Protocol

Protocol for a prospective multicenter intervention study (URivo study) assessing biomarkers in patients with previously treated advanced clear cell renal cell carcinoma receiving nivolumab

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ABSTRACT

Background: In recent years, immune checkpoint inhibitors have been introduced into routine clinical practice for treating patients with a wide variety of malignant tumors, including advanced renal cell carcinoma (aRCC), resulting in the significant improvement of the prognosis of these patients. However, a reliable biomarker predicting the clinical course in patients receiving nivolumab has not yet been developed; accordingly, the URivo study was planned to investigate the significance of various candidate biomarkers for aRCC patients treated with nivolumab.

Methods: This was designed as a prospective multicenter intervention study, and will include a total of 200 aRCC patients who are scheduled to receive nivolumab followed by treatment with either 1 or 2 tyrosine kinase inhibitors (TKIs). Using resected tumor tissues and serum samples prior to the introduction of nivolumab, the following assessments will be conducted: programmed death ligand-1 (PD-L1) and PD-L2 gene copy number gains by fluorescence in situ hybridization, serum concentrations of PD-L1 and PD-L2 by enzyme-linked immunosorbent assay, and expression of several proteins involved in apoptosis, epithelial-mesenchymal transition, signal transduction and immune reaction by immunohistochemical staining. The outcomes of these assays will be evaluated focusing on the association with the response to nivolumab, overall survival, progression-free survival and disease-specific survival.

Conclusions: The significance of various types of candidate biomarker, particularly PD-L1 and PD-L2, will be intensively investigated in this study, and this may offer unique information to determine the optimal indication of nivolumab for aRCC patients following the failure of TKIs.

Trial Registration: UMIN000030400; registered April 1, 2018.

Keywords: Advanced clear cell renal cell carcinoma, Nivolumab, Biomarker, PD-L1, PD-L2

INTRODUCTION

During the past 10 years, a paradigm shift has occurred in the field of systemic therapy for advanced renal cell carcinoma (aRCC) due to the introduction of various novel agents developed based on the molecular mechanisms mediating the progression of RCC.¹ These agents, which target vascular endothelial growth factor (VEGF) or mammalian target of rapamycin (mTOR), have significantly contributed to favorable clinical outcomes in patients with aRCC, particularly those sequentially receiving multiple agents.²,³ However, some
METHODS

Study design

The URivo study is an ongoing multicenter prospective open-label intervention single-arm study to identify reliable biomarkers in previously treated aRCC patients who will be treated with nivolumab. This study will evaluate whether the findings of several candidate biomarkers, which are described in detail below, are significantly correlated with the clinical outcomes of aRCC patients.

Endpoints

The primary endpoint of the URivo study is to investigate the impact of the findings of candidate biomarkers on the best overall response to nivolumab in the included aRCC patients. The tumor response to nivolumab will be assessed according to the response evaluation Criteria in Solid Tumors (RECIST) version 1.1.

The secondary endpoint is to assess the association between the findings of candidate biomarkers and prognostic outcomes, including OS, progression-free survival (PFS) and disease-specific survival (DSS), in these patients. Definitions of prognostic outcomes are as follows: OS, the time from the initiation of nivolumab to death due to any cause; PFS, the time from the initiation of nivolumab to first-demonstrated RECIST-defined tumor progression or death due to any cause; and DSS, the time from the initiation of nivolumab to death due to the progression of RCC.

Study population

Patients with aRCC, who were previously treated with either 1 or 2 TKIs and are going to receive nivolumab, are eligible for enrollment in the URivo study. After a full explanation of the study protocols, written informed consent from each participant will be obtained. Detailed eligibility and exclusion criteria for this study are listed in below.

Inclusion criteria

Inclusion criteria were unresectable or metastatic renal cell carcinoma; pathologically confirmed dominant component of clear cell carcinoma; previous history of treatment with 1 or 2 tyrosine kinase inhibitors; tissue specimens can be provided; measurable diseases according to RECIST version 1.1; major organ functions fulfilling the following criteria: neutrophil count ≥1,500/mm³, platelet count ≥ 75,000/mm³, hemoglobin ≥9.0 g/dl, aspartate aminotransferase ≤ 78 IU/l, alanine aminotransferase ≤ 85 IU/l, total bilirubin ≤ 1.5 mg/dl, creatinine ≤2.0 mg/dl; Age ≥20 years upon enrolment; Eastern Cooperative Oncology Group performance status 0 or 1; submission of written informed consent to participate in this study.
**Exclusion criteria**

Exclusion criteria were previous history of treatment with immune checkpoint inhibitors, including nivolumab; active infection; malignant tumors other than renal cell carcinoma; severe acute or chronic diseases other than infection or malignant tumors; woman who is pregnant or nursing; mental disease preventing participation in this study; intolerance to nivolumab; inappropriate characteristics for participating in this study other than those listed above.

