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SHORT REPORT

Absorption of the orally active multikinase inhibitor axitinib as a therapeutic index to guide dose titration in metastatic renal cell carcinoma

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Summary

Purpose Axitinib is an orally active multikinase inhibitor currently used to treat patients with metastatic renal cell carcinoma (RCC). This study examined the pharmacokinetics of axitinib and the relationship between peak drug concentration (C_{max}) and clinical outcomes in real-world practice. *Methods* Twenty patients with metastatic RCC treated with axitinib monotherapy were enrolled. Post-dose (1–4 h) blood samples were obtained, and axitinib C_{max} in plasma was measured by liquid chromatography–tandem mass spectrometry. Efficacy endpoints were best overall response (per RECIST 1.1) and progression-free survival (PFS). The safety endpoint was the cumulative incidence of dose-limiting toxicities (DLTs). *Results* Large inter- and intra-individual variability in dose-adjusted C_{max} was observed (0.02–11.2 ng/mL/mg). Axitinib absorption was significantly influenced by glucuronidation activity ($P = 0.040$). C_{max} at steady state was significantly higher in responders than in non-responders ($P = 0.013$). The optimal C_{max} cutoff to predict a clinical response was 12.4 ng/mL. The median PFS was significantly longer in patients who achieved an average steady state C_{max} above the threshold than in those who did not (799 vs. 336 days; $P = 0.047$). The cumulative incidence of DLTs was significantly higher in patients with $C_{max} \geq 40.2$ ng/mL than in other patients (sub-hazard ratio, 4.13; 95% confidence interval, 1.27–13.5; $P = 0.019$). *Conclusions* The potential therapeutic window of axitinib C_{max} in metastatic RCC was estimated at 12.4–40.2 ng/mL. Pharmacokinetically guided dose titration using therapeutic drug monitoring may improve the efficacy and safety of axitinib, warranting further investigation in a larger patient population.

Keywords Axitinib · Dose titration · Renal cell carcinoma · Therapeutic drug monitoring

Introduction

Axitinib is an oral, potent, and selective inhibitor of vascular endothelial growth factor (VEGF) receptor tyrosine kinases 1, 2, and 3 [1]. Axitinib is commonly used to treat patients with metastatic renal cell carcinoma (RCC) as a single agent or in combination with the immune checkpoint inhibitor pembrolizumab or avelumab [2–4]. The recommended starting dose of axitinib is 5 mg twice daily, and the dosage can be increased to 7 mg twice daily and then up to 10 mg twice daily based on individual tolerability.

Axitinib is absorbed relatively rapidly, and the maximum drug concentration (C_{max}) is reached within 4 h post-dose [5]. Axitinib is metabolized primarily in the liver by cytochrome P450 (CYP) 3A4/5 and, to a lesser extent (<10% each), by CYP1A2, CYP2C19, and uridine diphosphate glucuronosyltransferase (UGT) 1A1 [6]. The major metabolites in human plasma are axitinib N-glucuronide (M7) and axitinib sulfoxide (M12), which are both considered pharmacologically inactive

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[7]. The *UGT1A1* genotype and the CYP2C19 inferred phenotype have no clinically relevant effects on the pharmacokinetics of axitinib [8].

Hypertension is one of the most common adverse effects of axitinib administration, and it is associated with increased drug exposure and improved efficacy [9]. A prospective, randomized, double-blind phase 2 trial in patients with previously untreated metastatic RCC showed that axitinib dose titration according to clinical criteria (blood pressure, ≤150/90 mmHg; tolerability, no grade 3/4 toxicities; treatment, no dose reduction and ≤ 2 antihypertensive medications) for 2 consecutive weeks resulted in a significantly higher objective response rate (ORR) than placebo titration (54% vs. 34%) [10]. However, the median progression-free survival (PFS) and the median overall survival (OS) did not differ significantly between the two groups [10, 11]. These data suggest that the titration scheme might benefit from further refinement, including pharmacokinetically guided dosing with therapeutic drug monitoring (TDM) to optimize axitinib exposure in individual patients.

Axitinib shows large interindividual variability in plasma exposure (e.g., C_{max} and area under the concentration–time curve [AUC]) [12], which may affect its clinical efficacy and/or safety. Previous studies demonstrated a relationship between axitinib exposure and response in patients with RCC [9, 13, 14]. The median PFS and median OS were significantly longer in patients achieving an AUC ≥300 ng*h/mL for 24 h (AUC_{24}) than in those who did not [9]. Therefore, TDM is considered an important strategy to improve clinical outcomes by individualizing the axitinib dose. However, a target therapeutic exposure to axitinib remains to be established, especially during dose titration. Furthermore, assessment of AUC requires multiple blood sampling, which may not be feasible for outpatients.

The objectives of this study were as follows: (1) characterize the pharmacokinetics of axitinib monotherapy in patients with metastatic RCC; (2) investigate the relationship between peak drug concentration (C_{max}) and clinical outcomes in real-world practice; and (3) determine a therapeutic window of axitinib C_{max} to guide dose titration.

Patients and methods

Study design and patients

This study was part of a single-center, prospective cohort study called PRecision dOsing of moLecular-targeted agents based On therapeutic drug monitoriNG (PROLONG) (clinical trial identifier, UMIN000036158). The study aimed to optimize targeted therapy with multikinase inhibitors through pharmacokinetically guided dosing with TDM in the real-world setting. The protocol was approved by the institutional ethics committee of Asahikawa Medical University (#15018). All patients provided written informed consent. Consecutive patients with advanced or metastatic RCC who started axitinib monotherapy between October 1, 2015 and February 29, 2020 were prospectively enrolled. The baseline characteristics of the patients are summarized in Table 1.

