Pharmacokinetic–Pharmacodynamic Analysis of Sunitinib-Induced Thrombocytopenia in Japanese Patients with Renal Cell Carcinoma

Masashi Nagata,* Yasuyoshi Ishiwata,† Yutaka Takahashi,* Hiromitsu Takahashi,* Kazutaka Saito,* Yasuhiro Fuji,† Kazunori Kihara,⁎ and Masato Yasuhara*†

*Department of Pharmacy, Medical Hospital, Tokyo Medical and Dental University; 1–5–45 Yushima, Bunkyo-ku, Tokyo 113–8519, Japan; †Department of Urology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University; 1–5–45 Yushima, Bunkyo-ku, Tokyo 113–8519, Japan; and ‡Department of Pharmaceutics and Pharmacodynamics, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University; 1–5–45 Yushima, Bunkyo-ku, Tokyo 113–8519, Japan.

Received September 4, 2014; accepted December 6, 2014

The aim of the present study was to clarify the therapeutic range and adequate dose of sunitinib in Japanese renal cell carcinoma patients by means of a pharmacokinetic–pharmacodynamic analysis of sunitinib-induced thrombocytopenia. Six patients with renal cell carcinoma were enrolled in this study. After starting the sunitinib treatment, between three and seven blood samples were obtained from each patient just before the administration of sunitinib. Serum concentrations of sunitinib and its active metabolite N-desethyl-sunitinib were fit to the 1-compartment model with first-order absorption. Changes in platelet counts were fit to the pharmacokinetic–pharmacodynamic model, in which the proliferation of platelet progenitor cells was assumed to be linearly inhibited by sunitinib and its metabolite. All patients using 50 mg as an initial dose of sunitinib developed grade 2 or 3 thrombocytopenia. The pharmacokinetic–pharmacodynamic model created successfully described the time course of sunitinib-induced thrombocytopenia and could predict changes in platelet counts after alterations to the dosage of sunitinib administered. The simulation results indicated that the total trough level of sunitinib to avoid severe thrombocytopenia should be <100 ng/mL, and also that the initial daily dose of sunitinib could be reduced to 37.5 mg or 25 mg in most Japanese patients. In addition to the pharmacokinetic-guided dosage adjustment, the careful monitoring of platelet counts is required for the safe use of sunitinib.

Key words sunitinib; pharmacokinetic–pharmacodynamic analysis; thrombocytopenia; renal cell carcinoma

Sunitinib is an oral multi-targeted tyrosine kinase inhibitor approved for the first-line treatment of metastatic renal cell carcinoma (RCC). The standard dosing schedule of sunitinib is 50 mg daily for 4 weeks, followed by 2 weeks without the drug (Schedule 4/2). However, the sunitinib treatment is often associated with severe toxicity, necessitating dose reductions or discontinuation.

The incidence of adverse events such as thrombocytopenia was previously shown to be greater in Asian patients from Asian sites than in non-Asian patients. A global study reported that the incidence of grade 3 or 4 thrombocytopenia due to treatments with sunitinib was <10%. On the other hand, Miyake et al. showed that dose reductions due to adverse events associated with sunitinib were necessary in 102 out of 110 Japanese patients (92.7%) with the same dosing schedule, and the most common adverse event corresponding to grade ≥3 was thrombocytopenia in 59 patients (53.6%). Yuasa et al. and Uemura et al. reported the same findings and demonstrated that approximately 50% of Japanese patients developed grade 3 or 4 thrombocytopenia. These findings suggested that the standard initial dose of 50 mg of sunitinib may be intolerable to most Japanese patients.

The efficacy and side effects of drugs are generally determined in two phases; pharmacokinetics (PK) and pharmacodynamics (PD). Genetic variations in ABCG2, the efflux transporter in normal human tissues such as the small intestine, were shown to affect the PK of sunitinib, and sunitinib concentrations were greater in patients with a variant in ABCG2 421C>A than in wild-type patients. This genetic polymorphism has been associated with sunitinib-induced toxicities. In addition, the allele frequency of ABCG2 421C>A was found to be higher in Asians than in non-Asians, such as Caucasians and African-Americans. These findings suggested that the ABCG2 polymorphism may represent one of the reasons for the ethnic differences reported in sunitinib PK and toxicity. However, it currently remains unknown whether variability exists in the PD of sunitinib-induced toxicity.

