Title Page

Augmented clearance of nivolumab is associated with renal functions in chronic renal disease
model rats

Kazuaki Taguchi, Yukitaka Hayashi, Mayu Ohuchi, Hotaka Yamada, Shigehiro Yagishita, Yuki
Enoki, Kazuaki Matsumoto, Akinobu Hamada

Faculty of Pharmacy, Keio University of Pharmacy, Tokyo, Japan (K.T., Y.H., H.Y., Y.E.,
K.M.); Division of Molecular Pharmacology, National Cancer Center Research Institute, Tokyo,
Japan (M.O., S.Y., A.H.); Department of Medical Oncology and Translational Research,
Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan (M.O.,
A.H.).

K.T., Y.H., and M.O. contributed equally to this work.

Running Title Page

Running title: Accelerated blood clearance of nivolumab in renal impairment

Address correspondence to:

Akinobu Hamada, PhD.

Division of Molecular Pharmacology, National Cancer Center Research Institute.

Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan

Phone: +81-3-3542-2511

Fax: +81-3-3542-3815

E-mail: akhamad@ncc.go.jp

Number of text pages: 25

Number of tables: 1

Number of figures: 5

Number of references: 26

Number of words in the Abstract: 184

Number of words in Introduction: 402

Number of words in Discussion: 945

Abbreviations

AUC_{all}, area under the blood concentration-time curve; BUN, blood urea nitrogen; CKD, chronic kidney disease; CL, clearance; CRE, creatinine; CL_{cr}, creatinine clearance; CL_{er}, extrarenal clearance; CL_R, renal clearance; CL_{tot}, total clearance; IgG, immunoglobulin G

Abstract

The clinically approved dose of nivolumab is 240 mg Q2W. However, previous studies have shown that baseline nivolumab clearance (CL) is associated with treatment outcomes in patients with solid cancers, thus motivating researchers to identify prognostic factors and indices influencing nivolumab CL. This study used chronic kidney disease model rats to investigate whether chronic renal impairment affected nivolumab CL and explored the surrogate markers associated with nivolumab CL. We observed that the total CL for nivolumab (CL_{tot}) was approximately 1.42-times higher in chronic kidney disease model rats than that in sham rats with an increased urinary excretion. Additionally, CL_{tot} showed positive correlation with renal CL for nivolumab (CL_R), but not with extrarenal CL. Furthermore, the baseline levels of creatinine, blood urea nitrogen, creatinine CL, and urinary albumin/creatinine ratio based on laboratory data were also significantly correlated with CL_R . Our findings suggest that nivolumab CL increases as renal function deteriorates due to an increased excretion of nivolumab in the urine; additionally, laboratory data reflecting renal function may be a feasible index to qualitatively estimate nivolumab CL prior to nivolumab treatment under conditions of renal impairment.

Significance Statement

We demonstrated that nivolumab was rapidly eliminated from the circulation in chronic kidney disease model rats compared to sham rats with an increased urinary nivolumab excretion. Moreover, nivolumab clearance was significantly correlated with the baseline levels of certain laboratory parameters reflecting renal functions. These results indicate the potential applicability of baseline renal function as a prognostic index to qualitatively estimate nivolumab clearance prior to nivolumab treatment under conditions with renal impairment.

Introduction

Nivolumab, a fully human monoclonal immunoglobulin G₄, is an immune checkpoint inhibitor that targets programmed cell death receptor 1. The therapeutic efficacy of nivolumab alone or in combination with ipilimumab has been validated in patients with various solid cancers, such as melanoma (Robert et al., 2015), advanced gastric or gastroesophageal junction cancer (Kang et al., 2017), advanced renal cell carcinoma (Motzer et al., 2015), advanced non-small cell lung cancer (Borghaei et al., 2015), recurrent squamous cell carcinoma of the head and neck (Ferris et al., 2016), Hodgkin's lymphoma (Younes et al., 2016), and colorectal cancer (Azad et al., 2020). For nivolumab monotherapy in clinics, irrespective of the patient's physical conditions and underlying disorders, the fixed approved dose is 240 mg Q2W. Few clinical studies have shown that patients with a high baseline nivolumab clearance (CL) exhibit lower overall survival than patients with a low baseline nivolumab CL (Feng et al., 2017; Wang et al., 2019; Wang et al., 2020), indicating that nivolumab exposure is associated with treatment outcomes. Thus, the dosage adjustment of nivolumab that reflected baseline nivolumab CL may contribute to the improvement of therapeutic outcomes of nivolumab treatment. However, information on the factors that influence baseline nivolumab CL is limited.

