Elevated eosinophils as predictor of immune-related adverse events after ipilimumab and nivolumab treatment of advanced and metastatic renal cell carcinoma： a multicenter cohort study Eosinophil could be biomarkers for irAE

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Abstract

Purpose: Ipilimumab and nivolumab treatment against advanced and metastatic renal cell carcinoma (RCC) causes severe and lethal immune-related adverse events (irAEs). Predicting irAEs might improve clinical outcomes, however no practical biomarkers exist. This study examined whether eosinophils could be effective biomarkers for irAEs in RCC.

Methods: We retrospectively analyzed 75 patients with RCC treated with ipilimumab and nivolumab between August 2018 and March 2021 in a multicenter study. Eosinophils were examined before and two weeks after treatment, and immediately after irAE development. Median overall (mOS) and progression-free (mPFS) survival were examined by Kaplan–Meier method. The optimal cut-off value for irAE was determined by a receiver operating characteristic (ROC) curve. Univariate and multivariate analyses were undertaken to identify predictors of irAEs.

Results: The mOS and mPFS of patients who experienced irAEs (irAE group) were longer than those of the non-irAE group. Grade ≧2 irAEs were associated with poor mPFS. The eosinophil level two weeks after treatment was significantly upregulated in the irAE compared to non-irAE group (mean, 3.0% vs. 5.7%; $P < 0.05$). The ROC curve revealed the optimal cut-off value for eosinophil levels against ≧grade 2 irAE two weeks after treatment was 3.0% (area under the curve=0.699). In multivariate analyses, an eosinophil level ≧3.0% was a risk factor for ≧grade 2 irAE (odds ratio 4.18, 95% confidence interval 1.16–15.1).

Conclusion: An increased eosinophil level two weeks after treatment might be an effective biomarker for irAEs in patients with RCC treated with ipilimumab and nivolumab.

Introduction

Advanced and metastatic renal cell carcinoma (RCC) was previously recognized as one of the most aggressive human malignancies, in which the five-year survival rate was around 13% (Siegel, Miller, Fuchs, & Jemal, 2021). However, the approval and initiation of immune checkpoint inhibitor (ICI) therapy improved overall survival in this disease (Albiges et al., 2020; Choueiri et al., 2021; R. Motzer et al., 2021; R. J. Motzer et al., 2015; R. J. Motzer et al., 2019; Rini et al., 2019). The standard therapy for RCC of intermediate/poor risk according to the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) uses ipilimumab and nivolumab combination therapy as a first-line therapy. A four-year follow-up of the CheckMate 214 trial showed that median overall survival (mOS) was 48.1 months and the overall survival (OS) probability at four years in patients with RCC treated with ipilimumab and nivolumab was 53.4% (Albiges et al., 2020). However, many patients with RCC who were treated with ipilimumab and nivolumab inevitably developed immune-related adverse events (irAEs) with treatment discontinued or death due to the onset of irAE.

Immune-related adverse events occur when the immune system becomes non-specifically activated and can affect most organ systems. The CheckMate 214 trial demonstrated that 46% of patients had suffered

from a grade 3–4 irAE (R. J. Motzer et al., 2018). In particular, comprehensive studies have shown that ipilimumab and nivolumab therapy was associated with higher mortality rates than ICI monotherapy (D. Y. Wang et al., 2018). Therefore, the development of an effective biomarker to predict the onset of irAE is imperative. However, to date, no such practical biomarker has been reported in RCC treated with ipilimumab and nivolumab.

In this study, our aim was to identify peripheral blood biomarkers that were associated with the onset of irAE in patients with RCC that underwent ipilimumab and nivolumab therapy in a retrospective, multicenter study.

Materials And Methods

Patients and treatment

Data were retrospectively obtained from 75 patients who had been diagnosed with RCC and had undergone ≥ 1 course of treatment with ipilimumab and nivolumab (1 mg/kg ipilimumab and 240 mg/body nivolumab on day 1) between Aug 2018 and April 2021 in a multicenter study. Diagnosis of pancreatic cancer was determined histologically by experienced pathologists. We evaluated primary and metastasis tumor site such as bone, liver, lung or others (lymph node, brain, adrenal glands, abdominal wall, tooth ridge, iliopsoas muscle and pleura) by using Computed Tomography and Magnetic Resonance Imaging. The classification of responses was determined by experienced physicians based on radiology reports or imaging reviews using the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 (Eisenhauer et al., 2009) such as a complete response (CR), partial response (PR), stable disease, and progressive disease (PD). The characteristics of patients are listed in Table 1.