To optimize dosing schedules, the relationship between drug concentrations and clinical toxicity needs to be clarified in more detail. Hansson et al. reported that PK–PD modeling could be used for the early monitoring of adverse effects and clinical responses in sunitinib-treated patients with gastrointestinal stromal tumors. The aim of the present study was to clarify the therapeutic range and adequate dose of sunitinib in Japanese RCC patients with a PK–PD analysis of sunitinib-induced thrombocytopenia.

MATERIALS AND METHODS

Patients and Study Design Between June 2012 and October 2013, Japanese RCC patients treated with sunitinib at the Medical Hospital of Tokyo Medical and Dental University were enrolled in this study. The Helsinki Declaration and ethical guidelines for clinical research in Japan were followed throughout the study, and its protocol was approved by the Ethical Committee of the Faculty of Medicine, Tokyo Medical
and Dental University. Written informed consent was obtained from all patients. All patients received 25 to 50 mg of sunitinib (Sutent® Capsule, Pfizer Japan Inc., Tokyo, Japan) once daily on days 1–28. If the patient developed grade ≥3 toxicity (National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03) or intolerable toxicity despite supportive care at any time point during the study, the sunitinib treatment was interrupted until adequate recovery was achieved. During the sunitinib treatment, blood samples were obtained once or twice a week just before the administration of sunitinib, and blood sampling was continuously performed for 1–2 weeks after the sunitinib treatment. Additional blood samples were obtained in two patients (Patient Nos. 1, 5) at 2–3 cycles of chemotherapy with reduced dose of sunitinib. Serum was separated by centrifugation and stored at −20°C until analysis. Platelet counts for each patient were obtained through an electronic medical database.

**Determination of Sunitinib and Its Active Metabolite**

The sunitinib (Toronto Research Chemicals Inc., Toronto, Canada) and N-desethyl sunitinib (Toronto Research Chemicals Inc., Toronto, Canada) assays were performed according to a previously described method14) with minor modifications. One milliliter of serum, 100 µL of an internal standard (clonazepam, 1 µg/mL in methanol), and 1 mL 0.1 N NaOH were mixed vigorously for 10 s. This mixture and 3 mL ethyl acetate were shaken for 10 min and then centrifuged at 1700 × g for 10 min. The supernatant were evaporated to dryness in vacuo at room temperature. The residue was reconstituted in 125 µL of the mobile phase, and 50 µL of the mixture was then injected into the HPLC column. The HPLC apparatus was a Shimadzu Prominence LC-20 A system (Shimadzu Co., Kyoto, Japan). The column was a TSKgel ODS-80TM (5 µm, 4.6 mm i.d. ×150 mm; TOSOH, Japan) and was kept at 40°C. The mobile phase was 0.05 M phosphate buffer (pH 3.0)–acetonitrile (65:35, v/v). The flow rate was 1.0 mL/min, and the column effluent was monitored by a UV detector set at 431 nm (sunitinib and N-desethyl sunitinib) or 320 nm (clonazepam).

**PK–PD Analysis**

Serum concentration–time profiles for sunitinib and N-desethyl sunitinib were analyzed using a one compartment model with first-order absorption (Fig. 1). The differential equations describing the model in Fig. 1 were:

\[
\frac{dX_s}{dt} = -k_a \cdot X_s
\]

\[
\frac{dX}{dt} = k_a \cdot X_s - k_el \cdot X
\]

\[
\frac{dX_m}{dt} = k_m \cdot X - k_em \cdot X_m
\]

where \(X_s\), \(X\), \(k_a\), \(k_el\), \(k_m\), and \(k_em\) represent the amount of sunitinib at the absorption site, the amount of sunitinib in the body, the amount of N-desethyl sunitinib in the body, and the first order absorption rate constant of sunitinib, the first order elimination rate constant of sunitinib, the first order metabolism rate constant of sunitinib, and the first order elimination rate constant of N-desethyl sunitinib, respectively. The Laplace transform method yielded the following equations:

\[
C_{\text{trough},n} = \frac{F \cdot D \cdot k_s}{V_d \cdot (k_a - k_d)} \left( \frac{1 - e^{-nk_a\tau}}{1 - e^{-nk_d\tau}} \right)
\]