Blood samples and pharmacokinetic assessment

During hospitalization, serial blood samples (before the morning dose and at 2, 4, 8, and 12 h post-dose) were collected on the first week of treatment to obtain the full pharmacokinetic profile at steady state. For pharmacokinetic assessment in the outpatient setting, blood samples were longitudinally collected from remnant blood specimens from each visit at 1–4 h after the morning dose (corresponding to the absorption phase). These samples were used to measure individual axitinib C_{max} values.

Plasma samples (100 μL) were deproteinized with acetonitrile, and the supernatant was diluted in 20 mM di-n-butylamine acetate to increase the recovery of axitinib metabolites during solid-phase extraction with Oasis HLB cartridges (Waters, Tokyo, Japan). Axitinib concentration was measured by *hydrophilic interaction chromatography* combined with *tandem-mass spectrometry (HILIC–MS/MS)* (lower limit of quantification, 0.1 ng/mL). The inter- and intra-assay coefficients of variation were < 5%. Because M7 is the most abundant circulating metabolite [7], we analyzed the level of M7 in plasma. Analyses were performed in the multiple reaction monitoring mode at ion transitions *m/z* 387 → 356 (axitinib), *m/z* 563 → 387 (M7), and *m/z* 394 → 278 (erlotinib, internal standard). Because an authentic reference standard for M7 was not available, we assessed glucuronidation activity toward axitinib by calculating the M7/axitinib metabolic ratio (peak area ratio) in each assay.

Treatment

Axitinib was administered orally at a starting dose of 5 mg twice daily with food. In patients with poor liver function or co-morbidities and in those with poor Eastern Cooperative Oncology Group performance status, the initial dose was reduced at the physician's discretion. If axitinib was well tolerated at a given dose, the dosage was titrated stepwise to a maximum of 10 mg twice daily. When unacceptable toxicities were present, the dose was reduced or interrupted temporarily, followed by resumption at a reduced dose. Axitinib treatment was discontinued at the discretion of the physician in cases of severe adverse events.

Based on the in vivo pharmacologically effective concentration (C_{eff}) of axitinib (unbound C_{eff}, 0.28–0.85 nmol/L) and its high protein binding (>99.5%), the estimated total C_{eff} in human plasma ranges between 21.6 and 65.7 ng/mL [1]. In addition,

Table 1 Baseline characteristics of the patients

Characteristic		N = 20
Sex, male/female, n (%)		10/10 (50/50)
Age, median (range), y		68 (40–78)
Body weight, median (range), kg		61 (36–137)
ECOG performance status, n (%)		
0/1		17 (85)
2		3 (15)
Histology, n (%)		
Clear cell RCC		19 (95)
Non-clear cell RCC		1 (5)
Prior systemic therapy, n (%)		
1		9 (45)
2		11 (55)
First-line treatment, n (%)		
Sunitinib		10 (50)
Pazopanib		7 (35)
Interferon-α		3 (15)
Second-line treatment, n (%)		
Nivolumab		8 (73)
Sunitinib		1 (9)
Pazopanib		1 (9)
Sorafenib		1 (9)
Sites of metastases, n (%)		
Lung		15 (75)
Bone		10 (50)
Pancreas		4 (20)
Lymph nodes		4 (20)
Liver		2 (10)
Adrenal gland		2 (10)
Genetic polymorphism, n (%)		
*CYP3A4*22* (intron 6 C > T)	CC	20 (100)
	CT	0
	TT	0
*CYP3A5*3* (6986A > G)	AA	1 (5)
	AG	7 (35)
	GG	12 (60)
ABCG2 421C > A	CC	13 (65)
	CA	7 (35)
	AA	0
Starting dose, n (%)		
10 mg/day		6 (30)
8 mg/day		1 (5)
6 mg/day		9 (45)
<6 mg/day		4 (20)

ECOG Eastern Cooperative Oncology Group, *RCC* renal cell carcinoma

according to pharmacokinetic data reported in a previous phase 1 study among Japanese patients with solid tumors [15], the mean C_{max} values of axitinib after single and continuous dosing of 5 mg twice daily are 20.7 and 27.0 ng/mL, respectively, which are both reached above 20 ng/mL. Taking these historical pharmacokinetic data and the estimated total C_{eff} into consideration, we set a $C_{max} > 20$ ng/mL as the provisional, clear threshold for target concentration of axitinib to obtain antitumor activity. The pharmacokinetic profile at steady state and individual C_{max} measurements obtained during outpatient visits were reported to the physicians (i.e., reached or not reached the provisional target) to support clinical decision-making regarding the need for dose escalation at the next visit. The physicians then determined the need for dose adjustment of axitinib based on the pharmacokinetic assessment in addition to individual tolerability and clinical and laboratory findings.

Genotyping

Genomic DNA was extracted from the peripheral blood of patients using NucleoSpin Blood QuickPure (Takara Bio, Kusatsu, Japan). Based on previous findings regarding pharmacogenetic determinants associated with axitinib metabolism and disposition, we examined the *CYP3A4*22*, *CYP3A5*3*, and *ABCG2* 421C > A polymorphisms [6, 16, 17]. Genotyping was performed using TaqMan SNP genotyping assays (Thermo Fisher Scientific, Tokyo, Japan).

