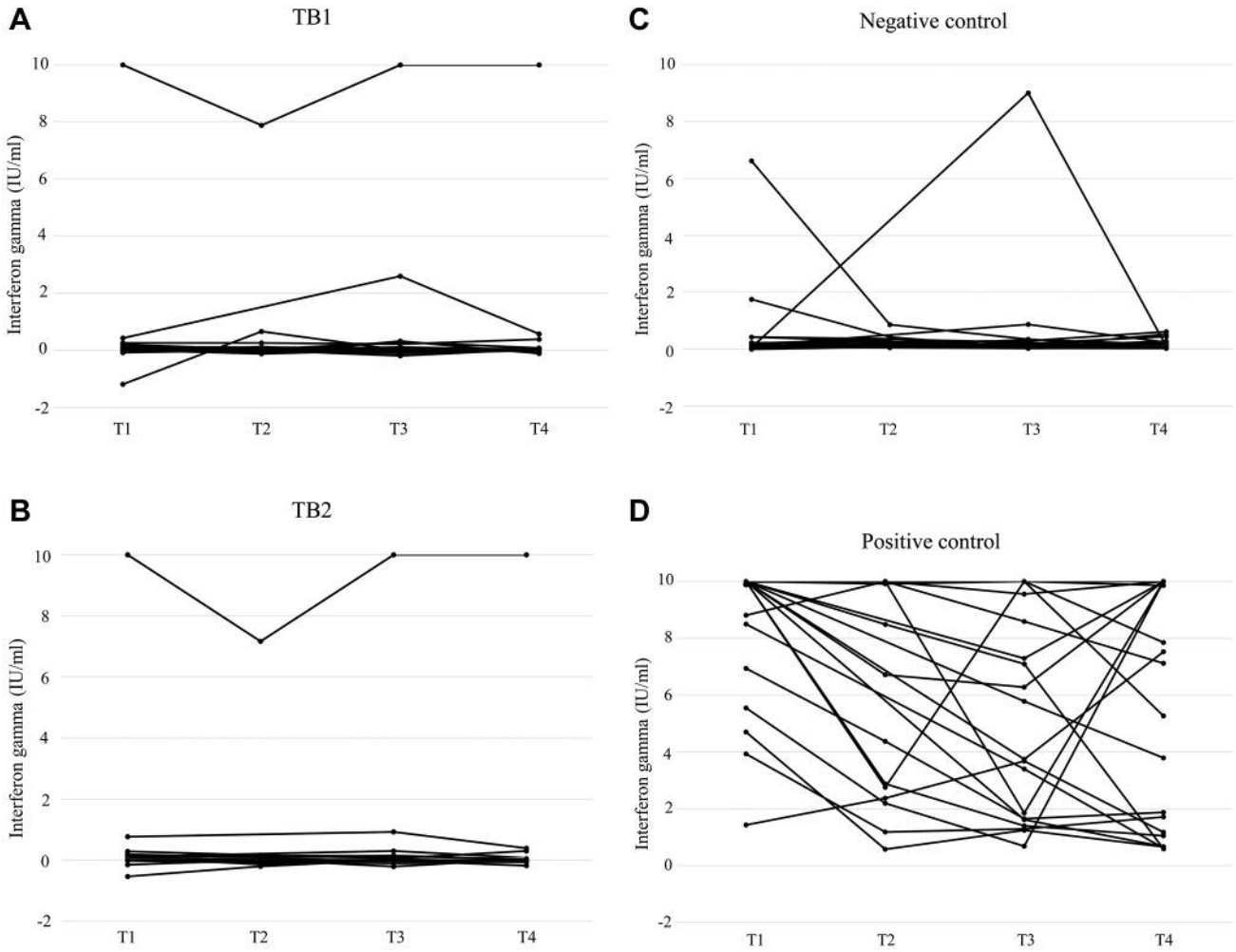

Figure 1. *Polygonal line graphs of interferon-gamma (IFN-γ) levels in patients receiving immune checkpoint inhibitors. A, Polygonal line graph of IFN-γ levels for TB1. One patient with both squamous non-small cell lung cancer and active pulmonary Mycobacterium tuberculosis infection showed a transient decrease in IFN-γ levels at T2. Another patient showed a transient increase at T3. B, Polygonal line graph of IFN-γ levels for TB2. Same patient as in part A showed a similar, transient decrease at T2. C, Polygonal line graph of IFN-γ levels in the negative control. One patient showed a rapid increase in IFN-γ levels at T3 and simultaneously experienced a rapid progression of liver metastases with Grade 2 continuous fever. Two patients showed increased IFN-γ levels at T1, which rapidly decreased at T2 to T4. D, Polygonal line graph of IFN-γ levels in the positive control. According to IFN-γ levels in the positive control, patients were categorized into three groups: patients (n=8) with <10 IU/ml at T1, those (n=12) with <10 IU/ml at T3 and/or T4, and those (n=9) without changes in IFN-γ levels. Each line in the plots represents one patient.*

according to the American Thoracic Society and Infectious Diseases Society of America guidelines (19) from July 17, 2017 to April 22, 2019. He received a left upper lobe resection on September 13, 2017 and adjuvant chemotherapy from November 1, 2017 to February 4, 2018. Because of cancer progression, he concurrently received pembrolizumab and anti-MTB agents from November 27, 2018. After starting the anti-*MTB* chemotherapy, his sputum *MTB* culture remained negative. He demonstrated a transient decrease in IFN-γ levels after pembrolizumab treatment as follows: ≥10, 7.88, ≥10, and ≥10 IU/ml for TB1, and ≥10, 7.17, ≥10, and ≥10 IU/ml for TB2 at T1, T2, T3, and T4, respectively. Afterwards, he did not experience *MTB* recurrence. The other patient had high IFN-γ levels (2.60 IU/ml) in TB1 at T3. Subsequently, IFN-γ levels were decreased to 0.57 IU/ml at T4. This patient did not develop *MTB*. As shown in Figure 1C, three patients presented with high IFN-γ levels in NC at T1 and T3. Of them, one patient presented with high IFN-γ levels (9.01 IU/ml) for NC at T3; this patient simultaneously experienced a rapid progression of liver metastases with Grade 2 continuous fever.