Table 1
Clinical features of patients

Characteristics, n (%)	All Patients	Non-irAE group	irAE group	*P* value
Total	75 (100)	27 (36.0)	48 (64.0)	
Age, n (%)				0.06
< 65 years	23 (30.7)	12 (44.4)	11 (22.9)	
≧ 65 years	52 (69.3)	15 (55.6)	37 (77.1)	
Gender				0.41
Male	56 (74.7)	22 (81.5)	34 (70.8)	
Female	19 (25.3)	5 (18.5)	14 (29.2)	
IMDC risk group				0.33
Intermediate	43 (57.3)	13 (48.1)	30 (62.5)	
Poor	32 (42.7)	14 (51.9)	18 (37.5)	
Karnofsky Performance Status				
30	1 (1.3)	0 (0.0)	1 (2.1)	0.44
40	1 (1.3)	1 (3.7)	0 (0.0)	
50	5 (6.7)	1 (3.7)	4 (8.3)	
60	3 (4.0)	1 (3.7)	2 (4.2)	
70	12 (16.0)	7 (25.9)	5 (10.4)	
80	14 (18.7)	4 (14.9)	10 (20.8)	
90	16 (21.3)	5 (18.5)	11 (22.9)	
100	20 (26.7)	5 (18.5)	15 (31.3)	
Not evaluable	3 (4.0)	3 (11.1)	0 (0.0)	
Histological subtype				
Clear cell	57 (76.0)	19 (70.4)	38 (79.2)	0.66
Non-clear cell	9 (12.0)	4 (14.8)	5 (10.4)	
Not evaluable	9 (12.0)	4 (14.8)	5 (10.4)	
Metastasis site, Bone				

IMDC: International Metastatic Renal Cell Carcinoma Database Consortium; irAE, immune-related adverse event

Characteristics, n (%)	All Patients	Non-irAE group	irAE group	*P* value
No	59 (78.7)	23 (85.2)	36 (75.0)	0.38
Yes	16 (21.3)	4 (14.8)	12 (25.0)	
Metastasis site, Liver				
No	65 (86.7)	22 (81.5)	43 (89.6)	0.48
Yes	10 (13.3)	5 (18.5)	5 (10.4)	
Metastasis site, Lung				
No	30 (40.0)	8 (29.6)	22 (45.8)	0.22
Yes	45 (60.0)	19 (70.4)	26 (54.2)	
Metastasis site, Others				
No	46 (61.3)	13 (48.1)	33 (68.8)	0.09
Yes	29 (38.7)	14 (51.9)	15 (31.2)	
Nephrectomy before ipilimumab and nivolumab				
No	42 (56.0)	17 (63.0)	25 (52.1)	0.33
Yes	31 (41.3)	9 (33.3)	22 (45.8)	
Not evaluable	2 (2.7)	1 (3.7)	1 (2.1)	
Number of courses				1.00
1	7 (9.3)	3 (11.1)	4 (8.3)	
2	9 (12.0)	3 (11.1)	6 (12.5)	
3	15 (20.0)	5 (18.5)	10 (20.8)	
4	44 (58.7)	16 (59.3)	28 (58.3)	
Response to ipilimumab and nivolumab				< 0.01
Complete response	3 (4.0)	3 (11.1)	0 (0.0)	
Partial response	28 (37.3)	4 (14.8)	24 (50.0)	
Stable disease	15 (20.0)	4 (14.8)	11 (22.9)	
Progressive disease	21 (28.0)	12 (44.4)	9 (18.8)	

IMDC: International Metastatic Renal Cell Carcinoma Database Consortium; irAE, immune-related adverse event

Characteristics, n (%)	All Patients	Non-irAE group	irAE group	*P* value
Not evaluable	8 (10.7)	4 (14.8)	4 (8.3)	
Steroids before ipilimumab and nivolumab				0.36
Use of steroids	1 (1.3)	1 (3.7)	0 (0.0)	
No use of steroids	74 (98.7)	26 (96.3)	48 (100.0)	

IMDC: International Metastatic Renal Cell Carcinoma Database Consortium; irAE, immune-related adverse event

Immune-related adverse events

An irAE was defined as an adverse effect that promotes immune system activity and requires intensive monitoring or treatment with steroids. We divided patients into two groups: irAE and non-irAE; depending on whether they had experienced an irAE or not, respectively. In addition, we analyzed the association with clinical outcome. Each irAE was divided into six disease groups, such as endocrine, gastrointestinal, skin, pulmonary, and others, and graded according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0. A profile of irAEs is listed in Table 2.