Outcomes

Efficacy endpoints were best overall response per RECIST 1.1 and PFS. For safety assessment, all adverse events were graded according to Common Terminology Criteria for Adverse Events version 4.03. The cumulative incidence of dose-limiting toxicities (DLTs), including grade 3/4 adverse events leading to treatment discontinuation and grade 2 hand–foot skin reaction (HFSR) requiring dose interruption, was estimated by adjusting for competing risks (e.g., death or treatment discontinuation due to disease progression) using the Fine and Gray model [18]. The data cutoff date was March 31, 2020.

Statistical analysis

The statistical significance of differences in non-parametric values between two groups was analyzed with the Mann–Whitney *U* test. The ORR between two groups was compared with Fisher's exact probability test. A receiver operating characteristic (ROC) curve was constructed, and the area under the ROC curve (AUC_{ROC}) was calculated to estimate an optimal cutoff value of axitinib C_{max} for predicting clinical response. The median PFS was estimated using the Kaplan-Meier method, and the difference between two groups was examined using the log-rank test. A two-sided $P < 0.05$ was considered statistically significant. All statistical analyses were performed using STATA software, version 16 (StataCorp LLC, Texas, USA).

Results

Patients and axitinib dose and exposure

Twenty patients with metastatic RCC receiving axitinib monotherapy were enrolled (Table 1). Axitinib treatment was discontinued because of adverse events or disease progression ($n = 7$ each). Regarding the data cutoff, axitinib therapy was ongoing at the time of the study in five patients, and one patient was censored because of transfer to another hospital. The median (range) follow-up period was 598 (54–2052) days. No pharmacokinetic data were obtained in one patient who discontinued treatment early because of unacceptable toxicities (e.g., severe fatigue and HFSR) within the first 2 weeks of therapy at 10 mg/day; however, the patient was included in the safety analysis.

Most patients (14/20 [70%]) started axitinib treatment at a lower dose than the recommended 5 mg twice daily. The median starting dose was 6 mg/day, and the median maximum dose reached was 10 mg/day, although the median tolerable maintenance dose was 8 mg/day (Fig. 1a). In patients whose maintenance dose was equal to the starting dose, the mean C_{max} was comparable between the first and last assessments (12.8 and 15.4 ng/mL, respectively) (Fig. 1b). In patients requiring dose reduction from the starting dose, the mean initial C_{max} was relatively higher (28.1 ng/mL) and decreased to 16.8 ng/mL at the last assessment (Fig. 1b). In patients achieving dose escalation above the starting dose, the mean C_{max} at the first assessment was relatively lower (8.4 ng/mL) and increased to 15.3 ng/mL at the last assessment (Fig. 1b). Overall, similar C_{max} values were maintained at approximately 16 ng/mL in the three groups.

Figure 2 shows the distribution of C_{max} values measured in all patients throughout the course of axitinib treatment (total no. of observations, 171). The median (range) C_{max} was 13.3 (0.2–66.9) ng/mL with an upper adjacent value of 40.2 ng/mL.

Axitinib pharmacokinetics

A full pharmacokinetic profile of axitinib at steady state was obtained in one patient during hospitalization (Supplementary Fig. S1). The peak level (20.8 ng/mL) was confirmed at 4 h after administration, and systemic exposure to axitinib during a dosing interval (AUC_{12}) was calculated at 178 ng*h/mL using the trapezoidal rule, which was consistent with data reported in previous phase 1 studies [15, 19]. This case supported the provisional target C_{max} of ≥20 ng/mL, which yields half of the effective AUC_{24} of ≥300 ng*h/mL (i.e., AUC_{12} ≥ 150 ng*h/mL) [9].

Inter- and intra-patient variability in dose-adjusted C_{max}, which is a measure of the ability to absorb the drug, was large (0.02–11.2 ng/mL/mg; Fig. 3a). Patients were classified into two groups according to axitinib absorption (i.e., poor and good), based on the middle value of the median of individual series (1.78 ng/mL/mg). The genotype distribution of *CYP3A5*3* and *ABCG2* 421C > A did not differ between the two groups. No *CYP3A4*22* alleles were detected in the