Recently, Bajaj et al. reported a pivotal population pharmacokinetic analysis that used integrated data obtained from 11 clinical studies of nivolumab conducted in patients with solid cancers (Bajaj et al., 2017). This study clearly showed that the final population pharmacokinetic model included baseline estimated glomerular filtration as a covariant of nivolumab CL, estimated using the Chronic Kidney Disease Epidemiology Collaboration equation. Additionally, another population pharmacokinetic analysis of nivolumab in patients with gastric or gastroesophageal junction cancer also included estimated glomerular filtration as a covariant of nivolumab CL (Osawa et al., 2019). Although these findings indicate that nivolumab CL is more or less affected by renal functions, the direct impact of renal impairment on nivolumab CL

is unclear because a small number of patients with renal impairment have been enrolled in clinical trials and most of them presented with mild to moderate renal impairment.

Therefore, to elucidate the influence of renal impairment on nivolumab CL, we conducted pharmacokinetic experiments in sham and chronic kidney disease (CKD) model rats and compared the blood retention and urinary excretion of nivolumab between the two groups. Furthermore, we also investigated the clinically available laboratory data associated with nivolumab CL under conditions with renal impairment.

Materials and Methods

Chemicals and reagents

Nivolumab (Opdivo[®]) was purchased from Ono Pharmaceutical Co., Ltd. (Osaka, Japan), and trastuzumab (Herceptin[®]) was purchased from Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). nSMOL[™] Antibody BA Kit was purchased from Shimadzu Co., Ltd. (Kyoto, Japan). LBIS Rat Albumin ELISA Kit was obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). All other reagents and solvents were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), Nacalai Tesque (Kyoto, Japan), or Sigma-Aldrich (St. Louis, MO, USA).

Surgical preparation of CKD model rats

Eleven Sprague-Dawley rats (male, 6 weeks old, 181.2 ± 9.5 g) were purchased from Sankyo Labo Service (Tokyo, Japan). All animals were housed in a temperature-controlled conventional room with a 12-h dark/light cycle, and they were provided with food and water *ad libitum*. Under conditions of subjection to isoflurane anesthesia, nephrectomized model rats (n = 6), which are generally used as CKD model rats, were surgically prepared by resecting two-thirds of the right kidney and the whole left kidney, as per methods previously reported (Shimoishi et al., 2007; Kadowaki et al., 2009). In sham rats (n = 5), the kidneys were taken out from the abdominal cavity, and then the kidneys were returned without resection. All CKD model rats were used for pharmacokinetic studies at 4 weeks after the left kidney resection. All animal experiments were reviewed and approved by the Animal Care and Use Committee of Keio University (Approval #: 18069-(0)).

Pharmacokinetic study

A day before the commencement of the pharmacokinetic study, a cannula filled with heparin solution was fixed (100 IU/mL) into the right jugular vein of all rats for nivolumab

administration and blood collection. Blood samples were collected to determine plasma baseline levels of creatinine (CRE), blood urea nitrogen (BUN), and albumin, and urine samples were collected from rats in a metabolic cage at 24 h for calculation of CRE CL (CL_{cr}) and quantification of baseline urinary albumin levels. Sham-operated (body weight: 436.4 ± 26.6 g, CRE: 0.17 ± 0.02 mg/dL, BUN: 15.3 ± 2.6 mg/dL) and CKD rats (body weight: 343.1 ± 54.4 g, CRE: 0.67 ± 0.32 mg/dL, BUN: 50.2 ± 16.1 mg/dL) were subjected to continuous administration with nivolumab solution (10 mg/kg) using a syringe pump for 60 min. After 1, 2, 4, 6, 24, 48, and 72 h of administration, venous blood samples (0.3 mL) were collected from a fixed cannula using a heparinized syringe. Blood samples were centrifuged at 1,200 rpm for 10 min to collect the plasma samples. All rats were housed in metabolic cages to collect urine samples until they were sacrificed. Urine samples were collected, and the volume was measured every 24 h. All plasma and urine samples were stored at -80 °C until use. All surgical procedures were performed under conditions of subjection to isoflurane anesthesia.