\[
\approx \frac{F \cdot D \cdot k_s}{V_d \cdot (k_a - k_d)} \left( \frac{1 - e^{-nk_d\tau}}{1 - e^{-nk_a\tau}} \right)
\]
\[
C_{n,\text{rough},a} \approx \frac{F \cdot D \cdot k_a \cdot k_u}{V_{\text{dm}} \cdot (k_{\text{em}} - k_{\text{d}})} \times \left( \frac{1 - e^{-k_{\text{tr}}t}}{1 - e^{-k_{\text{d}}t}} \right) \left( \frac{e^{-k_{\text{tr}}t}}{(k_a - k_{\text{d}})} \right) + \left( \frac{C_{\text{m,rough},a} - V_{\text{dm}} \cdot (k_{\text{em}} - k_{\text{d}}) \cdot C_{\text{t,rough},a}}{V_{\text{dm}} \cdot (k_{\text{em}} - k_{\text{d}})} \right) \left( e^{-k_{\text{d}}t} - e^{-k_{\text{tr}}t} \right)
\]

where \( C_{\text{t,rough},a}/C_{n,\text{rough},a} \) is the trough concentration of sunitinib/N-desethyl sunitinib after the \( n \)th dose, \( F \) is bioavailability, \( D \) is the sunitinib dose, \( V_d \) is the distribution volume of sunitinib, \( V_{\text{dm}} \) is the distribution volume of N-desethyl sunitinib, \( n \) is the number of doses, and \( r \) is the dosage interval (24 h). The term \( e^{-k_{\text{tr}}t} \) was neglected because \( e^{-k_{\text{tr}}t} \) was less than 0.01 (\( k_{\text{tr}} = 0.2/h \)).

Sunitinib \( (C_{\text{fin},a}) \) and N-desethyl sunitinib \( (C_{\text{m,fin},a}) \) concentrations at any time \( t \) 24 h after the final dose were described by

\[
C_{\text{fin},t} = C_{\text{t,fin},t} \cdot e^{-k_{\text{d}}t}
\]

\[
C_{\text{m,fin},t} = \frac{V_d \cdot (k_a - k_{\text{d}})}{V_{\text{dm}} \cdot (k_{\text{em}} - k_{\text{d}})} \cdot C_{\text{m,fin},t} \cdot e^{-k_{\text{d}}t}
\]

where \( C_{\text{t,fin},t}/C_{\text{m,fin},t} \) is the trough concentration of sunitinib/N-desethyl sunitinib after the final dose. The time course of sunitinib and N-desethyl sunitinib were simultaneously fit by a nonlinear least square program MULTI with the Damping Gauss Newton method algorithm. \( k_a \) was fixed at the value reported \( (0.2/h) \) because of a lack of sampling points in the absorption phase. Therefore, the structural model parameters to be estimated were \( k_{\text{tr}}, V_d/F, k_{\text{tot}}, \) and \( V_{\text{dm}}/F/k_{\text{tot}} \).

The PK–PD model developed by Friberg et al. \(^{17}\) was used with minor modifications. The model (Fig. 1) consisted of five compartments that represented platelet progenitor cells \( (\text{Prog}) \), three transit compartments with maturing cells \( (\text{Transit}) \), and circulating platelets \( (\text{Circ}) \). The differential equations describing the model in Fig. 1 are:

\[
\frac{d\text{Prog}}{dt} = k_{\text{pol}} \cdot \text{Prog} \cdot (1 - \text{Slope} \cdot C_{\text{total}}) \left( \frac{C_{\text{circ}}}{C_{\text{circ}}} \right)^{\gamma} - k_a \cdot \text{Prog}
\]

\[
\frac{d\text{Transit}_1}{dt} = k_a \cdot \text{Prog} - k_a \cdot \text{Transit}_1
\]

\[
\frac{d\text{Transit}_2}{dt} = k_a \cdot \text{Transit}_1 - k_a \cdot \text{Transit}_2
\]

\[
\frac{d\text{Transit}_3}{dt} = k_a \cdot \text{Transit}_2 - k_a \cdot \text{Transit}_3
\]

\[
\frac{d\text{Circ}}{dt} = k_a \cdot \text{Transit}_3 - k_{\text{circ}} \cdot \text{Circ}
\]

