Serum Lactate Dehydrogenase Before Nivolumab Treatment Could Be a Therapeutic Prognostic Biomarker for Patients With Metastatic Clear Cell Renal Cell Carcinoma

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Abstract. *Background/Aim: The purpose of this retrospective study was to identify the predictive biomarkers of response to pretreatment for patients with metastatic renal cell carcinoma (mRCC) treated with nivolumab. Materials and Methods: The subjects were 54 patients treated with nivolumab for mRCC with a clear cell component (mccRCC) between September 2016 and February 2018. We analyzed the impact of serum biomarkers (lactate dehydrogenase [LDH], neutrophil–to–lymphocyte ratio, and C-reactive protein) on patients treated with nivolumab. We adopted the International Metastatic Renal Cell Carcinoma Database Consortium prognostic model using six clinical factors (0=favorable, 1 or 2=intermediate, 3 to 6=poor risk groups, respectively). Results: The prognostic risk classification (non-poor vs. poor) and serum LDH levels were correlated with the objective response of nivolumab treatment for mccRCC. Elevated serum LDH levels at baseline were an independent biomarker for progression-free survival (PFS) of mccRCC patients receiving nivolumab [HR=2.268 (95%CI=1.014-5.051), p=0.046]. Notably, high LDH levels were associated with a poorer PFS for patients in the favorable-risk group. Conclusion: Serum LDH levels at baseline before nivolumab treatment were associated with the objective response and clinical outcome of nivolumab treatment.*

Nivolumab is an immune checkpoint inhibitor (ICI). It is a highly selective anti-programmed death 1 (PD-1) human monoclonal IgG4 antibody that potentiates T-cell responses by blocking the binding of PD-1 on activated T cells with its ligands, PD-L1 and PD-L2, expressed on antigen-presenting cells and on some tumor cells (1). Advanced and metastatic renal cell carcinomas (mRCC) are currently treated with molecular targeted therapy (MTT) agents as a first-line standard of care (2). In a phase III trial [CheckMate 025 trial (3)], the overall survival (OS) of patients treated with nivolumab was significantly prolonged compared to those treated with everolimus for metastatic clear cell RCC (mccRCC) patients who had failed prior MTT. Furthermore, various trials have reported excellent results using combination therapy of MTTs and /or ICIs for mccRCC (4, 5).

Despite these clinical successes, ICI for mccRCC does not achieve long-lasting responses for all patients. Buder-Bakhaya *et al.* have reviewed the current status of prognostic and predictive biomarkers (including clinical, tissue, blood, and stool biomarkers, as well as imaging biomarkers) in ICI mainly for melanoma and other malignancies (6). Blood biomarkers such as serum lactate dehydrogenase (LDH), C-reactive protein (CRP), and the neutrophil-to-lymphocyte ratio (NLR), have been identified as possible biomarkers for response to ICI (6). However, there is only a small number of reports on mRCC patients. The purpose of this retrospective study was to identify the predictive biomarkers at pretreatment in terms of response of patients with mccRCC treated with nivolumab.

Materials and Methods

Ethics and patient selection. This retrospective study received approval from the Institutional Review Board of Saitama Medical University International Medical Center (SIMC, approval #: 14-049). SIMC (n=31) and Keio University School of Medicine (n=23) provided data on 54 patients treated with nivolumab for mRCC with a clear cell component (mccRCC) between September 2016 and February 2018. Table I shows a comparison of patient characteristics.

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Routine examination and follow-up. In order to predict the outcomes of mccRCC patients at the initiation of first line therapy, we stratified them into three groups using six factors (time from RCC diagnosis to systemic therapy initiation, Karnofsky performance status (KPS), anemia, neutrophilia, thrombophilia, and serum corrected calcium) based on the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) model (0=favorable, 1 or 2=intermediate, 3 to 6=poor risk groups, respectively) (7). Basically, patients with mRCC who had failed MTTs received intravenously 3 mg/kg nivolumab, every two weeks. Laboratory values were standardized according to the institutional upper and lower limits of normal values. Radiographic evaluations using computed tomography (CT) and/or magnetic resonance imaging (MRI) were performed every two to three months, while additional CT and elective bone scans were performed when clinically indicated. Patients without neurological symptoms were generally not subjected to brain imaging tests.

Clinical outcome. Overall survival (OS) was defined as the time from initiation of first line targeted therapy to the date of death from any cause or date of censorship from last follow up. Progression-free survival (PFS) was defined as the time between nivolumab treatment initiation and radiographical progression or cancer-related death. Based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (8), we originally classified patients into two groups: A responder group of patients with complete response (CR), partial response (PR), or long stable disease (SD) for over 6 months and a non-responder group with progression disease (PD) or short SD for less than 6 months.

