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Correlation between serum adenosine deaminase activity and efficacy of anti-programmed cell death-1 antibody

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ABSTRACT

Objective: Serum adenosine deaminase (ADA) activity is a marker of immune reaction to several diseases. We evaluated changes in serum ADA in patients with lung cancer undergoing chemotherapy or anti-programmed cell death-1 (PD-1) therapy to examine the correlation between serum ADA and the therapy efficacy.

Materials and methods: We assessed 50 patients with advanced lung cancer receiving chemotherapy or anti-PD-1 therapy. Serum ADA was measured before and on day 7 of the first treatment cycle and day 0 of subsequent cycles. Correlations between ADA change and efficacy of treatment were evaluated.

Results: Of the 50 patients, 20 were treated with chemotherapy and 30 were treated with anti-PD-1 therapy. Serum ADA decreased significantly between baseline and day 7 of the first cycle in patients undergoing chemotherapy, regardless of response (partial response [PR] or stable disease [SD]: −23% [−38 to +32; p = 0.002]; progressive disease [PD]: −12% [−42 to +6; p = 0.500]). Conversely, in patients undergoing anti-PD-1 therapy, serum ADA increased significantly between baseline and 7 days after the first dose and before subsequent doses in patients who had PR or SD. (day 7 of first cycle: +6% [−10 to +34; p = 0.034], day 0 of second cycle: 8% [−5 to +37; p = 0.002], day 0 of third cycle: 9% [−3 to +55; p = 0.002]). However, serum ADA did not significant change in PD patients undergoing anti-PD-1 therapy. Furthermore, early increases in serum ADA were associated with longer progression-free survival in patients receiving anti-PD-1 therapy (p = 0.006).

Conclusion: Changes in serum ADA could be used to predict clinical benefit from anti-PD-1 therapy in patients with lung cancer. The association between changes in serum ADA and the efficacy of ant-PD-1 therapy thus remains inconclusive and requires further study.

1. Introduction

Immune checkpoint inhibitors represent an important development in the treatment of advanced cancers. In cases of non-small cell lung cancer (NSCLC), the effectiveness of anti-programmed cell death-1 (PD-1) antibody therapies such as nivolumab [1] or pembrolizumab [2] has been demonstrated in several clinical trials. However, the response to anti-PD-1 therapy differed from responses to previous treatments, which included durable response, slow response, pseudoprogression and hyperprogression. Such unconventional response patterns made it difficult to differentiate patients who responded to treatment from those who did not respond on the basis of imaging results in the early stages of treatment. Thus, the discovery of biomarkers that influence clinical response to anti-PD-1 therapy is important for maximizing benefits and avoid excessive treatment and unnecessary toxicities of these agents in clinical practice. The anti-PD1 antibody is thought to act by inhibiting the binding of PD-1 expressed on the surface of T cells to a ligand expressed by tumor cells and eliciting a cancer-specific immune response that results in antitumor effects in which the T cell plays a significant role. Specific T cell activation may be a good biomarker for predicting the effectiveness of the anti-PD-1 antibody.

Adenosine deaminase (ADA) is a purine catabolic enzyme, capable

Abbreviations: PD-1, programmed cell death 1; ADA, adenosine deaminase; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; Adeno, adenocarcinoma; Sq, squamous cell carcinoma; PD-L1, PD-1 ligand 1; ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; PR, partial response; SD, stable disease; PD, progressive disease; A2AAR, A2A adenosine receptor

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of catalyzing the deamination of adenosine, resulting in inosine [3]. The most important biological activity of ADA is related to lymphoid tissue and is necessary for the proliferation and differentiation of T lymphocytes. ADA levels of T lymphocytes are approximately 10 times higher than that of B lymphocytes. ADA activity varies depending on cell proliferative status and maturity [4]. Increases in serum ADA have been reported in several inflammatory and autoimmune diseases, including systemic lupus erythematosus, celiac disease, Behcet's disease, Graves' disease, rheumatoid arthritis, ulcerative colitis and tuberculosis [4,5]. While, it is known that deletion of the ADA gene causes severe combined immunodeficiency in heritable form. It is believed that serum ADA activity may be altered as a result of T cell activation. We hypothesize that ADA may become a biomarker predicting clinical response in anti-PD-1 therapy. But there has been no previous study on the association of serum ADA and anti-PD-1 therapy.