The area under the blood concentration-time curve (AUC_{all}) from 0 h to 72 h following nivolumab administration was analyzed using a non-compartmental model, with the Phoenix[®] WinNonlin[®] software program (Version 8.0; Certara LP, Princeton, NJ, USA). The apparent half-life was estimated from the elimination rate constant using the 24-72 h time points. Total CL (CL_{tot}), renal CL (CL_R), extrarenal CL (CL_{eR}), and renal excretion ratio for nivolumab were calculated using the following equation:

$$CL_{tot} = \text{Dose}/AUC_{all}$$

$$CL_R = X(u)_{0 \rightarrow 72}/AUC_{all}$$

$$CL_{eR} = CL_{tot} - CL_R$$

$$\text{Renal excretion ratio (\%)} = CL_R/CL_{tot} * 100$$

where X(u)_{0→72} represents the cumulative excreted amount of nivolumab in urine from 0 h to 72 h after nivolumab administration.

Clinical biochemistry assessments in plasma and urine

Plasma concentrations of BUN, CRE, and albumin and the urine concentration of CRE were analyzed using Fuji DRI-CHEM 7000Z (FUJIFILM Wako Pure Chemical Corp., Tokyo, Japan). Urine microalbumin levels were determined using the LBIS Rat Albumin ELISA Kit. CLcr was calculated based on the CRE levels in plasma and in 24-h cumulative urine before nivolumab administration.

Quantification of nivolumab concentration in plasma and urine using liquid chromatography-mass spectrometry

Nivolumab concentrations in plasma and urine were quantified using the target signature peptides ASQSVSSYLAWYQQKPGQAPR (m/z: 785.0 > 940.2) and LLIYDASNR (m/z: 532.9 > 838.2), respectively, using liquid chromatography-electrospray ionization-mass spectrometry with a triple quadrupole mass spectrometer (Nexera X2 and LCMS-8050, Shimadzu), as per previously reported protocols (Ohuchi et al., 2021). These signature peptides were extracted from plasma and urine samples using the nSMOL™ Antibody BA Kit as follows: the plasma and urine samples were filtered using Ultrafree-MC SV (Merck Millipore, Billerica, MA, USA) and centrifuged at 1,800 g for 5 min. Subsequently, the supernatant (5 µL of plasma or 40 µL of urine) was subjected to treatments using the nSMOL™ Antibody BA Kit according to the nSMOL proteolysis method. As an internal standard, trastuzumab signature peptides IYPTNGYTR (543.3 > 404.8) and FTISADTSK (485.2 > 721.1) were used for nivolumab quantification in plasma and urine, respectively. The optimized analytical conditions of mass spectrometry for each peptide are listed in Supplementary Table 1. The urinary selectivity and reproducibility of nivolumab and trastuzumab surrogate peptides are shown in Supplementary Fig. 1 and Supplementary Table 2.

Statistical analysis

All data are expressed as mean $\pm$ standard deviation. Statistical analyses were performed using the unpaired Student's *t*-test or Welch *t*-test. Pearson's test was used for conducting correlation analyses. Data analysis was performed using the GraphPad Prism® version 8.4.2 (GraphPad, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

Results

Plasma concentration and pharmacokinetic parameters of nivolumab in sham and CKD model rats

Figure 1 and supplementary Figure 2 show the time course of nivolumab plasma concentration in sham and CKD model rats after the performance of a continuous intravenous administration (60 min) of nivolumab at a dose of 10 mg/kg (a semi-logarithmic plot and a linear plot, respectively), and Table 1 lists the pharmacokinetic parameters calculated based on the plasma concentration curve. Nivolumab was rapidly cleared from the circulation in CKD model rats compared to sham rats. Along with the changes in plasma concentration, CL_{tot} was approximately 1.42-times higher in CKD model rats than that in sham rats, and AUC_{all} and estimated half-life was approximately 0.72-times and 0.39-times lower in CKD model rats than that in sham rats, respectively.

Relationship between nivolumab CL and renal functions

As shown in Figure 1 and supplementary Figure 2, the ingravescence of renal function influences nivolumab CL. Correlations between CL_{tot} for nivolumab and plasma CRE or BUN concentration were analyzed to further assess the association of nivolumab CL with renal functions. We observed that nivolumab CL_{tot} was positively correlated with the baseline plasma levels of CRE (Fig. 2A; $r = 0.782$, $p = 0.0045$) and BUN (Fig. 2B; $r = 0.826$, $p = 0.0017$), indicating that nivolumab CL increased as the renal functions deteriorated. In contrast, the plasma baseline levels of albumin, which is a covariant of nivolumab CL in population

pharmacokinetic analysis conducted for patients with solid tumors (Osawa et al., 2019), were not correlated with CL_{tot} for nivolumab (Fig. 2C; $r = -0.367$, $p = 0.266$).