Table 2
Profile of irAEs

	Number of Patients	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)	Grade 5 (%)
Profile of irAE, n (%)	68 (100)	28 (41.2)	11 (16.1)	19 (27.9)	5 (7.4)	5 (7.4)
Endocrine	17 (25.0)	6	4	5	1	1
Thyroid dysfunction	10	6	3	1	0	0
Primary adrenal insufficiency	4	0	1	1	1	1
Pituitary dysfunction	3	0	0	3	0	0
Gastrointestinal	14 (20.6)	4	2	6	0	2
Colitis	7	2	2	1	0	2
Hepatitis	3	0	0	3	0	0
Decreased appetite	3	2	0	1	0	0
Nausea	1	0	0	1	0	0
Skin	11 (16.2)	8	2	1	0	0
Rash	7	4	2	1	0	0
Pruritus	4	4	0	0	0	0
Pulmonary	9 (13.2)	1	2	3	1	2
Pneumonitis	7	1	1	3	1	1
Pulmonary embolism	1	0	0	0	0	1
Asthma	1	0	1	0	0	0
Others	17 (25.0)	9	1	4	3	0
Fever	4	4	0	0	0	0
Infusion reaction	2	1	1	0	0	0
Myocarditis	2	0	0	0	2	0
Renal disorder	2	2	0	0	0	0
Encephalitis	1	0	0	1	0	0
Myasthenia gravis	1	0	0	0	1	0

irAE: immune-related adverse event

	Number of Patients	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)	Grade 5 (%)
Myoclonus	1	0	0	1	0	0
Demyelinating polyneuropathy	1	0	0	1	0	0
Optic neuritis	1	0	0	1	0	0
Elevation of serum creatine kinase	1	1	0	0	0	0
Dysgeusia	1	1	0	0	0	0
irAE: immune-related adverse event						

Data collection

In order to identify a predictive biomarker, we examined peripheral blood parameters after blood sampling immediately after the occurrence of an irAE (irAE sample), before one course of treatment with ipilimumab and nivolumab (baseline samples), two weeks after treatment with one course of ipilimumab and nivolumab (two-week samples), and before two course of treatment with ipilimumab and nivolumab (two-course samples), including hemoglobin, white blood cells, neutrophils, eosinophils, lymphocytes, monocytes, platelets, lactate dehydrogenase, calcium, C-reactive protein, and creatinine. This study was undertaken according to the World Medical Association Declaration of Helsinki and approved by an institutional review board (approval number 60-19-0196).

Statistical analyses

Data are presented as boxplots. A middle horizontal line was drawn inside each box that signifies the median value. The 25th and 75th percentiles were represented by the bottom and top of each box, respectively. The ends of the whisker plots signify the minimum and maximum of the data for that plot, respectively. *P*-values of statistical significance are indicated as $*P < 0.05$; $**P < 0.01$. Differences in the quantified data between groups were compared using one-way ANOVA, followed by a Bonferroni post hoc test, or t-test. Fisher's exact test was used to assess differences in the characteristics of patients. The optimal cut-off points for potential peripheral blood biomarkers to predict irAE onset were determined form the analysis of receiver operating characteristic (ROC) curves. Overall survival and progression-free survival (PFS) were calculated using a Kaplan–Meier method and log-rank test. Univariate and multivariate logistic regression analyses were used to assess risk factors for irAE. All reported *P*-values were two-sided. Statistical analyses were performed using GraphPad Prism 9 software, and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan, (Kanda, 2013)).

Results

Patient characteristics and frequency of onset of irAEs

Patient characteristics are shown in Table 1. Of the 75 patients, 48 (64%) had experienced an irAE (irAE group) while 27 (36%) had not experienced an irAE (non-irAE group). The age, ratio of genders, distribution of IMDC risk, Karnofsky Performance Status, histological subtype, metastasis site (bone, liver, lung and others), Nephrectomy before ipilimumab and nivolumab, number of courses, and use of steroids before ipilimumab and nivolumab did not significantly differ between the two groups. The irAE group showed a significantly better response to ipilimumab and nivolumab than the non-irAE group ($P < 0.01$).

The irAE profiles are shown in Table 2. A total of 68 irAEs were observed. The most affected systems were endocrine (n = 17; 25.0%), followed by gastrointestinal (n = 14; 20.6%), skin (n = 11; 16.2%), and pulmonary (n = 9; 13.2%), as well as others (n = 17; 25.0%). Of the irAE group, 58.8% of patients had experienced an irAE of grade ≧ 2.

IrAE of grade ≧ 2 is related to poor clinical outcome

The mOS and median (m)PFS of the irAE group was significantly longer than that of the non-irAE group (mOS, $P < 0.05$; mPFS, $P < 0.05$, respectively; Fig. 1a and b). The mOS of patients in the irAE group who experienced an irAE of grade ≧ 2 was no different to that of patients in the irAE group who experienced an irAE of grade 1 ($P = 0.61$; Fig. 1c). Notably, the mPFS of patients in the irAE group who tolerated an irAE of grade ≧ 2 was significantly shorter than of patients in the irAE group who tolerated an irAE of grade 1 ($P < 0.05$; Fig. 1d), indicating that irAE onset of grade ≧ 2 was associated with a poor clinical outcome.