In the previous study, plasma ADA isoform 2 was evaluated in cancer patients undergoing chemotherapy. ADA2 decreased from an average of 22.7 ± 10.5 U/L for baseline to 15.0 ± 4.6 U/L after 7–10 days first dose chemotherapy [6]. ADA may be an indicator of therapeutic efficacy in chemotherapy for cancer. Because of different mechanism of antitumor effect, it is thought that there were different ADA changes in patients with chemotherapy and anti-PD-1 therapy. Furthermore, ADA change in patients treated with anti-PD-1 therapies may be different with treatment efficacy. Therefore, we evaluated changes in serum ADA in patients with lung cancer who were undergoing chemotherapy or anti-PD-1 therapy and examined the correlation between serum ADA and the efficacy of the therapy.

2. Materials and methods

2.1. Patients and serum ADA measurements

Twenty patients undergoing chemotherapy and 30 patients undergoing anti-PD-1 monotherapy (nivolumab or pembrolizumab) at Department of Thoracic Medical Oncology, the Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Tokyo, Japan between September 2017 and November 2017 were included in the study. We measured serum ADA before and at day 7 of the first cycle and at day 0 of subsequent cycles. Serum ADA was measured at a commercial laboratory (SRL Inc., Tokyo, Japan). The reference value was set to 5.0–20.0 U/L. Changes (%) in serum ADA from baseline were calculated at day 7 of the first cycle and at day 0 of subsequent cycles. All the patients underwent computed tomography every 2–3 months. Efficacy of treatment was retrospectively evaluated according to the Response Evaluation Criteria in Solid Tumors v1.1 guidelines. Correlations between serum ADA changes and the efficacy of treatment were evaluated. Progression-free survival (PFS) was defined as the number of months between the first treatment and death or progression, whichever occurred first. This retrospective study was approved by the Institutional Review Board of our hospital.

2.2. Statistical analysis

Mann–Whitney U test was used compare serum ADA in each point among chemotherapy group and anti-PD-1 group. The Wilcoxon signed-rank test was used to compare changes in serum ADA during treatment among the partial response (PR) or stable disease (SD) group and progressive disease (PD) group. The Kaplan–Meier method was applied to assess the PFS curve, and the groups were compared using the log-rank test. The capacity percentage changes in serum ADA in predicting PR or SD patients was analyzed using receiver operating characteristic (ROC) curve analysis. We performed all statistical analyses using the EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

Table 1
Baseline characteristics of patients.

Characteristics	Chemotherapy (n = 20)	Anti-PD-1 therapy (n = 30)
Age (years)		
Median (range)	67.5 (46–81)	72.5 (35–83)
Gender: n (%)		
Female	4 (20)	5 (17)
Male	16 (80)	25 (83)
ECOG PS: n (%)		
0	5 (25)	4 (13)
1	14 (70)	26 (87)
2	1 (5)	0 (0)
Smoking status: n (%)		
Current or former	16 (80)	24 (80)
Never	4 (20)	6 (20)
Stage: n (%)		
IIIA	5 (25)	1 (3)
IIIB	5 (25)	2 (7)
IV	8 (40)	18 (60)
Recurrence	2 (10)	9 (30)
Treatment line: n (%)		
1	16 (80)	11 (37)
2	3 (15)	11 (37)
≥3	1 (5)	8 (27)
Histologic features: n (%)		
Adenocarcinoma	10 (50)	17 (57)
Squamous cell carcinoma	3 (15)	12 (40)
NSCLC	5 (25)	1 (3)
SCLC	2 (10)	0 (0)
EGFR status: n (%)		
Mutant	2 (10)	2 (7)
Wild	15 (75)	27 (90)
Unknown	3 (15)	1 (3)
ALK status: n (%)		
Translocation	0 (0)	0 (0)
Wild	17 (85)	30 (100)
Unknown	3 (15)	0(0)
PD-L1 status: n (%)		
0	6 (30)	4 (13)
1–49	4 (20)	6 (20)
≥50	5 (25)	17 (57)
Unknown	5 (25)	3 (10)
Treatment regimen: n (%)		
Chemoradiotherapy	9 (45)	
Chemotherapy	11(55)	
Nivolumab		13 (43)
Pembrolizumab		17 (57)

Abbreviations: *ALK*: anaplastic lymphoma kinase; ECOG PS: Eastern Cooperative Oncology Group performance status; *EGFR*: epidermal growth factor receptor; NSCLC: non–small cell lung cancer; PD-1: programmed cell death 1; SCLC: small cell lung cancer.