Urinary excretion of nivolumab

The urinary excretion of nivolumab every 24 h was compared between the sham and CKD model rats. The results showed that nivolumab excretion every 24 h significantly increased in CKD model rats compared to sham rats until 72 h after nivolumab administration (Fig. 3A). Additionally, CL_R in the CKD model rats remarkably increased as compared to that in sham rats during the 72-h observation period while no significant change was observed in CL_{eR} between the two groups (Table 1). Furthermore, CL_{tot} for nivolumab was positively correlated with CL_R for nivolumab (Fig. 3B; $r = 0.931$, $p < 0.0001$); however, no correlation was observed between CL_{tot} and CL_{eR} ($r = -0.018$, $p = 0.958$).

Relationship between nivolumab CL and baseline urinary parameters

In addition to the plasma laboratory data reflecting renal impairment, as shown in Fig. 2, the relationship between nivolumab CL and urinary parameters reflecting renal function was also evaluated. We observed that CL_{tot} for nivolumab decreased as the baseline CL_{Cr} calculated from the 24 h-accumulated urine sample increased (Fig. 4A; $r = -0.867$, $p = 0.0006$). In addition to CL_{Cr} , urinary albumin/CRE ratio was also correlated with CL_{tot} for nivolumab (Fig. 4B; $r = 0.847$, $p = 0.001$).

Relationship between nivolumab renal CL and relevant parameters for renal function

The relationship between the CL_R for nivolumab and the plasma urinary parameters reflecting renal function were evaluated. The relationship between the CL_{tot} for nivolumab and the relevant parameters for renal function is shown in Fig. 2 and Fig. 4. The CL_R for nivolumab was markedly correlated with CRE (Fig. 5A; $r = 0.905$, $p = 0.0001$), BUN (Fig. 5B; $r = 0.928$, p

< 0.0001), baseline CL_{cr} (Fig. 5D; $r = -0.852$, $p = 0.0009$), and urinary albumin/CRE ratio (Fig. 5E; $r = 0.871$, $p = 0.0005$). However, the plasma baseline levels of albumin did not correlate with CL_R for nivolumab (Fig. 5C; $r = -0.37$, $p = 0.262$).

Discussion

This study reports two major novel findings regarding the pharmacokinetics of nivolumab. One finding indicates that renal impairment is an important factor influencing nivolumab CL due to an increased urinary excretion. Another finding indicates that nivolumab CL may be qualitatively estimated using plasma and urinary laboratory data reflecting renal functions prior to nivolumab treatment.

Previous studies have suggested that nivolumab, exhibited long retention time in blood circulation (CL in human: 7.9-9.5 mL/hr) with a linear elimination (0.1-10 mg/kg) as well as endogenous IgG, and other IgG preparations (Kontermann, 2011; Desnoyer et al., 2020). This is because IgG preparations, including nivolumab, can be salvaged from intracellular catabolic degradation by lysosomes via actions of the neonatal Fc receptor (Ryman and Meibohm, 2017; Datta-Mannan, 2019). Additionally, proteins with molecular weights greater than 50 kDa, such as nivolumab (molecular weight: approximately 145 kDa), block glomerular filtration due to histological characteristics of the kidney, such as size and charge barrier (Tryggvason and Wartiovaara, 2005). However, the enhanced plasma retention of nivolumab could not observed in the CKD model rats compared to the sham rats (Fig. 1, Suppl. Fig. 2 and Table 1). It is known that structural disruptions of glomeruli with a loss of size and charge barrier occur when renal impairment is induced, resulting in the facilitation of protein leakage into the urine. In fact, it has been reported that high-molecular-weight protein preparations and dimerized albumin (MW > 130 kDa) are excessively and rapidly excreted into the urine under conditions of renal impairment (Taguchi et al., 2010). In this study, the urinary excretion of nivolumab was significantly higher in the CKD model rats compared to the sham rats (Fig. 3A). Additionally,

CL_R for nivolumab, but not CL_{eR} , was significantly correlated with CL_{tot} for nivolumab (Fig. 3B), indicating that nivolumab CL was dependent on CL_R under conditions of renal impairment. Taken together, the findings suggest that the facilitation of renal elimination rather than catabolic degradation may mainly contribute to the loss of blood retention of nivolumab in CKD model rats.