Table II. *Immune-related adverse events grouped according to interferon-gamma status in positive control.*

	Total (n=29)			Group-1 (n=8)			Group-2 (n=12)			Group-3 (n=9)		
Number of patients with immune-related adverse events (%)												
Yes	16 (55.2)			4 (50.0)			8 (66.7)			4 (44.4)		
No	13 (44.8)			4 (50.0)			4 (33.3)			5 (55.6)		
Number of patients according to immune-related adverse events (n)												
Grade of immune-related adverse events[a]	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3
Fever	4	1	0	0	0	0	2	1	0	2	0	0
Eruption	6	1	0	2	1	0	3	0	0	1	0	0
Diarrhea	1	2	0	0	0	0	1	1	0	0	1	0
Interstitial pneumonitis	1	2	2	1	0	0	0	2	2	0	0	0
Thyroid disorder	7	0	0	2	0	0	2	0	0	3	0	0
Diabetes mellitus	0	0	1	0	0	1	0	0	0	0	0	0

[a]There were no treatment-related deaths.

After administration of corticosteroid, the fever improved with a decrease in IFN-γ levels in NC at T4. In the remaining two patients, the IFN-γ levels in NC increased to 6.62 and 1.75 IU/ml at T1. The former was the same patient who concurrently received pembrolizumab and anti-*MTB* chemotherapy, as mentioned above. The later did not have specific disease complications. In these two patients, IFN-γ levels in NC decreased to 0.86 and 0.43 IU/ml at T2, respectively. The IFN-γ levels in the other 25 patients did not change for TB1, TB2, and NC, through all sampling points.

In contrast, as shown in Figure 1D, the IFN-γ levels for PC varied before and after ICI treatments. The patients were divided into three groups according to these IFN-γ levels as follows: Group-1 (Gr-1, n=8) with <10 IU/ml at T1, Group-2 (Gr-2, n=12) with at least one decrease to <10 IU/ml at T3 and/or T4, and Group-3 (Gr-3, n=9) with no changes in IFN-γ levels. One patient with IFN-γ levels that transiently decreased to 9.92 IU/ml for PC at only T2 was categorized as Gr-3.