3. Results

3.1. Patient characteristics

Patient characteristics are described in Table 1. Of the 50 patients included in this study, 20 were undergoing chemotherapy (chemotherapy group), 30 were undergoing anti-PD-1 antibody monotherapy (anti-PD-1 group: nivolumab [n = 13] and pembrolizumab [n = 17]). The clinical stage IIIA/IIIB/IV/ recurrences were 5 (25%)/5 (25%)/8 (40%)/2 (10%) in the chemotherapy group and 1 (3%)/2 (7%)/18 (60%)/9 (30%) in the anti-PD-1 group, respectively. Sixteen patients (80%) were receiving first-line treatment in the chemotherapy

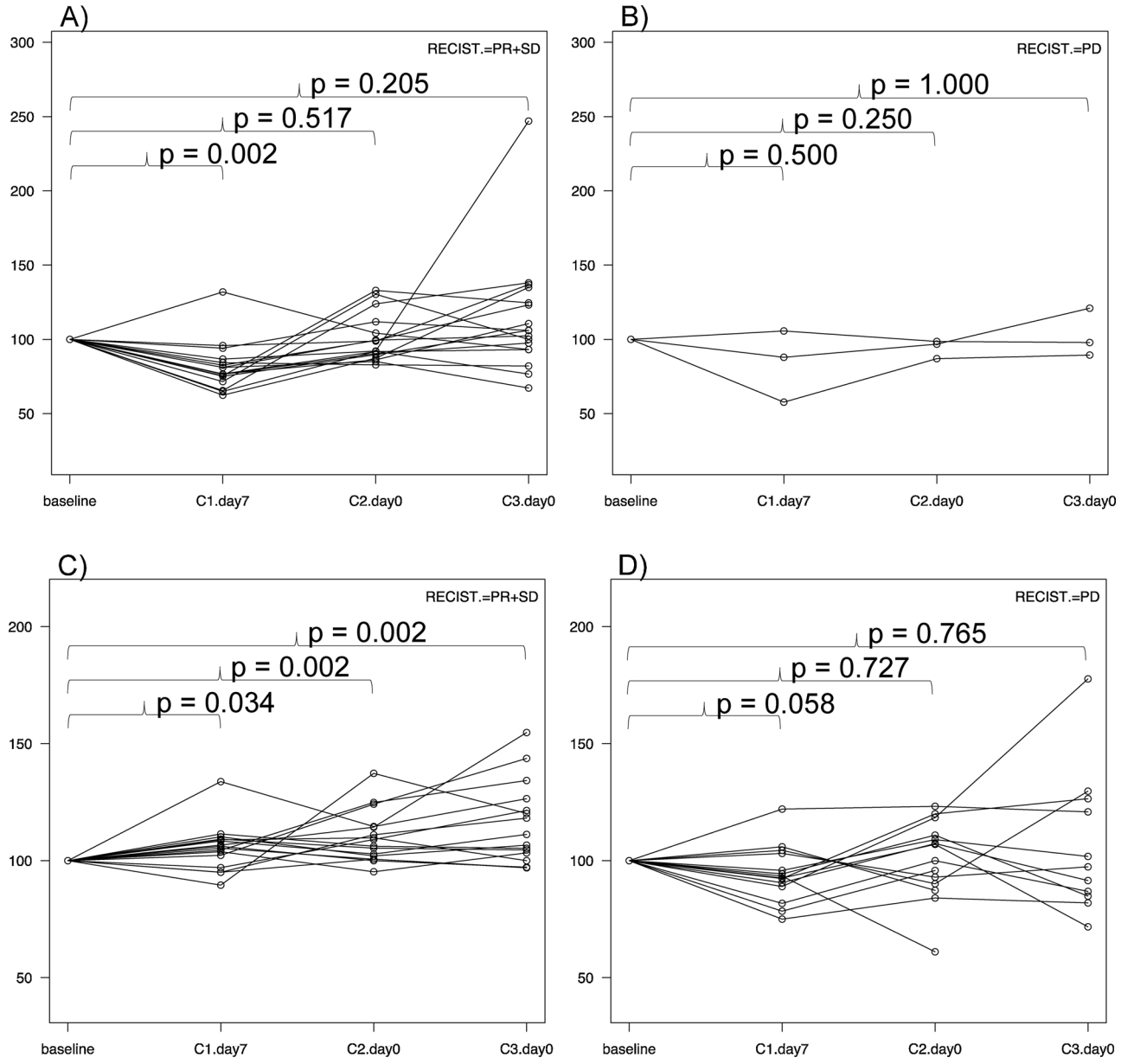

Fig. 1. Trend of adenosine deaminase (ADA) change (%) in each group.
The Wilcoxon signed-rank test was used to compare changes in serum ADA during treatment after baseline in the group of patients with partial response (PR) or stable disease (SD) and in the group of patients with progressive disease (PD). (A) PR or SD in the patients receiving chemotherapy; (B) PD in the patients receiving chemotherapy; (C) PR or SD in the patients receiving anti-programmed cell death-1 (PD-1) therapy; (D) PD in the patients receiving anti-PD-1 therapy.

group; 1 (37%) were receiving first-line treatment and 11 patients (37%) were receiving second-line treatment in the anti-PD-1 group. There were more progressive patients in anti-PD-1 group than chemotherapy group. And