**Study procedures**

The flowchart of the URivo study is presented in Figure 1. After obtaining informed consent by the attending physician, patients will undergo screening examinations, and their eligibility to be included in this study will be evaluated. Screening examinations will consist of patients’ characteristics (gender, age, height, body weight, complications, Eastern Cooperative Oncology Group performance status, Union for International Cancer Control TNM classification, Memorial Sloan-Kettering Cancer Center risk classification for previously treated aRCC patients and histopathological information of RCC), blood tests (red blood cell count, hemoglobin, hematocrit, white blood cell count, neutrophil count, platelet count, total protein, albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, amylase, blood urea nitrogen, creatinine, uric acid, creatine phosphokinase, C-reactive protein, blood glucose, sodium, potassium, chlorine and calcium) and radiological examinations for the assessment of target lesions (enhanced computed tomography or magnetic resonance imaging).12

Then, screened information on each patient judged to be eligible will be entered in the electronic data capture (EDC) system of this study, and case registration will be regarded as completion by re-conformation through the EDC system. Within 28 days after the registration, it will be necessary to initiate the treatment with nivolumab at a dose of 3 mg/kg as an intravenous infusion every 2 weeks, until disease progression or the development of intolerable AEs.7 Dose interruption of nivolumab based on the proper use guide (https://www.opdivo.jp/drug_info_files/drug_info/opdivo/tekisei/20000157/guide_rcc.pdf) will be permitted.

In addition, each patient will provide a 20 mL blood sample and formaldehyde-fixed, paraffin-embedded tumor tissue sample before starting treatment with nivolumab, and these samples will be stored for the future investigation of biomarkers.

**Follow-up schedule**

Following the initiation of treatment with nivolumab, each participant is required to visit the attending physician every 2 weeks for the administration of nivolumab and the assessment of AEs by blood examinations. Radiological examinations for the assessment of target lesions will be performed every 8 weeks within 24 weeks after starting nivolumab therapy, and be continued every 12 weeks thereafter.

**Biomarker analyses**

The URivo study includes the following biomarker assays using tissue and serum specimens obtained from participants, that will be performed while blinded to their clinicopathological data.

**FISH**

FISH analyses will be performed using tissue specimens to investigate copy numbers of PD-L1 and PD-L2 genes, as previously described.13

**ELISA**

Concentrations of PD-L1 and PD-L2 in serum samples will be measured using ELISA kits (ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions.

**Immunohistochemical staining**

Immunohistochemical staining of tissue specimens will be conducted using antibodies targeting the following proteins, as previously described:14 Akt, phosphorylated (p)-Akt (Cell Signaling Technology, Danvers, MA, USA), Bax (Abcam, Cambridge, United Kingdom), Bcl-2 (Dako, Carpinteria, CA, USA), Bcl-xl (Santa Cruz Biotechnology, Santa Cruz, CA, USA), E-cadherin, N-cadherin (Dako), β-catenin (BD Transduction Laboratories, Franklin Lakes, NJ, USA), CD4, CD8 (Dako), clusterin (Santa Cruz Biotechnology), heat shock protein27 (HSP27), HSP90 (Novocastra Laboratories, Newcastle, United Kingdom), e-Jun N-terminal kinase (JNK), p-JNK (Cell Signaling Technology), Ki-67 (Dako), mitogen-activated protein kinase (MAPK), p-MAPK (Cell Signaling Technology), matrix metalloproteinase (MMP)2, MMP-9 (Daiichi Fine Chemical, Toyama, Japan), Mcl-1 (Santa Cruz Biotechnology), p53 (Novocastra Laboratories), PD-L1, PD-L2 (Dako), PTEN (Proteintech, Rosemont, IL, USA), signal transducers and activation of transcription 3 (STAT3), p-STAT3 (Cell Signaling Technology), Slug, Snail (Abcam), vimentin (Santa Cruz Biotechnology) and ZO-1 (Cell Signaling Technology).

**Sample size, follow-up period and power calculations**

The URivo study plans to recruit 200 participants, and the duration of participant recruitment will be 2 years. The follow-up period will be 3 years from enrollment of the final participant. Therefore, the total duration of this study will be up to 5 years.
In this study, the ratio of responders to non-responders to nivolumab is considered to be 2 to 1 based on the outcomes of the CheckMate 025 study in order to determine an optimal sample size. When it is estimated that the area under the curve (AUC) of the receiver operating characteristic curve (ROC) for a certain parameter as a predictor of endpoints of this study is 0.50 and 0.65 under the null and alternative hypotheses, respectively, 196 patients have to be included to achieve a power of 0.90 using a significance level of 0.025 based on a one-sided test. Accordingly, a total of 200 participants are scheduled to be included in this study after considering the presence of those who will drop out.

**Research organization**

A complete list of principal investigators and their institutions involved in the URivo trial is provided in Table 1.