where \( k_{\text{pol}}, k_a, k_{\text{circ}}, \) and \( \gamma \) represent the first order proliferation rate constant of platelet progenitor cells, the first order transition rate constant, the first order degradation rate constant of circulating platelet, and feedback parameter, respectively. \( C_{\text{circ}} \) represents the platelet count at baseline. At steady state, \( d\text{Prog}/dt = 0 \), and therefore \( k_{\text{pol}} = k_a \). In addition, to minimize the number of parameters to be estimated, it was assumed in the modeling that \( k_{\text{a}} = k_{\text{circ}} \). The proliferation of platelet progenitor cells was assumed to be linearly inhibited by sunitinib and its primary metabolite. Since the primary metabolite exhibited similar potency to sunitinib in the \textit{in vitro} tyrosine kinase inhibition assays, the pharmacological activity of the primary metabolite was assumed to be equal to sunitinib.\(^{18}\) Therefore, the drug effect was included in the model as follows: \( k_{\text{pol}} \times (1 - \text{Slope} \cdot C_{\text{total}}) \). Where \( C_{\text{total}} \) was the predicted total sunitinib \( \text{(sunitinib+N-desethyl sunitinib)} \) concentration. This inhibitory effect was also assumed to have a threshold value, and \( C_{\text{total}} \) less than 30 mg/mL was set to zero. If the total sunitinib concentration exceeded this threshold value, \( C_{\text{total}} \) was the total sunitinib concentration minus 30. PD parameters were estimated by fitting the platelet counts–time profiles using MULTI (RUNGE) with the Damping Gauss Newton method algorithm.\(^{19}\) The structural model parameters to be estimated were \( k_{\text{a}} \) (the first order transition rate constant), \( \gamma \) (the feedback parameter), and \( \text{Slope} \).

Simulation of Sunitinib Concentrations and Platelet Counts Based on the estimated PK–PD parameters, time \textit{versus} platelet count curves were simulated in two patients (Patient Nos. 1, 5) after dose reductions based on the estimated PK–PD parameters in each patient. Furthermore, the maximum total trough level \( (\text{TTL}) \) of sunitinib and platelet counts in each patient were simulated in the daily dose range of sunitinib from 12.5 to 50 mg at first cycle on Schedule 4/2 or a schedule of 2 weeks of treatment/1 weeks off (Schedule 2/1).

RESULTS

Patient Characteristics and Treatment Toxicity Six Japanese RCC patients treated with sunitinib were enrolled in this study \( (\text{Table 1}) \). In initial treatment, four patients \( (\text{Nos. 1, 2, 4, and 5}) \) received the standard dose \( (50 \text{mg/d}) \), and two patients \( (\text{Nos. 3, 6}) \) received the lower dose \( (25 \text{mg/d}) \) at a physician’s discretion. Five out of the six patients discontinued the sunitinib therapy within 18 d because of adverse events. All patients using \( 50 \text{mg} \) as the initial dose developed grade 2 \( (<7.5 \times 10^9/\mu \L) \) or grade 3 \( (<5 \times 10^9/\mu \L) \) thrombocytopenia.

PK in Japanese RCC Patients Figure 2 and Table 2 shows serum sunitinib concentration–time profiles and estimated PK parameters in Japanese RCC patients. The PK model developed successfully described the time course of sunitinib concentrations. Based on these PK parameters, we simulated the TTL in each patient with the standard dosing schedule of sunitinib \( (50 \text{mg daily for 28 d}) \) \( (\text{Table 3}) \). The estimated TTL at a steady state showed large interindividual variability \( (100–261 \text{ng/mL}) \).