Blood sampling immediately after occurrence of grade ≧ 2 irAE

Differential outcomes in PFS between irAE of grade ≧ 2 and grade 1, inspired us to examine the effective biomarkers for predicting occurrence of grade ≧ 2 irAE. To identify effective biomarkers associated with the onset to grade ≧ 2 irAE, we examined blood samples from patients of the irAE group collected immediately after the occurrence of irAE (irAE samples) with respect to: hemoglobin, white blood cells, neutrophils, lymphocytes, eosinophils, monocytes, platelets, lactate dehydrogenase, calcium, C-reactive protein, and creatinine. The eosinophil count in irAE samples derived from patients who had experienced grade ≧ 2 irAE, was significantly upregulated compared to that before one course of treatment with ipilimumab and nivolumab (baseline sample; $P < 0.05$, Fig. 2a), whereas other factors were not apparently different between baseline and irAE samples derived from patients who had experienced grade ≧ 2 irAE (Supplementary Fig. S1). The factors in irAE samples derived from patients who had experienced grade 1 irAE did not change in baseline samples (Supplementary Fig. S2)

Upregulation of eosinophil count two weeks after treatment predicts occurrence of grade ≧ 2 irAE

To elucidate whether the eosinophil level predicts the onset of grade ≧ 2 irAEs, we examined this at an early phase of treatment with ipilimumab and nivolumab. The eosinophil count in before two course of treatment with ipilimumab and nivolumab (two-course sample), was upregulated in irAE group derived from patients who had experienced grade ≧ 2 irAEs compared to that in non-irAE group (Supplementary Fig. S3a); however, this was not observed for baseline samples (Fig. 2b). To find out if it is predictable before two course of treatment, we next examined the eosinophil count specifically in two weeks after treatment with one course of ipilimumab and nivolumab (two-week sample). Notably, the eosinophil count of the irAE group derived from patients who had experienced grade ≧ 2 irAE, was found to be significantly higher than that of the non-irAE group and irAE group derived from patients who had experienced grade 1 irAE in two-week sample (Fig. 2c).

Cut-off value of eosinophil count in two-week samples for predicting occurrence of grade ≧ 2 irAE

The optimal cut-off value for the eosinophil count in two-week samples to differentiate the occurrence of grade ≧ 2 irAE was 3.0% as determined by ROC curve analysis (area under the curve = 0.699, 95% confidence interval = 0.54–0.84, sensitivity = 0.75, specificity = 0.64; Fig. 3a). Univariate and multivariate logistic regression analyses showed that an eosinophil count of ≧ 3.0% in two-week samples was found to be an independent risk factor for the development of grade ≧ 2 irAEs (Table 3). Consistently, an eosinophil count of < 3.0% in two-week samples correlated with a poor mOS and mPFS (mOS, $P < 0.05$; mPFS, $P < 0.05$, respectively; Fig. 3b and c).

Table 3
Univariate and multivariate logistic regression analysis of risk factors for the occurrence of ≧ grade 2 irAEs

	Univariate			Multivariate		
	OR	95%CI	*P* value	OR	95%CI	*P* value
Age: ≥65 years	1.20	0.44–3.23	0.71			
Gender: male	1.28	0.44–3.66	0.64			
IMDC risk group: Poor	0.52	0.20–1.33	0.17			
Karnofsky Performance Status: ≥80	3.68	1.23-11.0	< 0.05	3.3	0.79–13.7	0.10
Histological subtype, Clear cell: Yes	1.52	0.51–4.47	0.13			
Histological subtype, Non-clear cell: Yes	0.90	0.22–3.67	0.88			
Metastasis site, Bone: Yes	2.27	0.72–7.06	0.15			
Metastasis site, Liver: Yes	0.44	0.10–1.86	0.26			
Metastasis site, Lung: Yes	0.51	0.20–1.30	0.15			
Metastasis site, Others: Yes	0.44	0.16–1.16	0.09			
Nephrectomy before ipilimumab and nivolumab: Yes	1.79	0.69–4.56	0.22			
Eosinophil count: ≥3.0%	5.33	1.55–18.3	< 0.01	4.18	1.16–15.1	< 0.05

CI: confidence interval; IMDC: International Metastatic Renal Cell Carcinoma Database Consortium; irAE: immune-related adverse event; OR: odds ratio

Discussion

In this study, we demonstrated that upregulation of the eosinophil count ≧ 3.0% two weeks after treatment might be a predictive biomarker for the onset of irAEs of grade ≧ 2 in patients with RCC treated with ipilimumab and nivolumab. Recent comprehensive studies on onset to irAE induced by ipilimumab and nivolumab have shown that the median-time onset of all-grade irAEs ranged from 2.4 to 13.9 weeks (Tang et al., 2021), indicating the need to predict irAEs before two course of treatment with ipilimumab and nivolumab. Although several reports have suggested an association of blood cell count parameters with the prediction of irAEs in patients under ICI therapy (Hommes, Verheijden, Suijkerbuijk, & Hamann, 2020), no reports exist on effective biomarkers, especially in RCC treated with ipilimumab and nivolumab.

Eosinophils in two weeks after treatment might be one of the key determinants of irAEs onset of grade ≧ 2 in patients with RCC and who underwent ipilimumab and nivolumab therapy, as reported for other, different types of cancers. Our approach using eosinophils as an effective prediction biomarker might help to diagnose and treat irAEs.