in anti-PD-1 group, many patients were treated as second-line later. The chemotherapy group 3 (15%) patients with squamous cell carcinoma and 2 (10%) with small cell lung cancer (SCLC) and the anti-PD-1 group comprised 12 (40%) patients with squamous cell carcinoma and 17 (57%) with adenocarcinoma and no SCLC. Both the groups comprised two NSCLCs with mutations in epidermal growth factor receptor gene. PD-L1 expression was examined in 15 patients (75%) in the chemotherapy group and 27 patients (90%) in the anti-PD-1 group.

The median follow-up time was 90 days (44–154) in the chemotherapy group and 78.5 days (14–146) in the anti-PD-1 group, respectively. In terms of the treatment response, we observed 14 patients with PR, 3 with SD and 5 with PD in the chemotherapy group, and 11 with PR, 5 with SD and 15 with PD in the anti-PD-1 group, respectively. Disease control rate as 85% in the chemotherapy group and 54% in the anti-PD-1 group, respectively.

3.2. Serum ADA changes during treatment

Serum ADA of baseline, day 7 of first cycle and day 0 of second cycle evaluated in all patients in both groups. But serum ADA of day 0 of

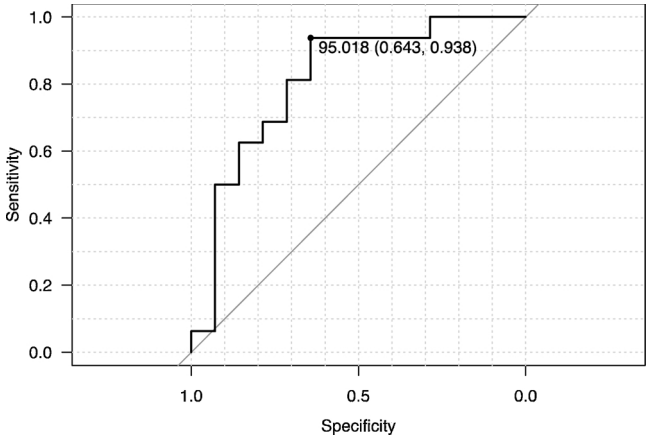

Fig. 2. Receiver operating characteristic curves for percentage change in serum adenosine deaminase in predicting which patients would have a partial response or stable disease.