Patient demographics and laboratory data according to IFN-γ status in PC before ICI treatments. As shown in Table I, among the three groups, the performance status of 2 tended to be more frequently observed in Gr-1 than in the other groups. The mean BMI, lymphocyte count, and serum albumin tended to be lower in Gr-1 than in the other groups. The NLR was higher in Gr-1 than in the other groups. The mean CRP was significantly higher in Gr-1 than in Gr-3 (p=0.0268) and it tended to be higher in Gr-1 than in Gr-2. The mean serum albumin was significantly lower in Gr-1 than in Gr-3 (p=0.0169) and it tended to be lower in Gr-1 than in Gr-2. None of the patients had autoimmune disease. Three of the 29 patients took corticosteroid and all of the three belonged to Gr-3. Of the three, one patient took 10 mg hydrocortisone for supplementation of adrenal dysfunction due to cytotoxic agents, one received 5 mg prednisolone for pemetrexed-induced interstitial pneumonitis that developed 11 months ago, and the other patient 5 mg prednisolone for chronic urticaria.

Immune-related adverse events according to IFN-γ status in PC. Table II shows immune-related adverse events (irAEs) according to IFN-γ status in the PC. The number of patients with irAEs and the number of irAEs were greater in Gr-2 than in the other groups. In particular, ICI-induced interstitial pneumonitis (ICI-IP) developed in five patients (17.2%), and specifically in four patients in Gr-2.

Computed tomography findings, BAL, and TBLB. BAL and TBLB were performed for three of the five patients with ICI-IP. Of these three patients, one was in Gr-1 (Case 1) and two were in Gr-2 (Cases 2 and 3).

Figure 2 shows the computed tomography findings before and after ICI treatment, and the pathological findings for Case 1 (Figure 2A, B, and C), Case 2 (Figure 2D, E, and F), and Case 3 (Figure 2G, H, and I). In Case 1, Grade 1 ICI-IP developed 29 days after starting atezolizumab. The BAL findings were as follows: a cell count of 44.0×10^5/ml; macrophages 35.4%, lymphocytes 52.8%, neutrophils 6.3%, and eosinophils 4.3%; and a CD4/CD8 ratio of 2.94. A TBLB specimen obtained from the left bronchus 3a (lt-B3a) and lt-B4a is shown in Figure 2C. The BMI, NLR, CRP, and serum albumin before ICI treatment were 24.5 kg/m^2, 4.16, 1.87 mg/dl, and 3.3 g/dl, respectively. This patient has been followed up without receiving corticosteroid.

In Case 2, Grade 2 ICI-IP developed 37 days after starting atezolizumab. The BAL findings were as follows: a cell count of 10.5×10^5/ml; macrophages 41.6%, lymphocytes 56.1%, neutrophils 1.2%, and eosinophils 1.0%; and a CD4/CD8 ratio