Eosinophils have a homeostatic role in the immune response. They combat several parasitic, bacterial, and viral infections by interacting with B cells, T cells, macrophages, and neutrophils. Eosinophils are further involved in the regulation of several diseases, including allergic asthma, esophagitis, myopathies, and autoimmune disorders due to directly or indirectly promoting tissue damage or altering the local immune status (Kita, 2011; Rosenberg, Dyer, & Foster, 2013; Wechsler et al., 2021). A recent study showed that upregulation of the eosinophil count in the peripheral blood is linked with a better prognosis and response to ICI therapy. In patients with recurrent or metastatic head and neck squamous cell carcinoma treated with nivolumab, a higher eosinophil count was associated with better survival (Minohara et al., 2021; Nishikawa et al., 2021; Nishikawa et al., 2018). The eosinophil count positively correlated with overall survival in patients with melanoma treated with ipilimumab (Martens et al., 2016). Indeed, eosinophils induced by ICI play an important role in the elimination of tumors. Mechanistically, functional experiments showed that anti-cytotoxic T-lymphocyte–associated protein 4 increased eosinophil infiltration into tumors. Activated tumor-infiltrating eosinophils produced chemokines and recruited CD4 + T cells and CD8 + T cells, which resulted in tumor elimination (Carretero et al., 2015; Jia-Nan Cheng & Zhihua Gong, 2021; Zheng et al., 2020). Furthermore, eosinophils associate with the onset of irAE induced by ICI. A recent case report described how specific tissue-infiltrating eosinophils occurred as an adverse effect, such as in eosinophilic fasciitis and eosinophilic pneumonia triggered by ICI (Chan et al., 2019; Jodai et al., 2019). Therefore, several studies have focused on the eosinophil count as an effective biomarker of the onset of irAEs (Adam Diehl, 2017; Chu et al., 2020; Nakamura et al., 2019). Our data showed that the upregulation of eosinophils is associated with the clinical outcome and onset of irAEs, as reported in different types of cancers. These results collectively indicated that the upregulation of eosinophils in peripheral blood reflects the efficacy and toxicity of ICI by tumor and tissue-infiltrating eosinophils.

Previous studies on eosinophils as a biomarker to predict irAEs have focused on levels before treatment or one month after treatment. For example, a high eosinophil count before or one month after nivolumab or pembrolizumab monotherapy increased the risk of occurrence of irAEs by 1.3 times in solid tumors (Adam Diehl, 2017). Additionally, upregulation of eosinophils before or one month after nivolumab or pembrolizumab monotherapy may be an effective biomarker to predict endocrine irAEs in patients with melanoma (Nakamura et al., 2019). Interestingly, our data revealed that patients in the irAE group who experienced endocrine, gastrointestinal, and skin disorders had a significantly higher eosinophil count in two-week samples than patients in the non-irAE group ($P < 0.05$ for all; Supplementary Fig. S3b–d). Patients in the irAE group who experienced pulmonary disease tended to show a higher eosinophil count than those in the non-irAE group ($P = 0.06$; Supplementary Fig. S3e), but not for other disorders ($P = 0.42$; Supplementary Fig. S3f). Furthermore, the eosinophil count in two-week samples of patients in the irAE group was significantly upregulated at the onset of irAE, not only during early courses (one or two

courses) but also during late courses (three or four courses) compared to that of patients in the non-irAE group ($P < 0.05$; Supplementary Fig. S3g and h), indicating that the eosinophil level two weeks after treatment was upregulated by the onset of any type of irAE, excluding other diseases, regardless of when the adverse event occurred. These results suggest the eosinophil count is regulated by different ICIs and types of cancers. Our data also suggest the necessity of examining the eosinophil count during one course of ipilimumab and nivolumab to predict the onset of irAEs.

In the past, most studies had shown ICI-mediated irAEs might be associated with an improved response to therapy and survival outcome in several types of cancers (Freeman-Keller et al., 2016; Y. Wang et al., 2018). However, especially in RCC, no consensus of evidence exists in the association between the onset of irAEs and clinical outcome. The CheckMate 214 trial showed that OS between patients with and without irAE did not reveal a significant difference in RCC after ipilimumab and nivolumab therapy (R. J. Motzer et al., 2020). In contrast, Ikeda et al. revealed that the onset of irAEs was significantly associated with an improvement of clinical outcome in RCC treated with ipilimumab and nivolumab (Ikeda et al., 2021). Our data revealed the significantly longer mOS of patients showing irAEs, along with the upregulation of eosinophils in peripheral blood, than that in patients without irAEs, as reported in different types of cancers. These controversial results might be reflected in different patient characteristics.