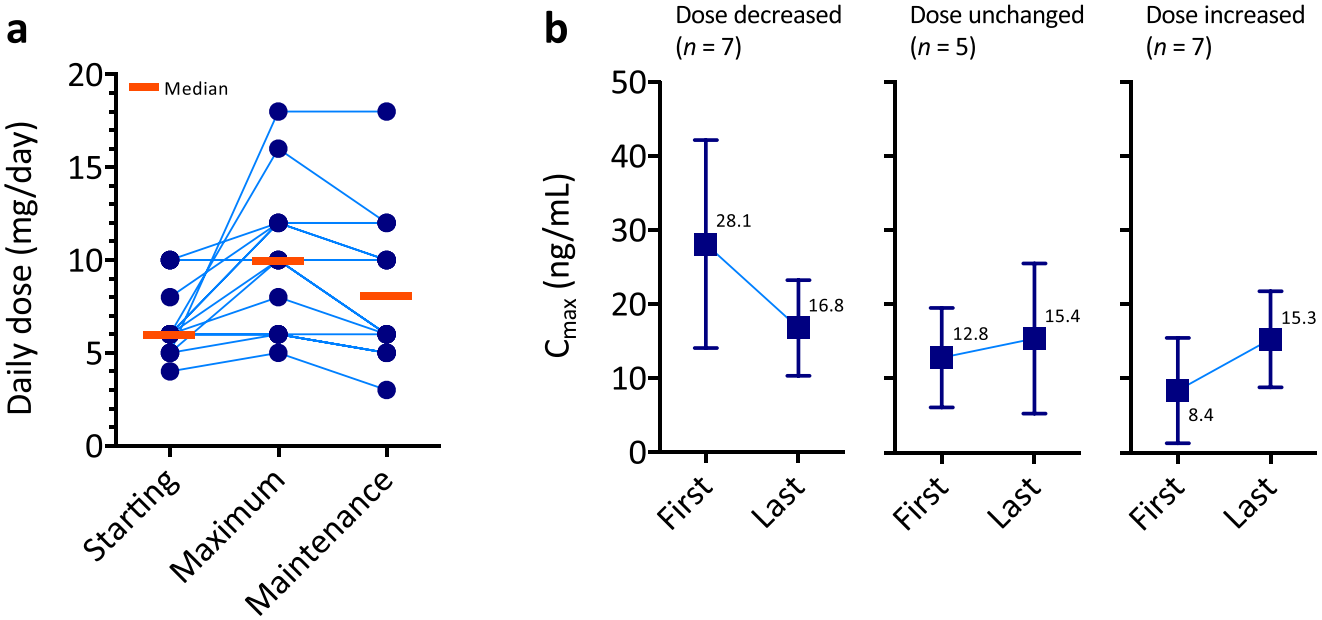

Fig. 1 **a** Changes in axitinib daily dose. Data from the same patients are connected. **b** Changes in peak plasma concentration (C_{max}) of axitinib between the first and last pharmacokinetic assessments in three groups (i.e., patients in which axitinib dose decreased below the starting dose, was unchanged, or increased above the starting dose in the end). Data are presented as the mean with standard deviation

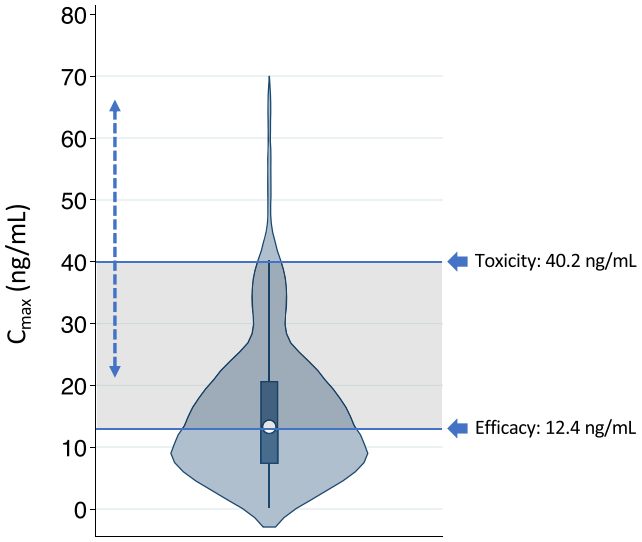

Fig. 2 Violin plot of peak plasma concentration (C_{max}) of axitinib in all patients throughout the treatment. The *box* within the plot area indicates the median (*circle*) and the interquartile range of the data, with whiskers representing the upper and lower adjacent values. The *vertical dotted line* with arrows shows the pharmacologically effective total axitinib concentration in human plasma estimated from a previous study [1], ranging from 21.6 to 65.7 ng/mL. The *shaded area* denotes the potential therapeutic window (12.4–40.2 ng/mL) suggested in the present study

cohort, which was consistent with previous findings in the Japanese population [20].

There were also large inter- and intra-individual differences in M7/axitinib metabolic ratio (Fig. 3a). The glucuronidation activity toward axitinib was significantly higher in patients with poor axitinib absorption than in those with good absorption of the drug ($P = 0.040$; Fig. 3b). The number of patients achieving a clinical response (i.e., complete response [CR] and partial response [PR]) was higher in the good axitinib absorption group than in the poor axitinib absorption group (ORR 80% vs. 33.3%; $P = 0.070$), although the difference was not statistically significant (Fig. 3a).

Axitinib exposure and efficacy/safety relationships

As shown in Fig. 4a, the response improved in correlation with increased axitinib exposure, which was defined as the mean value of individual C_{max} levels at steady state ($C_{max,ss}$). The $C_{max,ss}$ was significantly higher in responders achieving CR and PR than in non-responders with stable disease (SD) and progressive disease (PD) ($P = 0.013$; Fig. 4b). The optimal C_{max} cutoff value to predict clinical response was 12.4 ng/mL, with an AUC_{ROC} of 0.81 (95% confidence interval [CI], 0.59–1.00) (Fig. 4c). The median PFS was significantly longer in patients who achieved a $C_{max,ss}$ greater than or equal to the threshold than in those who did not (799 vs. 336 days; $P = 0.047$; Fig. 4d).