Statistical analysis. The variables of different groups were compared using the chi-square test or the Mann-Whitney *U*-test as appropriate. Laboratory values were standardized according to the institutional upper and lower limits of normal values. Values were presented as medians and interquartile ranges (IQR) or confidence intervals (CI) for continuous variables and as frequencies with percentages for categorical variables. Survival curves were constructed using the Kaplan–Meier method and compared using the log-rank test. Univariate and multivariate analyses were performed using the Cox proportional hazards model to identify factors associated with PFS. The level of significance was set at $p < 0.05$. The above analyses were done using the SPSS version 23.0 statistical software package.

Results

Patient characteristics (Table I). Fifty-four cases (44 males, 10 females) were treated with nivolumab for mccRCC in our cohort. The median follow-up duration from nivolumab initiation was 10.6 months (IQR=7.5-14.3). At initial systemic treatment for mccRCC, the status of IMDC risk classification was favorable in 12 (22.2%), intermediate in 34 (63.0%), and poor in eight patients (14.8%), respectively. Prior nephrectomy was performed in 49 patients (90.7%), and prior MTTs with more than three agents before nivolumab treatment was performed in 13 patients (24.1%).

During the first nivolumab treatment, the median age was

69 years (IQR=63-75), and 10 patients (18.5%) had KPS less than 80%. The metastatic sites were the lungs in 40 (74.1%), liver in 13 (24.1%), bone in 15 (27.8%), lymph nodes in 14 (25.9%), brain in six (11.1%), and pancreas in seven patients (13.0%), respectively. The median values of laboratory data were 2.89 (IQR=1.85-4.61) for NLR, 196 U/l (IQR=164-245) for LDH, and 0.67 mg/dl (IQR=0.19-1.91) for CRP, respectively.

Clinical outcome (Table I, Figure 1A-C). Best objective responses following nivolumab treatment were CR in two (3.7%), PR in 17 (31.5%), SD in 23 (42.6%), and PD in 12 patients (22.2%), respectively (Table I). The median PFS in the patients with SD was 6.0 months. During follow-up, 10 patients died, and 28 patients had disease progression. The median OS objective in the total cohort (n=54) was not reached (Figure 1A), but the median PFS was 7.9 months (95%CI=3.6-12.3, Figure 1B). In the sub-group analysis, the median PFS according to IMDC risk classification was 6.2 (95%CI=1.6-10.8) in the favorable risk group, 11.3 (5.4-17.3) in the intermediate risk group, and 1.8 months (1.0-2.7) in the poor risk group, respectively (Figure 1C).

Nivolumab treatment responders versus non-responders (Table I). We distinguished patients with SD into long and short SD groups based on the median PFS (6.0 months), and then we classified all patients into two groups [A responder group (n=31): CR, PR, and a long SD and a non-responder group (n=23): PD and short SD groups] as mentioned previously (see Material and Methods). Regarding IMDC risk classification, the responders included fewer patients with favorable risk along with poor risk patients and many of the intermediate risk group patients compared with the non-responders (favorable: 16.1 *vs.* 30.4%, intermediate: 77.4 *vs.* 43.5%, poor: 6.5 *vs.* 26.1%, *p*=0.029). As for laboratory data at nivolumab initiation, the median LDH levels were significantly lower in the responders than in the non-responders (178 *vs.* 229 U/l, *p*<0.001), and similarly NLR in the responders showed a lower tendency (2.46 *vs.* 3.33, *p*=0.060). Meanwhile, there were no significant differences in the CRP levels (*p*=0.944) or other factors (i.e. gender, prior nephrectomy, number of prior MTTs, age, KPS and metastatic sites at nivolumab initiation) between the two groups.

Predictive factors of therapeutic effect with nivolumab treatment (Table II, Figure 1D-F). We investigated the predictive factors of PFS in mccRCC patients treated with nivolumab based on the above results that compared the characteristics of the responders and non-responders. Univariate and multivariate analyses were performed using four indices: IMDC risk classification (non-poor *vs.* poor), and NLR, LDH, and CRP (low *vs.* high, median level as a

Table I. *Patients' characteristics*.