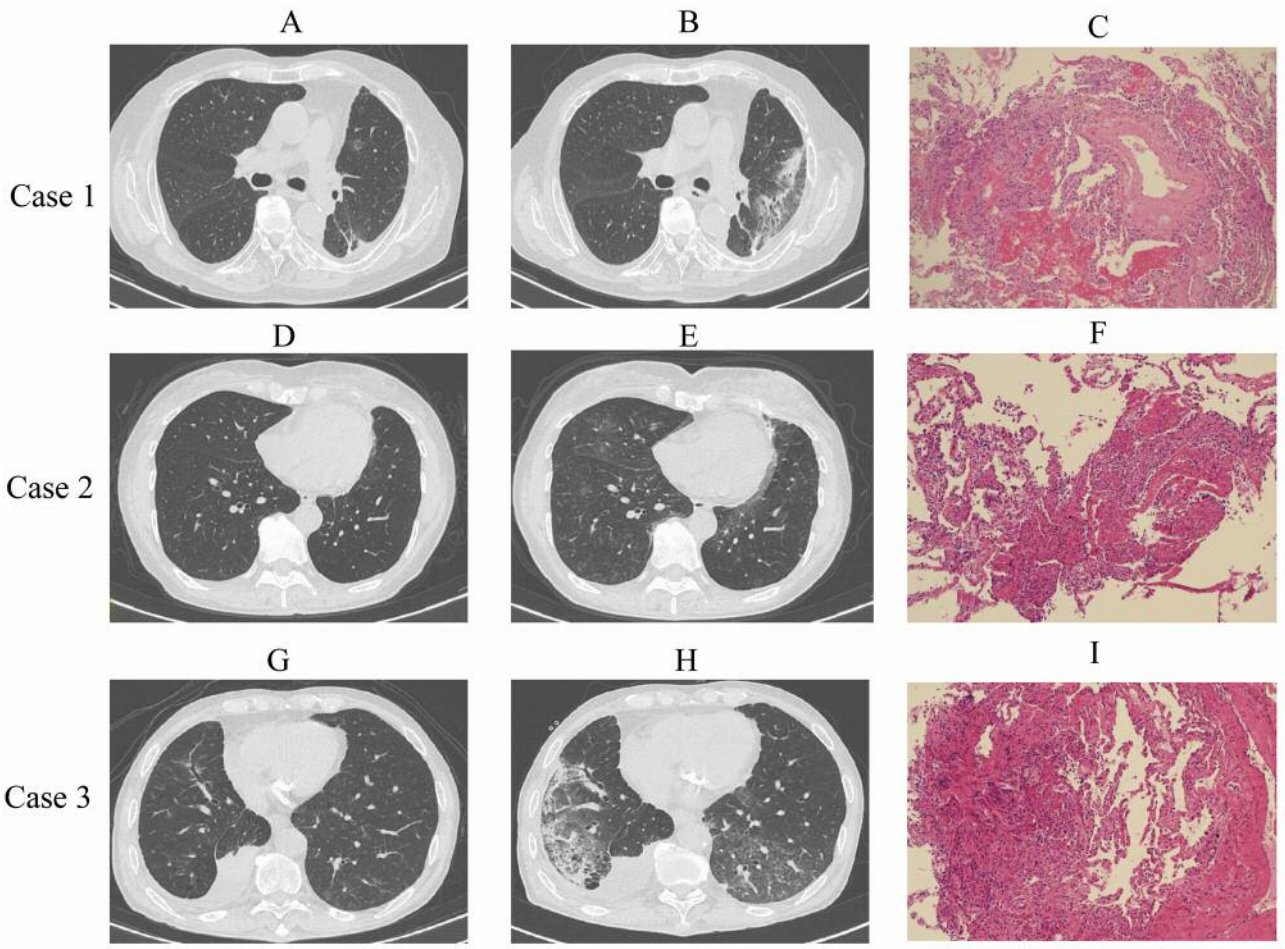

Figure 2. *Chest computed tomography (CT) scan findings and pathological findings of transbronchial lung biopsies. Case 1: A, Chest CT image before atezolizumab treatment. B, Chest CT image on day 29 of nivolumab treatment revealed ground grass opacity with linear consolidation in left residual lung after surgery. C, Pathological finding [Hematoxylin-Eosin staining (HE), ×100 magnification] mimicked organizing pneumonia with intra-alveolar collection of foamy cells and proliferation of atypical type II pneumocytes. Case 2: D, Chest CT image before atezolizumab treatment. E, Chest CT image on day 37 of atezolizumab treatment revealed diffuse ground grass opacity in both right lower lobe and left residual lung after surgery. F, Pathological finding (HE, ×100 magnification) showed pneumonia with apparent intra-alveolar exudates and infiltration of neutrophils. Case 3: G, Chest CT image before pembrolizumab treatment. H, Chest CT image on day 62 of pembrolizumab treatment revealed diffuse ground grass opacity with consolidation in right lung and diffuse ground grass opacity in left lung. I, Pathological finding (HE, ×100 magnification) showed organizing pneumonia with granulation tissue in the lumen of alveolar ducts and collection of macrophages in alveolar cavities.*

of 2.94. A TBLB specimen obtained from right bronchus 8a (rt-B8a), rt-B9a, and rt-B2b is shown in Figure 2F. Pathological analysis revealed pneumonia with apparent intra-alveolar exudate; however, we diagnosed this patient with ICI-IP based on the chest CT scan and BAL findings. The BMI, NLR, CRP, and serum albumin before ICI treatment were 19.9 kg/m^2, 4.13, 0.9 mg/dl, and 4.2 g/dl, respectively. This patient received orally 30 mg prednisolone. Afterwards, the ICI-IP improved without inflammatory scarring.