The most frequently observed adverse event of any grade was hypothyroidism (85%; Fig. 5a). No grade 5 toxicities were reported in this study. The most common grade 3/4 adverse events were hypertension and fatigue (20% each), followed by HFSR, anorexia, proteinuria, and increased alanine aminotransferase (5% each). Among the observed grade 3/4 adverse events, hypertension was manageable with antihypertensive medications. Grade 3/4 adverse events leading to treatment discontinuation included fatigue and anorexia, which were reported in four patients, and HFSR, interstitial pneumonitis, cerebral infarction, and renal failure (one patient each). Three patients reported grade 2 HFSR requiring dose interruption. The cumulative incidence of DLTs was significantly higher in patients with $C_{max} \geq 40.2$ ng/mL (upper adjacent value) than in others (sub-hazard ratio, 4.13; 95% CI, 1.27–13.5; $P = 0.019$; Fig. 5b).

Discussion

Axitinib is widely used in combination with the immune checkpoint inhibitor pembrolizumab or avelumab in patients with treatment-naïve metastatic RCC or as a single agent in patients with previously treated RCC [21, 22]. However, given the noncurative nature of molecular targeted drugs in metastatic RCC, axitinib therapy needs to be continued until there is no evidence of disease progression or unacceptable toxicity to prolong survival. Despite the improved antitumor activity of axitinib associated with the currently approved dosing titration, a survival benefit or a clinically meaningful improvement in efficacy has not been achieved even with the modified dose escalation and reduction approach [10, 11, 23]. Thus, establishing a more refined precision dosing of axitinib remains a clinical challenge.

For sunitinib and pazopanib, which are VEGF inhibitors used in metastatic RCC, the feasibility and clinical benefits of TDM using trough concentrations (C_{trough}) to individualize the dosage have been demonstrated [24]. However, a target concentration of axitinib remains to be established. Furthermore, limited data are available on the utility of axitinib TDM in the clinical setting [25, 26]. Regarding elimination, axitinib has a short effective plasma half-life ($t_{1/2}$) ranging from 2.5–6.1 h [5]. The $t_{1/2}$ values of axitinib are considerably shorter than those for sunitinib (41–86 h) and pazopanib (31 h), which accounts for the minimal accumulation of axitinib with relatively low C_{trough} levels at steady state compared with the drugs [5]. Therefore, it may be that the use of C_{trough} alone cannot accurately predict the systemic exposure to axitinib with variable oral absorption, as well as efficacy and safety. Actually, a significant but weak correlation is reported between C_{trough} and AUC_{0-12} in patients with RCC ($r^2 = 0.498$; $P < 0.001$) [27]. In contrast, AUC_{0-12} is correlated well with C_{max} among RCC patients in a neoadjuvant setting