Variables	Total cohort (n=54)		Responder (n=31)		Non-responder (n=23)		*p*-Value
	n	%	n	%	n	%	
Gender							0.728#
Male	44	(81.5)	26	(83.9)	18	(78.3)	
Female	10	(18.5)	5	(16.1)	5	(21.7)	
IMDC risk classification							0.029##
Favorable	12	(22.2)	5	(16.1)	7	(30.4)	
Intermediate	34	(63.0)	24	(77.4)	10	(43.5)	
Poor	8	(14.8)	2	(6.5)	6	(26.1)	
Prior nephrectomy							1.000#
Yes	49	(90.7)	28	(90.3)	21	(91.3)	
No	5	(9.3)	3	(9.7)	2	(8.7)	
Prior MTTs							0.620##
1	23	(42.6)	12	(38.7)	11	(47.8)	
2	18	(33.3)	12	(38.7)	6	(26.1)	
≥3	13	(24.1)	7	(22.6)	6	(26.1)	
Status at the first nivolumab treatment							
Age (median, IQR, years old)	69 (63-75)		71 (65-76)		67 (57-74)		0.106###
KPS (%)							0.407##
100	33	(61.1)	21	(67.7)	12	(52.2)	
90	11	(20.4)	6	(19.4)	5	(21.7)	
≤80	10	(18.5)	4	(12.9)	6	(26.1)	
Metastatic sites							
Lung	40	(74.1)	24	(77.4)	16	(69.6)	0.515##
Liver	13	(24.1)	6	(19.4)	7	(30.4)	0.346##
Bone	15	(27.8)	8	(25.8)	7	(30.4)	0.707##
Lymph node	14	(25.9)	9	(29.0)	5	(21.7)	0.545##
Brain	6	(11.1)	2	(6.5)	4	(17.4)	0.384#
Pancreas	7	(13.0)	5	(16.1)	2	(8.7)	0.685#
Laboratory data (median, IQR)							
Neutrophil (%)	66.6 (58.3-73.9)		63.4 (54.8-72.3)		69.1 (62.9-80.0)		0.074###
Lymphocyte (%)	23.6 (15.8-31.1)		26.4 (20.5-31.9)		20.6 (14.2-27.7)		0.061###
NLR	2.89 (1.85-4.61)		2.46 (1.74-3.61)		3.33 (2.27-5.68)		0.060###
LDH (U/l)	196 (164-245)		178 (147-204)		229 (195-334)		<0.001###
CRP (mg/dl)	0.67 (0.19-1.91)		0.79 (0.25-1.37)		0.51 (0.07-3.57)		0.944###
Best objective response							
CR	2	(3.7)	2	(6.5)	0	(0.0)	
PR	17	(31.5)	17	(54.8)	0	(0.0)	
SD	23	(42.6)	12	(38.7)	11	(47.8)	
PD	12	(22.2)	0	(0.0)	12	(52.2)	

IMDC: International metastatic renal cell carcinoma database consoritum; MTTs: molecular targeted therapies; IQR: interquartile range; KPS: Karnofsky performance status; NLR: neutrophil-to-lymphocyte ratio; LDH: lactate dehydrogenase; CRP: C-reactive protein; CR: complete response; PR: partial response; SD: stable disease; PD: progression disease; responder: PR, CR or SD (PFS>6.0 mo); non-responder: PD, SD (PFS≤6.0 mo). #Fisher, ##Pearson, ###Mann-Whitney *U*-test.

reference). As a result, serum LDH levels before nivolumab treatment was an independent predictive factor of PFS during nivolumab treatment in mccRCC patients [HR=2.268 (95%CI=1.014-5.051), *p*=0.046]. Interestingly, especially in the favorable group, the patients with high LDH [median PFS 3.2 months (95%CI=1.5-5.0)] had significantly worse PFS with nivolumab treatment than those with low LDH (not reached, *p*=0.037, Figure 1D). There were no significant associations between LDH levels before nivolumab treatment and therapeutic outcome for the intermediate (*p*=0.308, Figure 1E) or poor groups (*p*=0.549, Figure 1F).

Discussion

The current study showed that IMDC risk classification (non-poor *vs*. poor) and serum LDH levels were associated with radiographic objective response to nivolumab treatment for

Table II. *Predictive factors of progression-free survival during nivolumab treatment in mRCC patients.*

	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
IMDC risk classification						
Non-poor *vs.* poor	2.740	1.101-6.822	0.030	1.681	0.660-5.366	0.237
NLR						
Low *vs.* high	1.957	0.912-4.202	0.085	1.894	0.826-4.348	0.132
LDH (U/l)						
Low *vs.* high	2.494	1.144-5.435	0.021	2.268	1.014-5.051	0.046
CRP (mg/dl)						
Low *vs.* high	1.244	0.588-2.628	0.568	1.430	0.570-3.592	0.446

Median NLR 2.89; median LDH 196.5 U/l; median CRP 0.67 mg/dl.

mccRCC (Table I), and that elevated serum LDH levels at baseline were an independent biomarker for PFS of mccRCC patients receiving nivolumab (Table II); in particular, notably higher LDH levels were associated with worse PFS for patients in the favorable risk group (Figure 1D).