PK–PD Modeling of Sunitinib-Induced Thrombocytopenia The time courses of serum platelet counts following the administration of sunitinib, along with the fitted curves based on the nonlinear least squares methods, are shown in Fig. 3A. The individually fitted curves were well matched to the data obtained, and the observed platelet counts could be described well by our developed model \( (\text{coefficient of determination} (r^2) = 0.95, p < 0.01 \text{by analysis of variance in regression}) \) \( (\text{Fig. 3B}) \). The estimated PD parameters are shown in Table 2. Large interindividual variability was observed in these PD parameters. Thus, the curves drawn by the mean parameters of 6 patients were not well consistent with the observed platelet counts in some patients \( (\text{Fig. 3A}) \).
Fig. 2. Serum Concentration–Time Profiles of Sunitinib in RCC Patients
The initial dose of sunitinib was 50 mg (patient Nos. 1, 2, 4, 5) or 25 mg (patient Nos. 3, 6). ●, sunitinib; ○, N-desethyl sunitinib. Solid lines denote computer-fitted curves.
We simulated time versus platelet count curves in two patients (Patient Nos. 1, 5) after dose reductions based on the estimated PK–PD parameters in each patient with the 50 mg dose of sunitinib (Fig. 4A). There was a statistically significant relationship between the simulated and observed data ($r^2=0.57$, $p<0.01$ by analysis of variance in regression) (Fig. 4B).

Simulation of Sunitinib-Induced Thrombocytopenia Based on the Developed PK–PD Model Based on the estimated PK–PD parameters, 5 out of 6 patients were estimated to develop grade 3 or 4 thrombocytopenia with the 50 mg daily dose of sunitinib on Schedule 4/2 or 2/1, and large interindividual variability was observed in the initial dose (12.5–50 mg) to prevent severe thrombocytopenia (grade ≥3) in each patient (Table 3).

DISCUSSION

In the present study, all patients using 50 mg as an initial dose developed grade 2 or 3 thrombocytopenia, and 5 out of 6 patients discontinued the sunitinib therapy within 18 d because of adverse events (Table 1). This result was consistent with previous findings, and the standard initial dose of 50 mg of sunitinib may have been intolerable to most Japanese patients.

As to the pharmacokinetics of sunitinib, Houk et al. reported the population pharmacokinetic parameters using a two-compartment model with first-order absorption and first-order elimination. Apparent clearance/F for sunitinib was estimated to be 51.8 L/h and $V_d/F$ was estimated to be 2030 L for the central compartment (with interindividual variabilities (%CV) of 38% and 43%). The race was identified as a significant covariate for sunitinib clearance/F, which decreased by 13% in Asian patients. The simulated average concentration of total sunitinib in Caucasian male patient (body weight: 77 kg, 50 mg daily dose of sunitinib) was about 30–90 ng/mL, which decreased by 13% in Asian patients. The simulated average concentration of total sunitinib in Caucasian male patient (body weight: 77 kg, 50 mg daily dose of sunitinib) was about 30–90 ng/mL, which decreased by 13% in Asian patients. The simulated average concentration of total sunitinib in Caucasian male patient (body weight: 77 kg, 50 mg daily dose of sunitinib) was about 30–90 ng/mL, which decreased by 13% in Asian patients.
tion, serum drug concentrations would not be affected by the $k_a$ value. The $ABCG2$ 421C>A polymorphism may affect the $k_a$ for sunitinib. Yamasaki et al. demonstrated that the relative bioavailability and $k_a$ for sulfasalazine, a substrate of ABCG2, were increased in subjects with the $ABCG2$-A allele and the relative $k_a$ values in $ABCG2$-C/C, C/A, and A/A subjects were 1:1.8.21) Thus, patients with $ABCG2$ 421C>A variant might have faster $k_a$ for sunitinib. However, the increased $k_a$ has little effect on TTL. Therefore, the fixed $k_a$ value was used in the present study.

Although the mechanism responsible for sunitinib-induced thrombocytopenia currently remains unclear, this adverse effect may be associated with the inhibitory activity of sunitinib against c-kit and Flt-3,23) which are expressed on early hematopoietic progenitor cells and involved in their proliferation and differentiation.25) In a preliminary PK–PD analysis, the sunitinib inhibitory effect on the proliferation of platelet progenitor cells was described by either a linear function of $C_{total}$ or an $E_{max}$ model. Since both models described the observed platelet changes similarly (data not shown), we selected the simpler linear model in the following PK–PD modeling. Furthermore, the improvement in the description of the data was observed when a threshold value was added to the inhibitory linear function of $C_{total}$. Therefore, we hypothesized that the inhibitory effect of sunitinib had a threshold value, and the threshold values were determined in each patient. The mean threshold value of 6 patients was 30.9 ng/mL and the threshold value was fixed at 30 ng/mL in the final model. This PK–PD
model successfully described the time course of sunitinib-induced thrombocytopenia in each patient (Figs. 3A, B). Thus, this PK–PD model with the linear inhibition of platelet progenitor cell proliferation by sunitinib appears to be reasonable.