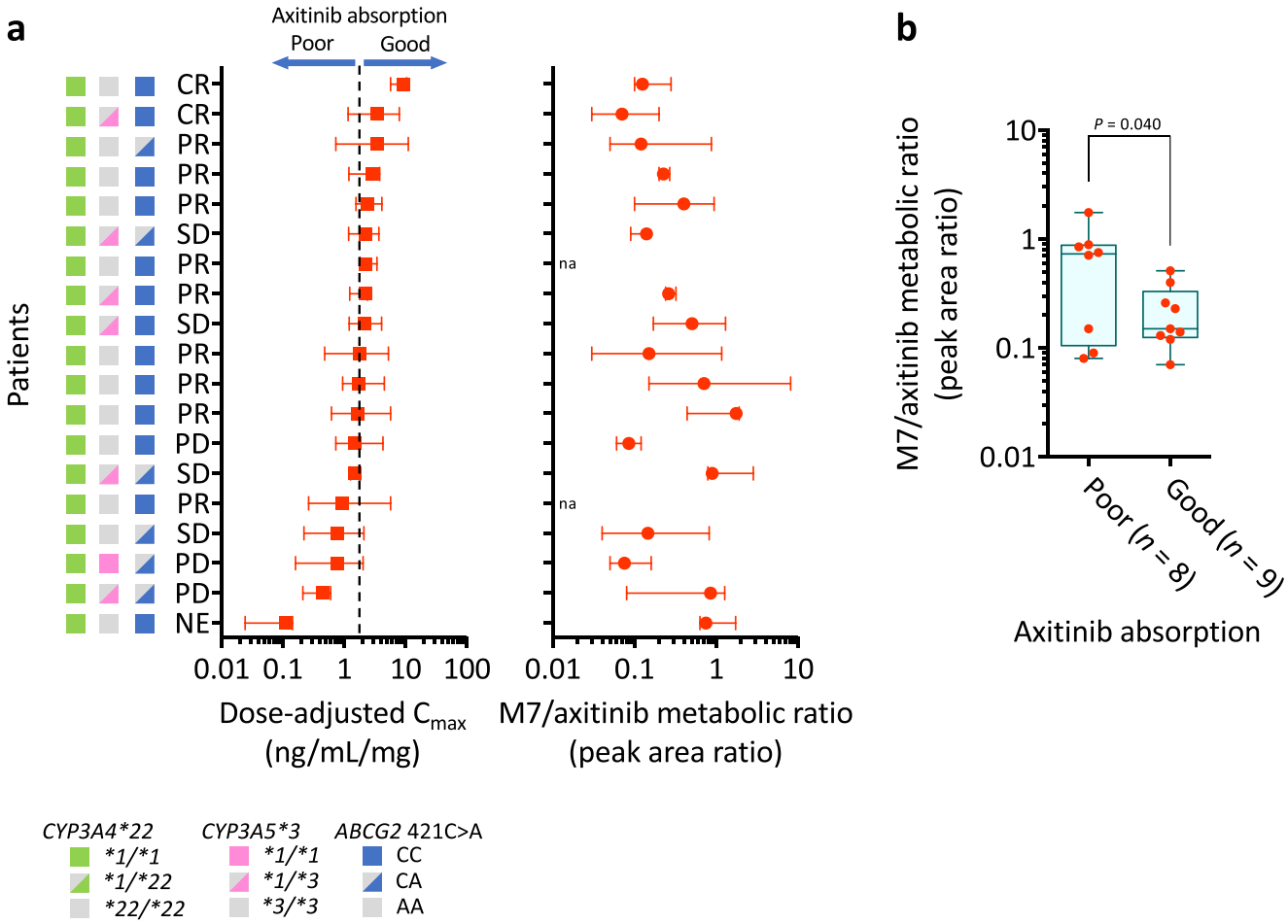

Fig. 3 **a** Dose-adjusted peak plasma concentration (C_{max}) of axitinib and axitinib glucuronide (M7)/axitinib metabolic ratio (peak area ratio). Data are presented as the median with range. The *vertical dotted line* indicates the middle value of the median of individual series. **b** Association between axitinib absorption and glucuronidation activity toward axitinib. Individual median values of M7/axitinib metabolic ratio were used for the analysis. The *boxes* indicate the median and the interquartile range of the data; the *whiskers* represent the minimum and maximum values. *CR*, complete response; *na*, not analyzed; *NE*, not evaluable; *PD*, progressive disease; *PR*, partial response; *SD*, stable disease

($P < 0.0001$) [28]. From these findings, C_{max} could be chosen as an alternative exposure parameter for axitinib TDM.

To the best of our knowledge, the present study provides the first real-world evidence of a potential therapeutic window of axitinib peak plasma concentration in metastatic RCC. Despite the small sample size, we observed clear relationships between axitinib exposure and efficacy and safety when the drug was used as monotherapy. The results suggested that the steady state C_{max} necessary for achieving a clinical response and survival benefit was ≥12.4 ng/mL (Fig. 4), whereas the upper threshold of C_{max} to avoid DLTs leading to treatment discontinuation or interruption was 40.2 ng/mL (Fig. 5b). As shown in Fig. 2, this potential therapeutic window between 12.4 and 40.2 ng/mL overlapped with the range of pharmacologically effective axitinib concentrations estimated from a previous study [1]. A population pharmacokinetic and pharmacodynamic model predicted that the concentration of axitinib necessary to achieve 50% of the maximum increase in diastolic blood pressure is 12.4 ng/mL [29]. Taken together, these findings indicate that an axitinib $C_{max} \geq 12.4$ ng/mL can lead to adequate activity that may also induce the off-target effect of hypertension, which is associated with efficacy [9].

However, higher axitinib concentrations may not further improve efficacy. Classification of axitinib exposure into four quartiles demonstrated that survival outcomes such as PFS and OS are better in quartiles 2 or 3 than in the other quartiles [13, 14]. Additionally, patients with the highest axitinib exposure (those in quartile 4) show the highest incidence of grade ≥ 3 adverse events [13]. In this study, a C_{max} higher than the upper adjacent value (40.2 ng/mL) was significantly associated with increased cumulative incidence of DLTs (Fig. 5b). These data suggest that excessive axitinib exposure is not generally tolerated, causing treatment discontinuation or dose reductions to potentially below previously tolerated level, which can ultimately result in decreased axitinib exposure and poor survival. Therefore, axitinib plasma concentrations need to be within a specific range to avoid under- and over-exposure to the drug and achieve a balance between efficacy