LDH is an enzyme required during the process of turning sugar into energy in human cells. This enzyme is present in many kinds of organs and tissues throughout the body, and is released by rapidly growing tumors. Therefore, serum LDH is often used as an index of organ injury and tumor burden. For melanoma, it has been indicated as a significant prognostic biomarker in the American Joint Committee on Cancer classification (9). Also for mRCC, the Memorial Sloan Kettering Cancer Center (MSKCC) risk model which consists of five prognostic factors including serum LDH levels at baseline, has generally been adopted for prognostic prediction of mRCC patients in the era of cytokines and MTTs (10). Recently, the IMDC risk model consisting of six prognostic factors excluding serum LDH levels at baseline, has been employed for therapeutic selection, as well as prognostic prediction for mRCC patients in the new era of ICIs (7, 11); however, our results suggest that pretreatment LDH levels could be a convenient blood biomarker of response to nivolumab treatment for mccRCC patients.

ICI treatments for mRCC have been approved following major phase III trials: the CheckMate 025 trial (using the MSKCC risk model), nivolumab *vs.* everolimus as sequential therapy in all risk groups (3); the CheckMate 214 trial (using the IMDC risk model), and nivolumab plus ipilimumab *vs.* sunitinib as first-line treatment in intermediate and poor risk groups (5). Interestingly, subgroup analyses of OS in both trials consistently showed that the clinical outcomes of ICI treatments in favorable risk groups were far from satisfactory, and our results were similar (median PFS 6.2 in the favorable, 11.3 in intermediate, and 1.8 months in poor group, respectively, Figure 1C). McDermott *et al.* studied the gene signature related to angiogenesis, immune and antigen presentation, and myeloid inflammation in RCC tumors, and found that highly angiogenic tumors were more responsive to antiangiogenic therapy, but not ICIs (12). High LDH serum levels as an angiogenic biomarker, indicating the overexpression of vascular endothelial growth factor (VEGF) and VEGF receptor (13), could be related to better outcomes in patients undergoing antiangiogenic treatment (14). Recently, the cancer immunogram has been investigated as a framework for personalized immunotherapy, suggesting that LDH levels could be a biomarker of inhibitory tumor metabolism (*e.g.* intratumoral lactic acid and low pH) and impairment of antitumor T-cell function (15). LDH levels at nivolumab initiation could be a therapeutic biomarker for mccRCC patients with favorable risk, who initially have few inflammatory markers.

Regarding other biomarkers, Shrotriya *et al.* reviewed the role of CRP as a prognosis biomarker for tumor recurrence and treatment response in adult solid tumors including mRCC (16); however, the database was compiled before the introduction of ICIs. Bilen *et al.* reported an association between NLR at baseline and outcome of mRCC patients receiving nivolumab (17). Among our small cohort, there was no significant association between CRP levels and clinical outcome, but a strong relation was found between NLR and objective response.

The present study has several limitations. First, it was limited by its retrospective nature, as well as by its short median follow-up and limited number of Japanese patients. The laboratory data were collected separately at each institution. Not all patients underwent prior nephrectomy or biopsy before being administered first-line targeted therapy. Furthermore, the systematic therapeutic strategy, such as surgical procedures (radical or cytoreductive), radiotherapy (for bone or brain), and medications (types or timing), were not standardized by the various physicians.