third cycle could not be evaluated in 3 patients in anti-PD-1 group due to disease progression. Median serum ADA of each point was significantly higher in anti-PD-1 group than chemotherapy group (baseline; 21.00 [11.8–56.0] versus 16.65 [10.2–27.5] [$p = 0.216$], day 7 of first cycle; 21.10 [11.2–49.8] versus 13.45 [7.3–25.2] [$p < 0.001$], day 0 of second cycle; 20.55 [10.3–66.3] versus 15.80 [11.1–23.4] [$p = 0.003$], day 0 of third cycle; 21.70 [11.9–99.5] versus 18.15 [10.2–41.0] [$p = 0.030$], Table A1).

Percentage change in serum ADA during chemotherapy is shown in Fig. 1A and 1B. In most patients of the chemotherapy group, serum ADA transiently decreased at day 7 of the first cycle from baseline, regardless of the therapeutic effect. In 17 patients with PR or SD observed −23% (−38% to +32%) change and in 3 patients with PD observed −12% (−42% to +6%) change in serum ADA from baseline (PR or SD: $p = 0.002$, PD: $p = 0.500$, Table A2).

Percentage changes in serum ADA in the anti-PD-1 group are shown in Fig. 1C and 1D. In 16 patients with PR or SD in the anti-PD-1 group, serum ADA gradually increased from baseline during treatment (Fig. 1C). A 6%, 8%, and 9% increase in serum ADA from baseline was observed at day 7 of the first cycle, and at day 0 of second and third cycle, respectively (p = 0.034, p = 0.002, p = 0.002, Table A1). No significant change in serum ADA was observed in 14 patients with PD in the anti-PD-1 group (Fig. 1D).

In predicting disease control (PR or SD), the percentage change in serum ADA expected to have a diagnostic accuracy with an area under the ROC curve of 0.812 (95% confidence interval [CI] 0.647–0.978). When the threshold is set to 95.018%, the specificity is 64.3% and the sensitivity is 93.8% (Fig. 2).

3.3. Serum ADA changes at day 7 of the first cycle and PFS

An increase in serum ADA at day 7 of the first cycle was observed in 2 of 20 patients in the chemotherapy group and in 16 of 30 patients in the anti-PD-1 group. PFS curves of 16 patients who experienced an increase and 14 patients who experienced a decrease in serum ADA are shown in Fig. 3. The PFS of patients with increase in serum ADA at day 7 was significantly longer than in patients with a decrease in serum ADA at day 7 in the anti-PD-1 group (not reached [95% CI: 1.8–not reached] versus 1.8 months [95% CI: 0.85–3.15] [p = 0.006]).

4. Discussion

This is the first study to examine change in serum ADA during lung cancer treatment, particularly during treatment with anti-PD-1 antibody. The change in ADA during treatment was clearly different between chemotherapy group and anti-PD-1 group. A transient decrease in serum ADA was observed regardless of the therapeutic effect in the chemotherapy group. Difference changes in serum ADA were observed between responder and non-responder in anti-PD-1 group. Responder was gradually increased from baseline during treatment, while non-responder was no significant change in serum ADA. Moreover, we have shown that patients who had elevated serum ADA had significantly longer PFS than those who had a decreased serum ADA at day 7 in the first cycle of anti-PD-1 therapy.

Median serum ADA of each points were significantly higher in anti-PD-1 group than chemotherapy group. Serum ADA has been shown to increase in several types of cancer, such as head and neck, esophagus, gastric, breast and ovarian, compared with normal healthy individuals. And serum ADA can be a useful parameter for diagnosing and for monitoring its progression [7]. Furthermore, serum ADA increases according to histopathological grade or carcinoma staging [8]. Because of increasing adenosine in the malignant tumor is operating serum ADA increasing. Due to the rapid growth, solid tumors were routinely experiencing severe hypoxia and necrosis, leading to adenine nucleotide degradation and adenosine release. Also, in cancer there is an increased turnover of malignant cells and an associated increase in nucleotide metabolism leading to an increase in purine metabolizing enzymes. ADA is particularly sensitive to stimulation by growth factors and cytokines during rapid tissue proliferation such as IL-2, IL12 and INF-γ, which increase during malignancy [9]. Difference of serum ADA of baseline between chemotherapy group and anti-PD-1 group were considered with difference of clinical stage and treatment lines, so in present study, we evaluated percentage change in serum ADA at each point.