In Case 3, Grade 3 ICI-IP developed 62 days after starting pembrolizumab. The BAL findings were as follows: a cell count of 19.5×10^5/ml; macrophages 25.2%, lymphocytes 20.5%, neutrophils 42.6%, and eosinophils 10.1%; and a CD4/CD8 ratio of 1.87. A TBLB specimen obtained from rt-B8a, rt-B9a, and rt-B2b is shown in Figure 2I. The BMI, NLR, CRP, and serum albumin before ICI treatment were 21.6 kg/m^2, 2.71, 6.22 mg/dl, and 2.9 g/dl, respectively. Since an intravenous pulse therapy with methylprednisolone 1000 mg/day for three consecutive days was not effective, this patient received cyclophosphamide pulse therapy (750 mg/day), as in a previous study (20). Afterwards, the ICI-IP improved with inflammatory scarring.

Table III. *Efficacy of immune checkpoint inhibitors grouped according to interferon-gamma status in positive control.*

	Total (n=29)	Group-1 (n=8)	Group-2 (n=12)	Group-3 (n=9)
Best response (n) (%)				
Complete response	0	0	0	0
Partial response	4 (13.8)	0	3 (25.0)	1 (11.1)
Stable disease	9 (31.0)	0	4 (33.3)	5 (55.6)
Progressive disease	15 (51.7)	7 (87.5)†	5 (41.7)	3 (33.3)
Not evaluable	1 (3.4)	1 (12.5)	0	0
Response rate (%)	13.8	0	25.0	11.1
Treatment cycles[a]				
Median (range)	3 (1-12)	2 (1-5)	3 (1-8)	4 (3-12)‡

[a]Number of treatment cycles was counted until May 31, 2019. †Group 1 *vs*. Group 2: p=0.0498; ‡Group 1 *vs*. Group 3: p=0.0080.

Efficacy of ICIs according to IFN-γ status in PC. As shown in Table III, the response rate (%) was 13.8, 0, 25.0, and 11.1 in all patients in Gr-1, Gr-2, and Gr-3, respectively. The frequency of PD in Gr-1 (87.5%) was significantly higher than that in Gr-3 (33.3%) (p=0.0498) and it tended to be higher in Gr-1 than in Gr-2 (41.7%) (p=0.0697). Especially, early progression was frequently observed in Gr-1. The number of treatment cycles [median (range)] in all patients, in Gr-1, Gr-2, and Gr-3 was 3 (1-12), 2 (1-5), 3 (1-8), and 4 (3-12), respectively. Gr-3 received significantly more ICI treatment cycles than Gr-1 (p=0.0080) and it tended to receive more than Gr-2 (p=0.092).

Discussion

This preliminary study aimed to verify our hypothesis that IFN-γ release from T lymphocyte would change after ICI treatment and to examine the usefulness of IFN-γ as biomarker for ICI treatment. Thus, we first reported that IFN-γ levels in the PC were dynamically changed after ICI treatment. According to the IFN-γ levels in the PC, the 29 NSCLC patients who received ICIs were divided into three groups. Gr-1 tended to have a higher level of both NLR and CRP and a lower level of both BMI and serum albumin than the other groups. Patients in Gr-1 with a PC of <10 IU/ml before treatment may have a poor immunological status including cancer-associated inflammation and malnutrition, as described in our previous study (21). Despite the fact that 62.5% of patients in Gr-1 had a PD-L1 Tumor Proportion Score ≥50%, seven patients (87.5%) in Gr-1 developed early cancer progression as mentioned previously (21). In Gr-2, IFN-γ levels were decreased after ICI treatment. Therefore, we hypothesize that the T lymphocytes are excessively activated in the tumor immune microenvironment by ICI treatment and overflow into the peripheral blood; they will not respond to PHA in the PC because they are already activated. These T lymphocytes would affect not only lung cancer but also various organs, especially the lungs. In Gr-3, IFN-γ levels were unchanged in the PC after ICI treatment. Therefore, we hypothesize that the T lymphocytes are not excessively activated in the tumor immune microenvironment and do not overflow into the peripheral blood. As a result, the patients in Gr-3 did not show severe irAEs and obvious responses. Some patients in Gr-3 continued with more treatment cycles than patients in the other groups. In this scenario, monitoring changes in the QFT-Plus PC would be useful for predicting the clinical course of patients treated with ICIs. In particular, patients with <10 IU/ml in the PC before treatment should avoid ICI treatment until cancer-associated inflammation and malnutrition have improved.