In conclusion, despite the small cohort with heterogeneous characteristics, our results showed that serum LDH levels at
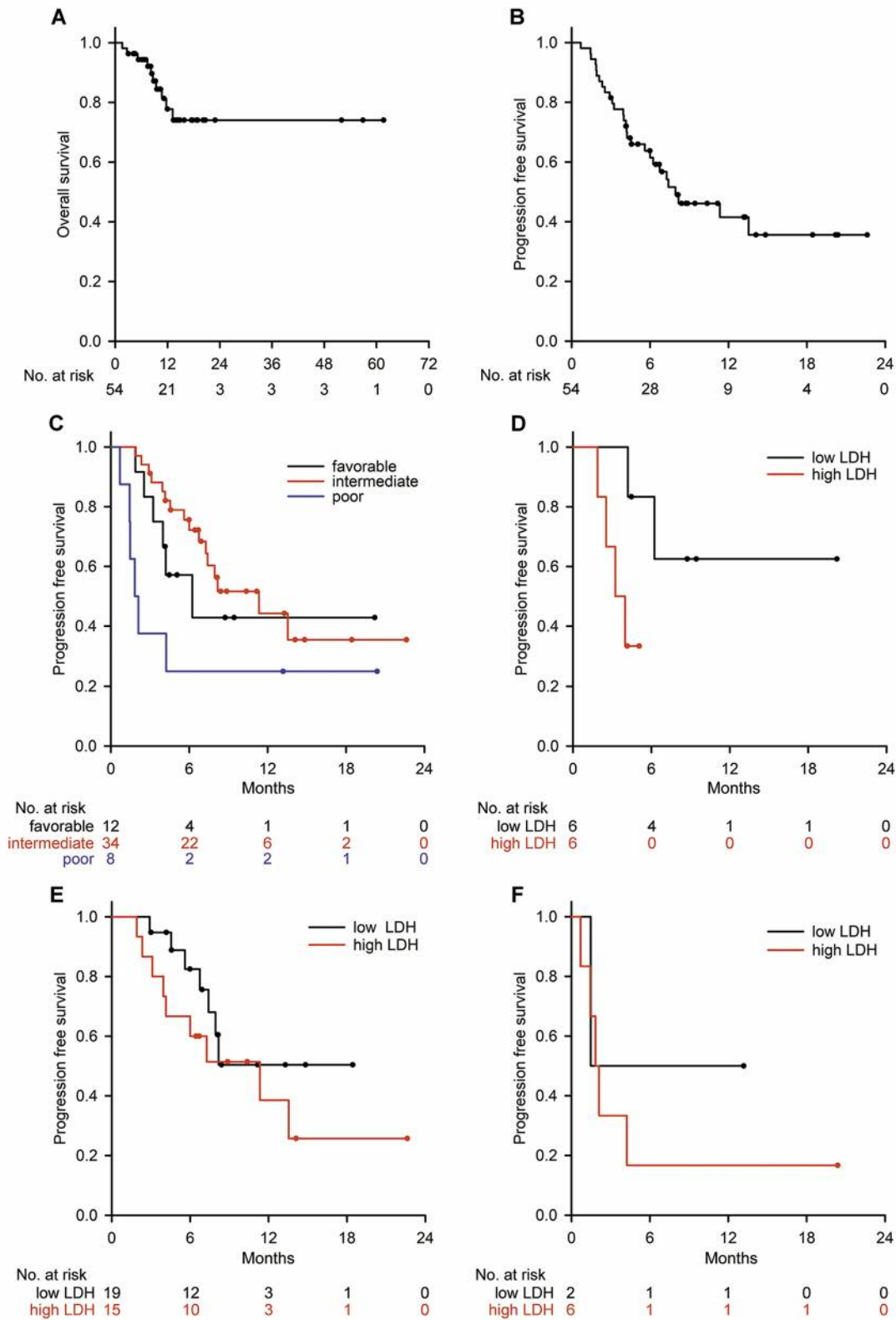
Figure 1. *Kaplan–Meier curves showing overall survival (OS, A) and progression-free survival (PFS, B) in the total cohort, as well as PFS according to the IMDC risk classification (C). Kaplan–Meier curves showing the association between PFS and serum LDH levels at baseline of nivolumab treatment for patients in the favorable (D), intermediate (E), and poor (F) risk groups, respectively.*

baseline before nivolumab treatment could be a therapeutic prognostic biomarker for patients with mccRCC. Further study is necessary to identify convenient and effective biomarkers to predict ICIs treatment response in order to select the optimal therapeutic strategy in the new era of ICIs.

Conflicts of Interest

All Authors declare that they have no conflicts of interest regarding this study.

Authors' Contributions

Conception and design of the study, Suguru Shirotake and Kimiharu Takamatsu; Collection and assembly of data, Suguru Shirotake, Kimiharu Takamatsu, Ryuichi Mizuno, and Go Kaneko; Analysis and interpretation of data, Suguru Shirotake, Kimiharu Takamatsu and Go Kaneko; Drafting of the article, Suguru Shirotake, Kimiharu Takamatsu and Go Kaneko; Critical revision of the article for important intellectual content, Ryuichi Mizuno, Koshiro Nishimoto, Mototsugu Oya and Masafumi Oyama; Final approval of the article, Suguru Shirotake. All Authors read and approved the version submitted for publication

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