Determination of nivolumab CL before the conduction of nivolumab-based therapy is clinically useful for dosage adjustment in patients with renal impairment because CL_{tot} for nivolumab is dependent on the extent of renal impairment. However, it is difficult to determine baseline nivolumab CL in individual patients without conducting post-treatment pharmacokinetic analysis. Thus, it is clinically important to identify the baseline levels of feasible clinical parameters that may enable the estimation of nivolumab CL. In this study, the baseline levels of plasma laboratory data (CRE and BUN) and urinary parameters (CL_{cr} calculated based on the 24-h urinary accumulation and albumin/CRE ratio) showed remarkable correlation with CL_{tot} and CL_R for nivolumab (Fig. 2A, 2B, Fig. 4 and Fig. 5A, 5B, 5D, 5E), suggesting that these parameters might be potential indicators for estimating nivolumab CL prior to nivolumab administration. Unfortunately, the plasma levels of CRE and BUN are influenced not only by renal functions but also by other factors such as muscle mass and meals. Furthermore, the 24-h urinary accumulation occurring before nivolumab treatment is a big burden from the perspective of improving the quality of life of patients. These findings suggest that the data on laboratory parameters are clinically unavailable and unfeasible for the estimation of nivolumab CL. However, the value of urinary albumin/CRE ratio can be determined without the 24-h urinary accumulation. It substantially reflects the renal functions without any interference from the pathophysiological factors. Therefore, the urinary albumin/CRE ratio before the nivolumab administration is a potential clinical indicator for estimating the nivolumab CL.

It has been reported that the plasma levels of albumin can be considered as a biomarker to estimate nivolumab CL in patients with solid tumors (Bajaj et al., 2017; Hirsch et al., 2020). Since hypoalbuminemia occurs as renal injury progresses, it is expected that baseline plasma albumin levels may be a feasible biomarker for the estimation of nivolumab CL under conditions with renal impairment. Contrary to our expectations, no correlation was observed between plasma baseline albumin levels and CL_{tot} and CL_R for nivolumab in this study (Fig. 2C and Fig. 5C). Based on previous findings, it has been hypothesized that high protein turnover, which is induced by cancer cachexia, is related to high CL of monoclonal antibody preparations (Bajaj et al., 2017). Additionally, cancer cachexia is frequently accompanied by hypoalbuminemia due to catabolic drive (Evans et al., 2008). Thus, hypoalbuminemia is associated with high nivolumab CL due to a high catabolic drive in cancer cachexia. Further studies using cachexia animal models are warranted to elucidate the mechanism.

In conclusion, for the first time, the present study showed that nivolumab CL increased in the CKD model rats compared to sham rats, and the baseline urinary albumin/CRE ratio might be a potential indicator to estimate nivolumab CL prior to nivolumab treatment under conditions of renal impairment. Although it has been recommended that no dosage adjustment of nivolumab is necessary in cancer patients with renal impairment (Sheng et al., 2017), our findings suggest that dosage adjustment of nivolumab may be performed depending on the patients' pathophysiological conditions. However, our findings are limited to a small number of CKD model rats investigated herein. The clearance of other antibody drugs (bevacizumab and panitumumab) was similar between the subjects with normal renal function and patients with end-stage renal disease (Garnier-Viougat et al., 2007; Krens et al., 2018). The difference in the outcomes between the present study and that from the previous study could be attributed to the presence of different types of CKD etiology or pathophysiology, such as the patients with end-stage renal disease undergoing hemodialysis being anuric. Thus, further animal experiments with different acute and chronic kidney injury models are warranted.

Authorship Contributions

Participated in research design: Taguchi, Matsumoto, and Hamada.

Conducted animal experiments: Taguchi, Hayashi, Yamada, and Enoki.

Contributed new reagents or analytic tools: Hayashi, Ohuchi, Yamada, Yagishita, and Hamada.

Performed data analysis: Taguchi and Ohuchi.

Wrote or contributed to the writing of the manuscript: Taguchi, Ohuchi, Matsumoto, and Hamada.