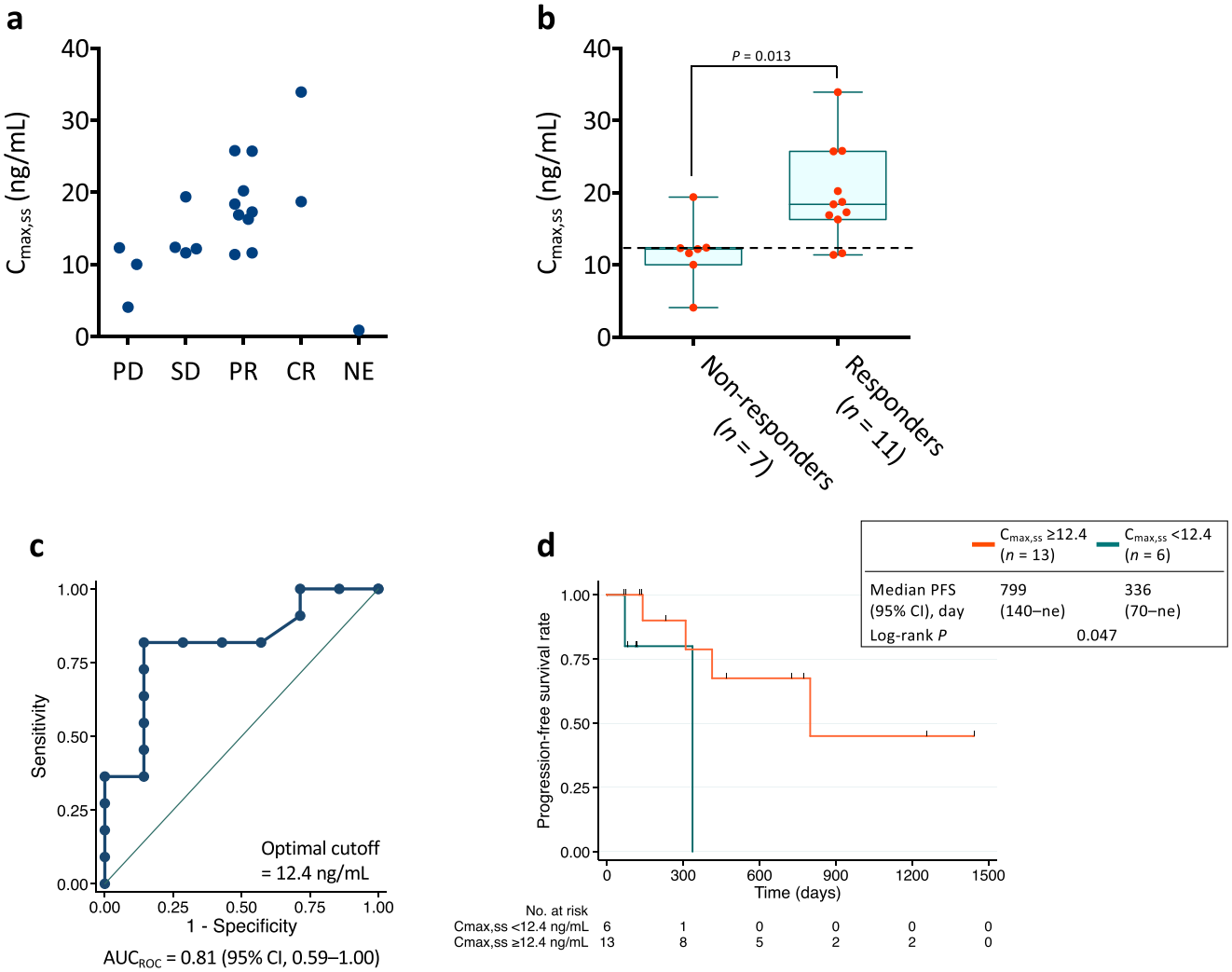

Fig. 4 **a** Best overall response in relation to the mean value of peak plasma concentration at steady state ($C_{max,ss}$) of axitinib. **b** Association between clinical response and axitinib $C_{max,ss}$. Responders included patients achieving complete response (CR) and partial response (PR). Non-responders included patients with stable disease (SD) and progressive disease (PD). One patient in whom response was not evaluable (NE) was excluded. The *boxes* indicate the median and the interquartile range of the data; the *whiskers* represent the minimum and maximum values. **c** Receiver operating characteristic (ROC) curve for predicting clinical response by axitinib C_{max} level. The optimal cutoff value was 12.4 ng/mL, which is shown as the *horizontal dotted line* in panel **b**. **d** Association between progression-free survival (PFS) and axitinib $C_{max,ss}$. AUC_{ROC}, area under the ROC curve; *CI*, confidence interval; *ne*, not estimable

and toxicity. The potential therapeutic window proposed in this study warrants further investigation in a larger patient population.

Schuck et al. [30] systematically evaluated response-guided titration of drugs as a therapeutic individualization strategy. The results showed that response-guided titration is most common for drugs used to treat metabolic and endocrine disorders, for which biomarkers are available for pharmacodynamic monitoring in clinical practice [30]. Among small molecular targeted drugs currently approved in oncology, dose escalation can be used for imatinib, dasatinib, bosutinib, and ruxolitinib if the response is insufficient [31]. For venetoclax, a ramp-up dosing schedule is used to gradually reduce tumor burden and decrease the risk of tumor lysis syndrome in chronic lymphocytic leukemia/small lymphocytic lymphoma [32]. In addition to axitinib, several drugs undergo dose titration based on tolerability. Brigatinib, which is indicated for anaplastic lymphoma kinase-positive metastatic non–small cell lung cancer, is administered at 90 mg orally once daily for the first 7 days; if tolerated, the daily dose may be increased to 180 mg [33]. For regorafenib, 160 mg orally once daily (3 weeks on/1 week off) is the approved dosage. Although not described in the labeling, the National Comprehensive Cancer Network Colon Cancer Guidelines recently included a weekly dose titration schedule based on individual tolerability, starting from 80 mg to 160 mg daily, as an option to treat patients with metastatic colorectal cancer [34]. This titration strategy starting from a reduced dose might be useful to prevent early discontinuation because of severe toxicities during targeted therapy. This includes axitinib