Interestingly, we found that having an irAE of grade ≧ 2 was associated with a poor clinical outcome, compared with having an irAE of grade 1 (mPFS; 16.7 months and 29.0 months, respectively; Fig. 1d). Consistently, in a study of 42-month results of the CheckMate 214 Trial, treatment-free survival of patients who experienced an irAE of grade ≧ 3 tended to be shorter than that of patients who did not experience an irAE of grade ≧ 3 (0.6 months and 6.1 months, respectively; (Regan et al., 2021)). Several studies have shown that the discontinuation of treatment by the onset of irAEs was associated with a poor clinical outcome (Naqash et al., 2020; Russano et al., 2021). Whereas mPFS of grade ≧ 2 irAE was shorter than that of Grade 1 irAE, mOS was not apparently different between Grade 1 and grade ≧ 2 irAEs. These results indicated that the follow-up period of OS may not be sufficient. Although further investigation is required, treatment discontinuation due to the onset of grade ≧ 2 irAEs might be related to a poor clinical outcome. Therefore, we need to predict and suppress the onset of irAEs, particularly irAEs of grade ≧ 2, to improve clinical outcomes. Our observations indicated that the prediction of grade ≧ 2 irAEs using the eosinophil level as an effective biomarker could further improve clinical outcome and prognosis due to treatment continuation.

The present study had several limitations. Only a small number of participants were involved in the study. In addition, because of the retrospective nature of the study, we could not control biases in the selection of patients. An interventional prospective study will be required to confirm our data.

In conclusion, our data provides a novel rationale for measuring the blood eosinophil level, with an elevated eosinophil count likely to be an effective biomarker for predicting grade ≧ 2 irAE onset in patients with RCC under ipilimumab and nivolumab therapy.

Abbreviations

CI
confidence interval
CR
complete response
ICI
immune checkpoint inhibitor
IMDC
International Metastatic Renal Cell Carcinoma Database Consortium
irAEs
immune-related adverse events
mOS
median overall survival
mPFS
median progression-free survival
OR
odds ratio
PD
progressive disease
PR
partial response
RCC
renal cell carcinoma
ROC curve
receiver operating characteristic curve
SD
stable disease

Declarations

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Competing interests

The authors have declared that no conflict of interest exists.

Authors' Contributions

Y.T., S.H., T. Yasui, and K.K. designed and directed the project. Y.T. analyzed most of the data with assistance from S.H., N.T., T. Naiki, T.E., K.T., S.I., N.M., Y.S., H.K., Y.N., Y. Hashimoto, T. Sakakura, M.A., Y.I., Y.K., Y.M., S.N., T.T., S.K., Y.M., K.O., A.O., and N.K. who carried out the acquisition of data. Y.S. conducted statistical analyses for the study. S.H. and Y.T. both wrote the manuscript. All authors discussed the results and made comments on the manuscript.

Data Availability

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

Ethics approval

This study was approved by the ethical review board at Nagoya City University Graduate School of Medical Sciences (approval number: 60-19-0196).

Consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. For retrospective study, the need for consent to participate was deemed unnecessary according to national regulations, “Ethical Guidelines for Medical and Health Research Involving Human Subjects.” The protocol summary was described on the hospital website, and the subjects were provided with the opportunity to opt-out.

Consent to publish

All authors consented the last version of this manuscript.

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Figures

Figure 1.