References

- Azad NS, Gray RJ, Overman MJ, Schoenfeld JD, Mitchell EP, Zwiebel JA, Sharon E, Streicher H, Li S, McShane LM, Rubinstein L, Patton DR, Williams PM, Coffey B, Hamilton SR, Bahary N, Suga JM, Hatoum H, Abrams JS, Conley BA, Arteaga CL, Harris L, O'Dwyer PJ, Chen AP, and Flaherty KT (2020) Nivolumab Is Effective in Mismatch Repair-Deficient Noncolorectal Cancers: Results From Arm Z1D-A Subprotocol of the NCI-MATCH (EAY131) Study. *J Clin Oncol* **38**:214-222.
- Bajaj G, Wang X, Agrawal S, Gupta M, Roy A, and Feng Y (2017) Model-Based Population Pharmacokinetic Analysis of Nivolumab in Patients With Solid Tumors. *CPT Pharmacometrics Syst Pharmacol* **6**:58-66.
- Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, Barlesi F, Kohlhäufel M, Arrieta O, Burgio MA, Fayette J, Lena H, Poddubskaya E, Gerber DE, Gettinger SN, Rudin CM, Rizvi N, Crinò L, Blumenschein GR, Jr., Antonia SJ, Dorange C, Harbison CT, Graf Finckenstein F, and Brahmer JR (2015) Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* **373**:1627-1639.
- Datta-Mannan A (2019) Mechanisms Influencing the Pharmacokinetics and Disposition of Monoclonal Antibodies and Peptides. *Drug Metab Dispos* **47**:1100-1110.

Desnoyer A, Broutin S, Delahousse J, Maritaz C, Blondel L, Mir O, Chaput N, and Paci A

(2020) Pharmacokinetic/pharmacodynamic relationship of therapeutic monoclonal antibodies used in oncology: Part 2, immune checkpoint inhibitor antibodies. *Eur J Cancer* **128**:119-128.

Evans WJ, Morley JE, Argilés J, Bales C, Baracos V, Guttridge D, Jatoi A, Kalantar-Zadeh K,

Lochs H, Mantovani G, Marks D, Mitch WE, Muscaritoli M, Najand A, Ponikowski P, Rossi Fanelli F, Schambelan M, Schols A, Schuster M, Thomas D, Wolfe R, and Anker SD (2008) Cachexia: a new definition. *Clin Nutr* **27**:793-799.

Feng Y, Wang X, Bajaj G, Agrawal S, Bello A, Lestini B, Finckenstein FG, Park JS, and Roy A

(2017) Nivolumab Exposure-Response Analyses of Efficacy and Safety in Previously Treated Squamous or Nonsquamous Non-Small Cell Lung Cancer. *Clin Cancer Res* **23**:5394-5405.

Ferris RL, Blumenschein G, Jr., Fayette J, Guigay J, Colevas AD, Licitra L, Harrington K,

Kasper S, Vokes EE, Even C, Worden F, Saba NF, Iglesias Docampo LC, Haddad R, Rordorf T, Kiyota N, Tahara M, Monga M, Lynch M, Geese WJ, Kopit J, Shaw JW, and Gillison ML (2016) Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. *N Engl J Med* **375**:1856-1867.

Garnier-Viougeat N, Rixe O, Paintaud G, Ternant D, Degenne D, Mouawad R, Deray G, and

Izzedine H (2007) Pharmacokinetics of bevacizumab in haemodialysis. *Nephrol Dial Transplant* **22**:975.

Hirsch L, Bellesoeur A, Boudou-Rouquette P, Arrondeau J, Thomas-Schoemann A,

Kirchgesner J, Gervais C, Jouinot A, Chapron J, Giraud F, Wislez M, Alexandre J,

Blanchet B, and Goldwasser F (2020) The impact of body composition parameters on severe toxicity of nivolumab. *Eur J Cancer* **124**:170-177.

Kadowaki D, Anraku M, Tasaki Y, Taguchi K, Shimoishi K, Seo H, Hirata S, Maruyama T, and

Otagiri M (2009) Evaluation for antioxidant and renoprotective activity of olmesartan using nephrectomy rats. *Biol Pharm Bull* **32**:2041-2045.

Kang YK, Boku N, Satoh T, Ryu MH, Chao Y, Kato K, Chung HC, Chen JS, Muro K, Kang

WK, Yeh KH, Yoshikawa T, Oh SC, Bai LY, Tamura T, Lee KW, Hamamoto Y, Kim

JG, Chin K, Oh DY, Minashi K, Cho JY, Tsuda M, and Chen LT (2017) Nivolumab in

patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or

intolerant of, at least two previous chemotherapy regimens (ONO-4538-12,

ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial.