The immunological responses of the three groups in this study resemble the "damage-response framework" against pathogens described by Casadevall and Pirofski (22), especially with *MTB*. They have indicated that too strong immunoreactions and too weak immunoreactions result in death of the host, but an appropriate immunoreaction results in a positive outcome for the host. In this study, the immunoreaction in Gr-3 may be an appropriate damage-response, which is important for better outcomes during ICI treatments. Teraoka *et al*. (23) have reported an appropriate damage-response so that early irAEs were associated with better outcomes after ICI treatment. Our patients in Gr-1 were immunosuppressed, leading to early progression, and those in Gr-2 had excessive immune responses, leading to favorable treatment responses and severe irAEs.

In the present study, the incidence of ICI-IP was 17.2%, which is higher than that reported in previous studies (24-26). However, a recent report by Suresh *et al*. (27) has indicated that 39 (19.0%) of 205 patients with advanced NSCLC experienced ICI-IP, similar to the percentage in our study. Suresh *et al*. have hypothesized that the increased incidence was partially explained by a greater awareness of this entity and increased pharmacovigilance with ICI administration. In

our study, 4 out of 5 patients with ICI-IP belonged to Gr-2. These results suggest that decreased IFN-γ levels in the PC after ICI treatment would be a useful marker for the early detection of ICI-IP. Furthermore, in our study, the mean CRP before ICI treatment was significantly higher in Gr-1 than in Gr-3 and it tended to be higher in Gr-1 than in Gr-2. In addition, the mean serum albumin before ICI treatment was significantly lower in Gr-1 than in Gr-3 and it tended to be lower in Gr-1 than in Gr-2. Gr-1 and Gr-2 represent a poorer immunological status than Gr-3. The incidence of ICI-IP in clinical practice may be higher than that in clinical trials because of higher numbers of patients with cancer-associated inflammation and malnutrition. Thus, the IFN-γ levels in the QFT-Plus PC before ICI treatment may be useful for the detection of a poor immunological status, including cancer-associated inflammation and malnutrition.

However, in a later publication, Suresh *et al*. (28) have reported that developing ICI-IP increased the risk of death, unlike extrapulmonary irAEs. Using experimental mouse models, Sakai *et al*. (9) have found that there was a difference in the contribution of IFN-γ for protecting from *MTB* infection between the lung and spleen (an extrapulmonary organ). Furthermore, they described that excessive IFN-γ is less protective in the lungs and eventually drives lethal disease. This mechanism could serve as a reference for irAEs due to ICIs.

Our study has several limitations that must be considered. First, the sample size in this study was small. Second, the population of patients enrolled in this study was heterogeneous. Third, we could not identify IFN-γ values of ≥10 IU/ml in the QFT-Plus assay. Fourth, we did not examine gene alterations such as tumor mutation burdens. Fifth, we did not examine cytokines and interleukins other than IFN-γ .

The above-mentioned limitations do raise questions about the usefulness of IFN-γ as a biomarker of ICI treatment, therefore, in the future, we will examine its usefulness as a biomarker in a prospective study that addresses the limitations listed above.

Conclusion

Changes in the PD-1/PD-l1 axis by ICI treatment affect INF-γ release by T lymphocytes. IFN-γ levels assayed by IGRA using QFT-Plus could be a novel, biomarker for the early detection of severe irAEs, including ICI-IP, and for patient selection for ICI treatment.

Conflicts of Interest

T.H. received honoraria and research funding from Ono Pharmaceutical Co. Ltd. (Osaka, Japan), Lilly Japan Co. Ltd. (Hyogo, Japan), AstraZeneca Co. Ltd. (Osaka, Japan), Taiho Pharmaceutical Co. Ltd. (Tokyo, Japan), Chugai Pharmaceutical Co. Ltd. (Tokyo, Japan), Merck Serono Co. Ltd. (Tokyo, Japan), MSD Oncology Co. Ltd. (Tokyo, Japan), Kyowa-Hakko Kirin, and Boehringer Ingelheim. The other Authors have no conflicts of interest to declare.

Authors' Contributions

All Authors were involved in the conception and design of the study, or acquisition of data, or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; and final approval of the version to be submitted. T.H., T.K, H.S., Y.S., Y.N., S.N., A.T, N.M., S.H., and N.O. collected clinical data. H.Y., A.M. and Y.T. performed measurement of QuantiFERON®-TB Gold Plus. H.K. performed flow cytometry. K.K made a pathological diagnosis. T.H., T.K., H.S., and T.T. performed the statistical analyses.

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