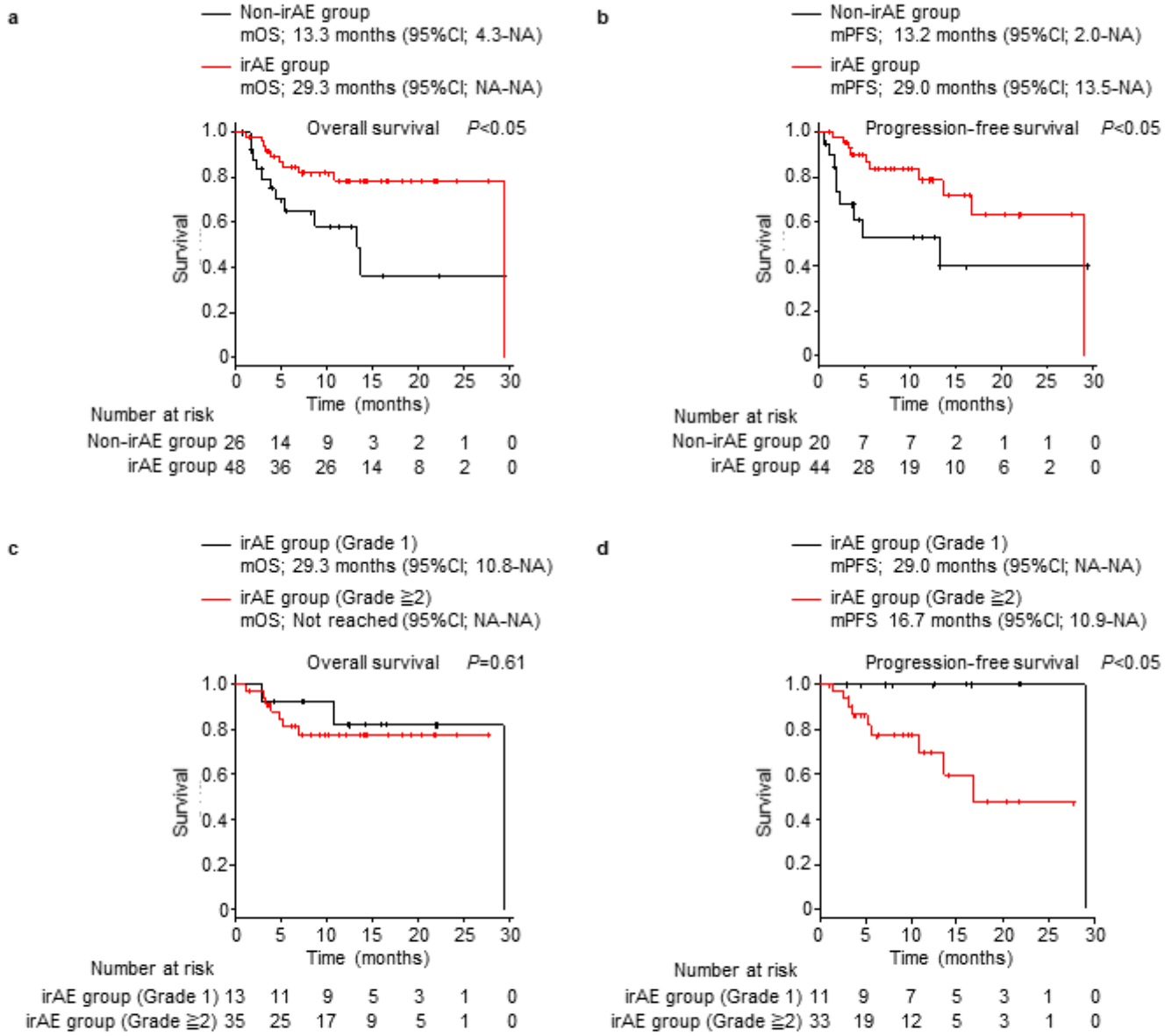

Figure 1

Occurrence of irAE associated with clinical outcome of treatment with ipilimumab and nivolumab

a–d, Kaplan–Meier survival curves for: (a) overall survival (non-irAE group; n=26, and irAE group; n=48); (b) progression-free survival (non-irAE group; n=20, and irAE group; n=44); (c) overall survival rate (irAE group [Grade 1]; n=13, and irAE group [Grade ≧2]; n=35); and (d) progression-free survival (irAE group [Grade 1]; n=11, and irAE group [Grade ≧2]; n=33) in patients. (a–d) log-rank test. CI, confidence interval; irAE, immune-related adverse events; mOS, median overall survival; mPFS, median progression-free survival; NA, not applicable.

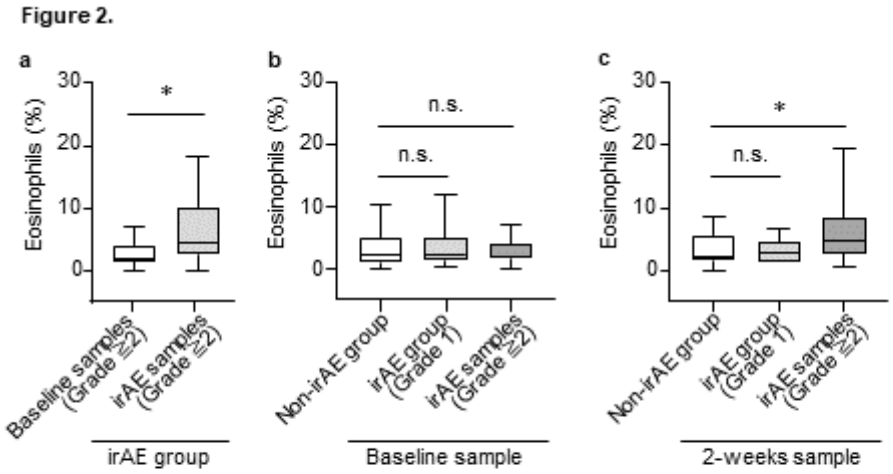

Figure 2

Upregulation of eosinophil count reflects onset of grade ≧2 irAE

a, Boxplots showing values for eosinophils (baseline samples, n=34, and irAE samples [Grade ≧2], n=24) in the blood samples of patients who had experienced irAE. **b,** Boxplot showing eosinophil counts in baseline samples; non–irAE (n=26) and irAE ([Grade 1]; n=13, [Grade ≧2]; n=34) groups. **c,** Boxplot showing eosinophil counts in two-week samples; non-irAE (n=19) and irAE ([Grade 1]; n=6, [Grade ≧2]; n=24) groups. The median value is represented by the middle horizontal line in each box. The bottom and top of each box indicate, respectively. The 25th and 75th percentiles are represented by the ends of the whiskers that indicate the minimum and maximum of all data, respectively. $^{*}P < 0.05$. (a) Unpaired *t*-test. irAE, (b–c) one-way ANOVA with Bonferroni post hoc tests, immune-related adverse events; n.s., not significant.