Lancet **390**:2461-2471.

Kontermann RE (2011) Strategies for extended serum half-life of protein therapeutics. *Curr Opin Biotechnol* **22**:868-876.

Krens LL, Baas JM, Guchelaar HJ, and Gelderblom H (2018) Pharmacokinetics and safety of panitumumab in a patient with chronic kidney disease. *Cancer Chemother Pharmacol* **81**:179-182.

Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, Tykodi SS, Sosman JA, Procopio G, Plimack ER, Castellano D, Choueiri TK, Gurney H, Donskov F, Bono P, Wagstaff J, Gauler TC, Ueda T, Tomita Y, Schutz FA, Kollmannsberger C, Larkin J, Ravaud A, Simon JS, Xu LA, Waxman IM, and Sharma P (2015) Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. *N Engl J Med* **373**:1803-1813.

Ohuchi M, Yagishita S, Taguchi K, Goto Y, Fukahori M, Enoki Y, Shimada T, Yamaguchi M, Matsumoto K, and Hamada A (2021) Use of an alternative signature peptide during development of a LC-MS/MS assay of plasma nivolumab levels applicable for multiple species. *J Chromatogr B Analyt Technol Biomed Life Sci* **1162**:122489.

Osawa M, Hasegawa M, Bello A, Roy A, and Hruska MW (2019) Population pharmacokinetics analysis of nivolumab in Asian and non-Asian patients with gastric and gastro-esophageal junction cancers. *Cancer Chemother Pharmacol* **83**:705-715.

- Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, Savage KJ, Hernberg MM, Lebbé C, Charles J, Mihalcioiu C, Chiarion-Sileni V, Mauch C, Cognetti F, Arance A, Schmidt H, Schadendorf D, Gogas H, Lundgren-Eriksson L, Horak C, Sharkey B, Waxman IM, Atkinson V, and Ascierto PA (2015) Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* **372**:320-330.
- Ryman JT and Meibohm B (2017) Pharmacokinetics of Monoclonal Antibodies. *CPT Pharmacometrics Syst Pharmacol* **6**:576-588.
- Sheng J, Srivastava S, Sanghavi K, Lu Z, Schmidt BJ, Bello A, and Gupta M (2017) Clinical Pharmacology Considerations for the Development of Immune Checkpoint Inhibitors. *J Clin Pharmacol* **57 Suppl 10**:S26-s42.
- Shimoishi K, Anraku M, Kitamura K, Tasaki Y, Taguchi K, Hashimoto M, Fukunaga E, Maruyama T, and Otagiri M (2007) An oral adsorbent, AST-120 protects against the progression of oxidative stress by reducing the accumulation of indoxyl sulfate in the systemic circulation in renal failure. *Pharm Res* **24**:1283-1289.
- Taguchi K, Urata Y, Anraku M, Watanabe H, Kawai K, Komatsu T, Tsuchida E, Maruyama T, and Otagiri M (2010) Superior plasma retention of a cross-linked human serum albumin

dimer in nephrotic rats as a new type of plasma expander. *Drug Metab Dispos*

38:2124-2129.

Tryggvason K and Wartiovaara J (2005) How does the kidney filter plasma? *Physiology*

(*Bethesda*) **20**:96-101.

Wang R, Shao X, Zheng J, Saci A, Qian X, Pak I, Roy A, Bello A, Rizzo JI, Hosein F, Moss

RA, Wind-Rotolo M, and Feng Y (2020) A Machine-Learning Approach to Identify a

Prognostic Cytokine Signature That Is Associated With Nivolumab Clearance in

Patients With Advanced Melanoma. *Clin Pharmacol Ther* **107**:978-987.

Wang R, Zheng J, Shao X, Ishii Y, Roy A, Bello A, Lee R, Zhang J, Wind-Rotolo M, and Feng

Y (2019) Development of a prognostic composite cytokine signature based on the

correlation with nivolumab clearance: translational PK/PD analysis in patients with

renal cell carcinoma. *J Immunother Cancer* **7**:348.

Younes A, Santoro A, Shipp M, Zinzani PL, Timmerman JM, Ansell S, Armand P, Fanale M,

Ratanatharathorn V, Kuruvilla J, Cohen JB, Collins G, Savage KJ, Trneny M, Kato K,

Farsaci B, Parker SM, Rodig S, Roemer MG, Ligon AH, and Engert A (2016)

Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell

transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2

trial. *Lancet Oncol* **17**:1283-1294.