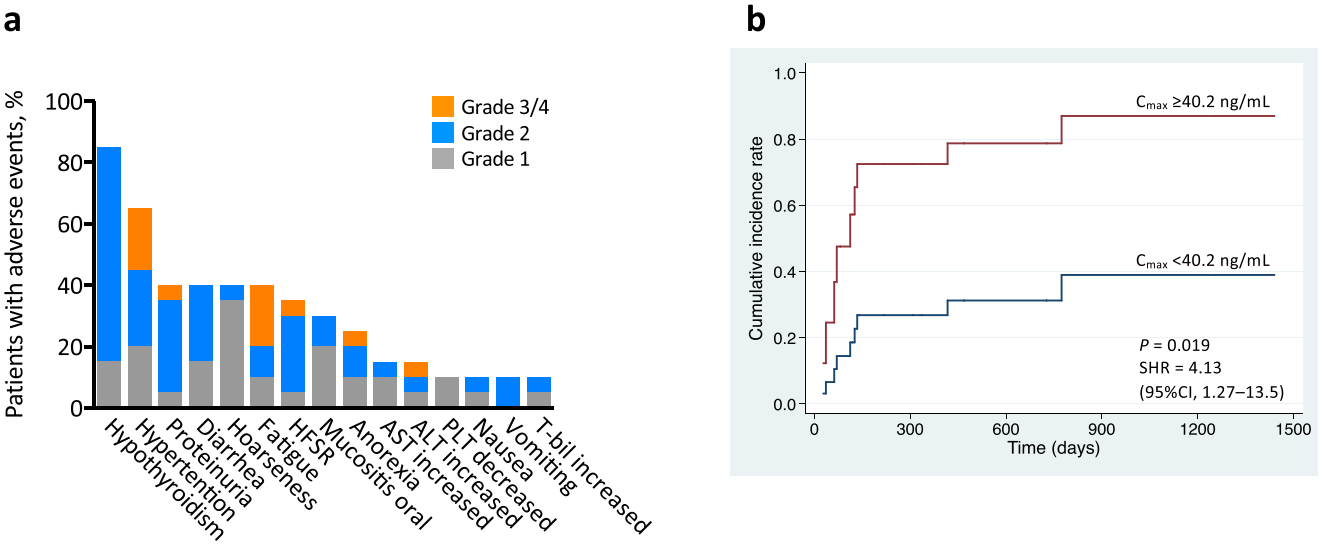

Fig. 5 **a** Adverse events occurring in ≥10% of the patients ($N = 20$). **b** Cumulative incidence of dose-limiting toxicities, including grade 3/4 adverse events leading to treatment discontinuation and grade 2 hand–foot skin reaction (HFSR) requiring dose interruption, according to axitinib C_{max} level. *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase; *CI*, confidence interval; *PLT*, platelet; *SHR*, sub-hazard ratio; *T-bil*, total bilirubin

therapy in certain patients, such as those with poor performance status, which may allow patients to continue treatment while maintaining disease control.

In this study population, the median PFS was 26.6 months (95% CI, 10.3–not estimable), which was comparable with that reported in a subgroup analysis of Japanese patients from the global phase 2 study of axitinib as first-line treatment (27.6 months) [35], and numerically longer than that of a Japanese cohort study of patients with previously treated RCC (13 months) [36]. In this study, the safety profile of axitinib was generally consistent with that reported previously [2, 10, 23], except hypothyroidism and proteinuria, which were more commonly observed in the present study (Fig. 5a). Furthermore, only one (5%) patient receiving the initial dose of 5 mg twice daily experienced early discontinuation because of DLTs within the first month. Most patients (70%) started axitinib therapy at a reduced dose, and the dosage was gradually increased if tolerated or reduced in cases of toxicity, with a smaller adjustment than that currently recommended (i.e., 1 mg increment). Similar to regorafenib, this "start low and go slow" approach may have contributed to a more tolerable and durable axitinib treatment course in this study. Additionally, the median maintenance dose was 8 mg/day in our patient population. The mean relative dose intensity of axitinib is 85.2% in real-world practice [36]. Considering these findings, a dosing titration scheme with a lower starting dose (e.g., 4 mg twice daily) based on tolerability and pharmacokinetic variability (e.g., C_{max}) may provide a safer, individualized axitinib regimen to prolong survival. The toxicity-based, pharmacokinetically guided dosing titration for axitinib should be validated in monotherapy studies, as well as in studies of axitinib in combination with pembrolizumab or avelumab.

Regarding the pharmacokinetics of axitinib, we first demonstrated that poor axitinib absorption was linked to its high glucuronidation activity (Fig. 3b), suggesting that first-pass liver metabolism mediated by UGT1A1 plays a role in the interindividual variability of axitinib absorption. This is supported by a previous result that poor metabolizers of UGT1A1 have a significantly higher axitinib exposure than the extensive metabolizers in patients with metastatic RCC, especially during the first 4 h post-dose [27]. Although the present study was limited by the lack of quantification of axitinib N-glucuronide (M7) in plasma, measuring M7/axitinib metabolic ratio (peak area ratio) will help predict the oral availability of axitinib, and rapid dose titration should be considered in patients with increased glucuronidation activity toward axitinib.

Conclusions

The present study suggests that the therapeutic window of axitinib peak plasma concentration is 12.4–40.2 ng/mL in patients with metastatic RCC. In addition to individual tolerability, TDM of axitinib C_{max} and M7/axitinib metabolic ratio will help guide dose titration to achieve therapeutic drug exposure and improve treatment outcomes in routine clinical practice, warranting larger, randomized studies.

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Authors' contributions Study concept and design: Masahide Fukudo, Seiji Matsumoto; Acquisition, analysis, or interpretation of data: all authors; Drafting of the manuscript: Masahide Fukudo; Critical revision of the manuscript for important intellectual content: all authors; Statistical analysis: Masahide Fukudo; Obtained funding: Masahide Fukudo; Administrative, technical, or material support: Gaku Tamaki, Makoto Azumi, Hidehiro Kakizaki; Supervision: Masahide Fukudo.

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Data availability The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

Compliance with ethical standards

Conflict of interest All authors have no conflicts of interest to declare.

Ethical approval The protocol of this study was approved by the institutional ethics committee of Asahikawa Medical University (#15018). The study was performed in accordance with the Declaration of Helsinki and its amendments.

Informed consent All patients provided written informed consent to participate in the study.

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