Figure 3.

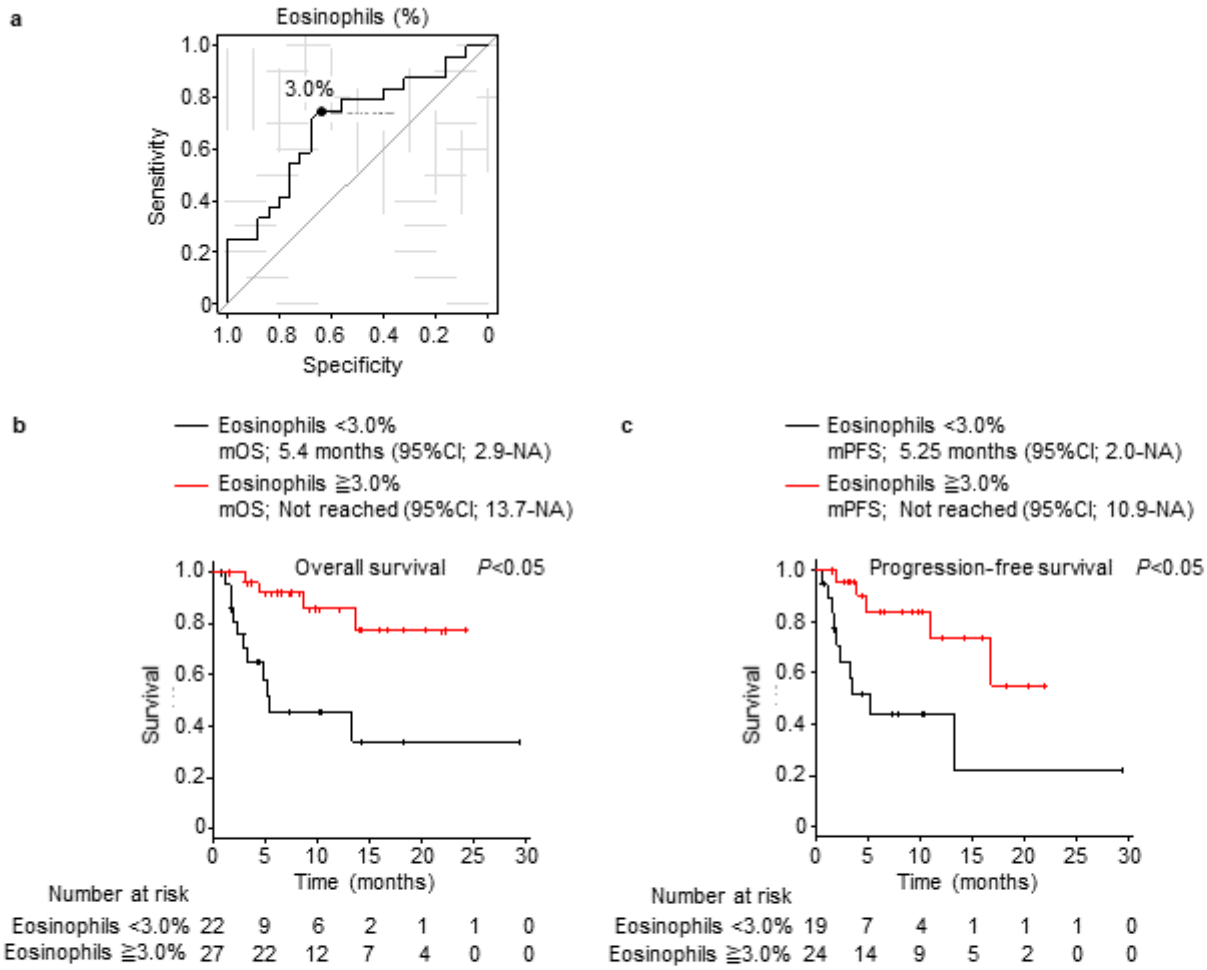

Figure 3

Optimal cut-off value of eosinophil count two weeks after treatment with ipilimumab and nivolumab

a, Receiver operating characteristic curve analysis of the eosinophil count for the occurrence of grade ≧2 irAE. **b–c,** Kaplan–Meier survival curve for (b) overall survival rate (eosinophils <3.0%; n=22, and eosinophils ≧3.0%; n=27) and (c) progression-free survival (eosinophils <3.0%; n=19, and eosinophils ≧3.0%; n=24) in patients. (b–c) log-rank test. CI, confidence interval; irAE, immune-related adverse events; mOS, median overall survival; mPFS, median progression-free survival; NA, not applicable.

Supplementary Files

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