Serum ADA has been reported to be a sign of a cell-mediated immune response, given biological activities of ADA are necessary for the proliferation and differentiation of T lymphocytes as well as maturation and function of blood monocytes and macrophages [10]. We

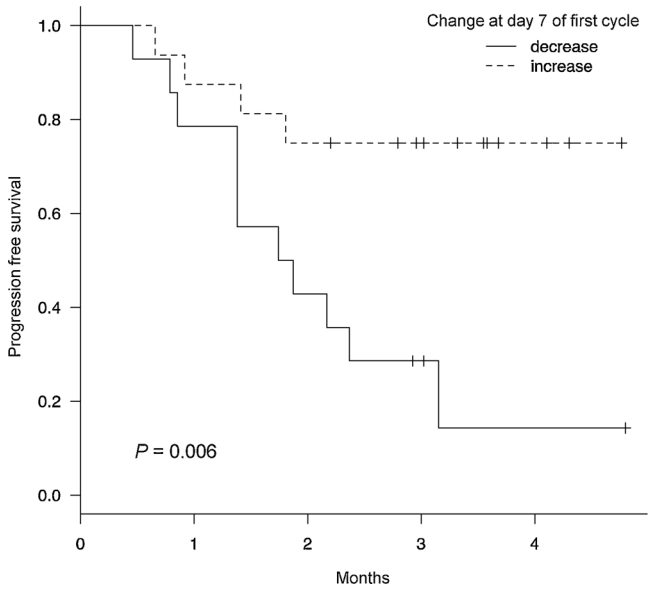

Fig. 3. Progression-free survival (PFS) among patients receiving anti-programmed cell death-1 therapy, according to increase or decrease in serum adenosine deaminase on day 7 in the first cycle.

hypothesized that the change of serum ADA may reflect immune reaction, particularly T cell activity during anti-PD-1 therapy. In fact, serum ADA decreased in patients who received chemotherapy, which is considered to be immunosuppressive, and serum ADA increased in most of the patients who responded to the anti-PD-1 therapy.

Recently, the analysis of several biomarkers, including peripheral T cells [11], circulating tumor cells [12], and serum cytokines [13], have been shown to be potentially useful for monitoring treatment response in patients with melanoma and NSCLC who are being treated with anti-PD-1 therapy. The analyzes of these blood biomarkers are less invasive than biopsies, and they can be repeated and sequentially studied. However, these methods are still under investigation and are not yet established in clinical practice. Routinely available blood and clinical markers that may predict response and toxicity to immune checkpoint inhibitors include lymphocyte count, eosinophil count, the neutrophil/lymphocyte ratio, lactate dehydrogenase and C-reactive protein, all of which have been reported to be correlated with the efficacy of anti-PD-1 therapy [14]. However, these markers do not directly reflect T cell immune response, and concurrent inflammatory conditions may also be affected. Serum ADA is a marker that is measurable in clinical practice and is believed to reflect immune response, especially T cell activity.

Although, in patients who were treated with anti-PD-1 therapy, there are showing remarkable effects, patients with insufficient effects are also seen, and it is surmised that immunosuppressive mechanisms of different routes are working. In recent years, the mechanism of immunosuppression of cancer cells through extracellular adenosine production has been revealed. Cancer cells produce adenosine through CD39/CD73 and suppress INF-γ production and cytotoxicity via A2A adenosine receptor (A2AAR) expressed on T cells and NK cells infiltrated into tumor tissue. Hypoxia in tumor tissue also induces CD73 derived from host and produces adenosine. Cancer cells are also considered to suppress antitumor immunity by lymphocytes using the host's endogenous adenosine production system [15]. ADA is present intracellularly as well as on the cell surface in association with CD26, metabolizing extracellular adenosine and reducing its biological activity. Cell surface ADA lowers local levels of adenosine and modulates adenosine-mediated suppression in T cells by preventing binding to the A2AAR. Furthermore, development of immunotherapy targeting A2AAR is also in progress. Changes in serum ADA may reflect immune suppression due to these mechanisms, and may be important indicators such as treatment choice and evaluation of efficacy.