Footnotes*

*This work was supported in part by the “Research on Regulatory Science of Pharmaceuticals and Medical Devices” fund from the Japan Agency for Medical Research and Development (AMED).

No author has an actual or perceived conflict of interest with the contents of this article.

Figure Legends

Figure 1

The semi-logarithmic plot of the time course for the plasma concentration of nivolumab after a continuous intravenous administration to sham (open circle) and CKD model rats (closed circle) at a dose of 10 mg/kg.

Sham (n = 5) and CKD rats (n = 6). Data were analyzed using an unpaired *t*-test. * $p < 0.05$, ** $p < 0.01$ vs. sham rats.

Figure 2

Correlation between CL_{tot} for nivolumab and (A) baseline CRE levels in plasma, (B) baseline BUN levels in plasma, and (C) baseline albumin levels in plasma.

Open and closed dots indicate data obtained from the analysis using sham and CKD model rats, respectively. Linear regression was calculated using Pearson's test. (A: $y = 0.632x - 0.77$, $r = 0.782$, $p = 0.0045$; B: $y = 41.6x - 45.2$, $r = 0.826$, $p = 0.0017$; C: $y = -0.451x + 3.162$, $r = -0.367$, $p = 0.267$)

Figure 3

(A) Cumulative nivolumab excretion in urine every 24 h after nivolumab administration to sham (open circle) and CKD model rats (closed circle).

Gray bars indicate the average values of each group. Data were analyzed using an unpaired *t*-test. ** $p < 0.01$. vs. sham rats.

(B) Correlation between CL_{tot} and CL_R for nivolumab.

Open and closed dots indicate data obtained from the analysis using sham and CKD model rats, respectively. Linear regression was calculated using Pearson's test. ($y = 1.007x - 1.558$, $r = 0.931$, $p < 0.0001$).

Figure 4

Correlation between CL_{tot} for nivolumab and (A) baseline CL_{cr} or (B) baseline urinary albumin/CRE ratio. Open and closed dots indicate data obtained from the analysis using sham and CKD model rats, respectively. CL_{cr} was calculated based on the CRE levels in plasma and in 24-h cumulative urine before nivolumab administration. Linear regression was calculated using Pearson's test. (A: $y = -20.2x + 59.3$, $r = -0.867$, $p = 0.0006$; B: $y = 3.03x - 4.81$, $r = 0.847$, $p = 0.001$)

Figure 5

Correlation between CL_R for nivolumab and (A) baseline CRE levels in plasma, (B) baseline BUN levels in plasma, (C) baseline albumin levels in plasma, (D) baseline CL_{cr} and (E) baseline urinary albumin/CRE ratio. Open and closed dots indicate data obtained from sham and CKD model rats, respectively. CL_{cr} was calculated based on the CRE levels in plasma and in 24 h cumulative urine before nivolumab administration. Linear regression was calculated using Pearson's test. (A: $y = 0.677x + 0.189$, $r = 0.905$, $p = 0.0001$; B: $y = 43.2x + 18.4$, $r = 0.928$, $p < 0.0001$; C: $y = -0.421x + 2.45$, $r = -0.37$, $p = 0.262$; D: $y = -18.4x + 27.4$, $r = -0.852$, $p = 0.0009$; E: $y = 2.88x - 0.0798$, $r = 0.871$, $p = 0.0005$)

Table

Table 1

Pharmacokinetic parameters of nivolumab in sham and CKD model rats.

AUC_{all} and CL_{tot} were analyzed using an unpaired *t*-test. X(u)_{0→72}, CL_R, and renal excretion ratio were analyzed using a Welch *t*-test. ** *p* < 0.01 vs. sham rats.

	Sham (n = 5)	CKD (n = 6)
AUC _{all} (h*μg/mL)	6411 ± 639	4585 ± 746**
X(u) _{0→72} (μg)	8.34 ± 6.37	958 ± 444**
CL _{tot} (mL/h/kg)	1.57 ± 0.16	2.23 ± 0.38**
CL _R (mL/h/kg)	0.003 ± 0.003	0.71 ± 0.45**
CL _{eR} (mL/h/kg)	1.57 ± 0.15	1.53 ± 0.21
Apparent half-life (h)	122.8 ± 119.6	47.5 ± 25.6
Renal excretion ratio (%)	0.19 ± 0.15	29.9 ± 15.5**

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5