**Table 1: Research organizations.**

<table>
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<th>Participating institutions</th>
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<tr>
<td>Hokkaido University</td>
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**DISCUSSION**

The recent introduction of immune checkpoint inhibitors into clinical practice has resulted in marked changes in the therapeutic strategy for patients with aRCC. In particular, nivolumab is currently regarded as a standard agent for aRCC patients previously received antiangiogenic therapies based on the results of the CheckMate 025 trial, demonstrating a significantly superior OS in previously treated aRCC patients receiving nivolumab to those receiving everolimus. Although the marked benefit of nivolumab has been widely accepted, there are several problems associated with the use of this agent for aRCC patients, such as the variable response rates and development of immune-related AEs. To date, however, there have been no biomarkers to precisely predict the clinical course of aRCC patients treated with nivolumab; therefore, the URivo study was designed to examine the utilities of several candidate biomarkers in aRCC patients who will receive nivolumab following the failure of either 1 or 2 TKIs.

Considering the mechanisms of action of immune checkpoint inhibitors blocking the binding of PD-L1 to its ligands, PD-L1 could theoretically be expected to be a useful biomarker for aRCC patients receiving these agents. However, similar to other types of malignant tumor, the role of PD-L1 expression by tumor cells and/or immune cells to predict treatment outcomes of immune checkpoint inhibitors, including nivolumab, in patients with aRCC remains unclear. For example, a prognostic benefit was shown for aRCC patients treated with nivolumab irrespective of the expression of PD-L1 in tumor tissues in the CheckMate 025 trial. The present inconsistencies regarding the significance of PD-L1 expression as a biomarker for patients treated with immune checkpoint inhibitors could be explained by several factors, such as the use of different antibodies for immunohistochemical staining, lack of a definitive cut-
off reference for PD-L1 positivity and various patterns of
the types of cells on which PD-L1 expression is
evaluated. Collectively, these findings strongly suggest
that it is important to comprehensively assess the
predictive value of PD-L1 in aRCC patients receiving
nivolumab; therefore, the copy number of the PD-L1
gene, serum concentration of PD-L1 and PD-L1 protein
expression will be assessed by FISH, ELISA and
immunohistochemical staining, respectively, in this
study. In particular, it will be of interest to investigate the
impact of PD-L1 copy number gains on the clinical
course of aRCC patients treated with nivolumab, since a
recent study by Inoue et al reported that an increase in the
PD-L1 gene copy number examined by FISH could be a
more feasible alternative biomarker than PD-L1 protein
expression for predicting the response to anti-PD-1/PD-
L1 therapy. Another point of interest is the exploration of candidate
biomarkers showing no association with the action
mechanism of immune checkpoint inhibitors. To date,
several studies indicated the more aggressive nature of
tumors with PD-L1 expression than that of those without
PD-L1 expression in RCC. For example, aRCC patients
with tumors showing ≥1% PD-L1 expression were shown
to have significantly shorter OS than those with tumors
<1% PD-L1 expression in the CheckMate 025 trial. We
also previously examined the expression pattern of
immune checkpoint-associated molecules in tumor
tissues in patients with aRCC treated with TKIs, and
showed that patients with positive PDL-L1 expression
had significantly unfavorable PFS and OS compared with
those without positive PD-L1 expression. Taken
traditional molecular biomarkers involved in the
malignant progression of RCC may have significant
impact on prediction of the clinical course of aRCC
patients receiving nivolumab; thus, the expression profile
of major proteins mediating the apoptosis, epithelial-
mesenchymal transition (EMT) and signal transduction in
addition to immunological reaction in tumor tissues will
be assessed by immunohistochemical staining in this
study.

Here, we would like to describe several limitations of the
protocol of the URivo study. Initially, it would be ideal to
perform genetic examinations of some other candidate
biomarkers involved in the response to immune
checkpoint inhibitors. For example, it may be useful to
assess the status of neoantigens, mutations encoding
immunologically active proteins that can be functional
targets of immune checkpoint inhibitors and lead to a
response to these agents. Secondly, more detailed
evaluation of the immunomodulatory activity of
nivolumab, such as tumor-associated lymphocytes and
serum chemokines, may provide insight into the
discovery of biomarkers for this agent. Finally, it will
be necessary to carefully consider the current as well as
future marked changes in first-line therapy for aRCC by
the introduction of immune checkpoint inhibitor-based
combined regimens, when reviewing the protocol of this
study.

As discussed above, despite several potential biomarkers
being examined to predict the response to nivolumab,
none have been introduced into clinical practice for
aRCC patients through prospective validation. We
believe that the outcomes of this proposed study will help
identify biomarkers to predict the likelihood of aRCC
patients benefiting from treatment with nivolumab.
Particularly, comprehensive assessments of the
significance of the PD-L1 expression status by multiple
assays may yield definitive information with respect to
whether the investigation of this molecule will provide
useful information for the selection of aRCC patients to
receive nivolumab.

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Conflict of interest: None declared
Ethical approval: The study was approved by the
Institutional Ethics Committee

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