Our study has some limitations. First, it was a single-centered and small-sized retrospective study. Because of retrospective analysis, patient characteristics were not consistent, and the number of patients may not have been sufficient for this examination. Second, changes in ADA were limited and could occur within the range of error. However, in the present study, a similar tendency was observed at several time points both of chemotherapy group and anti-PD-1 therapy group. The change in ADA was considered to be reproducible. Third, the present study was not adjusted to other known predictors, such as PD-L1 expression. ADA may be confounding with other predictors. The association between changes in serum ADA and the efficacy of anti-PD-1 therapy thus remains inconclusive and warrants further prospective study with larger cohorts.

5. Conclusions

In conclusion, our findings indicate that changes in serum ADA are associated with the efficacy of anti-PD-1 in patients with lung cancer. Further studies with larger numbers of patients and longer follow-up times are needed to validate our findings.

Conflicts of interest

Dr. Nishio received research funding from Novartis, ONO Pharmaceutical, Chugai Pharmaceutical, Bristol-Myers Squibb, TAIHO Pharmaceutical, Eli Lilly, Pfizer, Astellas Pharma and AstraZeneca, and honoraria from Pfizer, Bristol-Myers Squibb, ONO Pharmaceutical, Chugai Pharmaceutical, Eli Lilly, TAIHO Pharmaceutical and AstraZeneca. Dr. Horiike received research funding from Chugai Pharmaceutical and MSD, and honoraria from Pfizer, Chugai Pharmaceutical, Eli Lilly, Boehringer Ingelheim and AstraZeneca. Dr. Yanagitani received personal fees from Chugai Pharmaceutical. All the other authors have stated that they have no conflicts of interest.

Acknowledgments

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Appendix

Table A1
Change in serum adenosine deaminase activity.

	Chemotherapy (n = 20)		Anti-PD-1 therapy (*n* = 30)		*p*
	Median (U/L)	Range (U/L)	Median (U/L)	Range (U/L)	
Baseline	16.65	10.2–27.5	21.00	11.8–56.0	0.216
Day 7 of first cycle	13.45	7.3–25.2	21.1	11.2–49.8	< 0.001
Day 0 of second cycle	15.8	11.1–23.4	20.55	10.3–66.3	0.003
Day 0 of third cycle	18.15	10.2–41.0	21.7	11.9–99.5	0.03

PD-1, programmed cell death-1.

Table A2
Change in serum ADA activity due to therapeutic effect.

	Chemotherapy (n = 20)			Anti-PD-1 therapy (n = 30)		
	PR or SD (n = 17)	PD (n = 3)	p	PR or SD (n = 16)	PD (n = 14)	p
Serum ADA activity						
Baseline						
Median	16.7	14.2	0.874	21.0	20.0	0.819
Range	10.2–27.5	12.4–24.6		13.1–37.0	11.8–56.0	
Day 7 of first cycle						
Median	12.7	14.2	0.791	22.1	19.5	0.493
Range	7.3–25.2	10.9– 15.0		12.0–40.3	11.2–49.8	
Day 0 of second cycle						
Median	16.3	14.0	0.596	21.7	20.4	0.454
Range	11.1–23.4	12.0–21.4		13.8–40.6	10.3–66.3	
Day 0 of third cycle						
Median	18.5	15.0	0.634	22.8	21.5	0.521
Range	10.2–41.0	13.9–22.0		13.7–37.5	11.9–99.5	
% of ADA change from baseline						
Day 7 of first cycle						
Median	77	88	0.634	106	93	0.004
Range	62–132	58–106		90–134	75–122	
Day 0 of second cycle						
Median	92	97	0.634	108	103	0.129
Range	83–133	87–99		95–137	61–123	
Day 0 of third cycle						
Median	106	98	0.56	109	97	0.152
Range	67–247	89–121		97–155	72–178	

Abbreviations: ADA: adenosine deaminase; PD-1: programmed cell death-1; PR: partial response; SD: stable disease.

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