The results of the PK–PD analysis allowed the platelet count-time profiles with several dosing schedules of sunitinib to be predicted in each patient. The predicted platelet counts could be compared with the observed data in two patients (Patient Nos. 1, 5), whose blood samples were additionally obtained after the dose reduction. There was a statistically significant relationship between the simulated and the observed data in these two patients (Fig. 4B). Therefore, this PK–PD model may be used as a platform to optimize the dosing of sunitinib to prevent severe thrombocytopenia in clinical use.

Recent findings demonstrated that increased exposure to sunitinib was associated with improved clinical outcomes.24) Meanwhile, the target TTLs of sunitinib have not been established in clinical studies. In an in vivo study in mice, the minimum plasma concentrations required to block the target receptor tyrosine kinases such as vascular endothelial

---

Fig. 4. Simulation of Sunitinib-Induced Thrombocytopenia with Reduced Doses of Sunitinib

(A), Simulated serum concentration–time profiles of sunitinib and platelets in RCC patients (Patient Nos. 1, 5) with reduced doses of sunitinib. Dashed lines were simulated by the PK–PD parameters obtained from each patient with 50 mg of sunitinib (Table 2). ●, platelet; ○, total sunitinib (sunitinib + N-desethyl sunitinib). (B), Observed versus simulated platelet counts. The solid line and the dashed line denote the line of identity and the regression line.
growth factor receptor-2 were estimated to be in the range of 50–100 ng/mL.25 In a retrospective clinical study, Faivre et al. reported that most patients with severe toxicity had TTL ≥100 ng/mL, and therapeutic efficacy was lower in patients with TTL <50 ng/mL than in patients with TTL ≥50 ng/mL.26 However, our simulation results indicated that 3 out of 6 Japanese patients (Nos. 1, 5, 6) with RCC were estimated to develop severe (grade 3 or 4) thrombocytopenia when target TTLs were in the range of 50–100 ng/mL on Schedule 4/2 with the daily dose of 37.5 mg (Table 3). Therefore, the TTL to avoid severe thrombocytopenia may be <100 ng/mL in most Japanese patients.

A recent study reported that a treatment with sunitinib on Schedule 2/1 was associated with significantly decreased toxicity in patients who developed grade 3/4 toxicity on Schedule 4/2.27 However, 83% patients were estimated to develop grade 3 or 4 thrombocytopenia with the 50 mg daily dose of sunitinib on Schedule 2/1 in our simulation results (Table 3). Therefore, to prevent the severe thrombocytopenia, the initial daily dose of sunitinib should be reduced to 37.5 or 25 mg even on Schedule 2/1.

The present study showed the presence of large interindividual variability in both PK and PD of sunitinib (Table 2). Therapeutic drug monitoring involves using drug concentrations, pharmacokinetic principles, and pharmacodynamics criteria to optimize drug therapy in individual patients. Lankheet et al. reported that a PK-guided dosing strategy of sunitinib was useful to reach the target TTL (>50 ng/mL) because the PK of sunitinib showed large interindividual variations.28 In addition, they demonstrated that the frequency of severe toxicities (grade >3) did not correlate with TTL. Furthermore, the estimated PD parameters to sunitinib-induced thrombocytopenia showed large interindividual variability in our study (Table 2). These results suggest that the simple monitoring of serum sunitinib concentrations is not sufficient to completely prevent sunitinib-induced thrombocytopenia, and the careful monitoring of platelet counts is needed for the safe use of sunitinib.

In conclusion, the present study showed that the TTL of sunitinib to avoid severe thrombocytopenia should be <100 ng/mL, and also that the initial daily dose of sunitinib could be reduced to 37.5 mg or 25 mg in most Japanese patients. In addition to the PK-guided dosage adjustment, the careful monitoring of platelet counts is required for the safe use of sunitinib. Since the number of patients who participated in this study was limited, further investigations are necessary to confirm our results.

Acknowledgment This work was supported by a Grant-in-Aid for the Encouragement of Scientists [23928021] from the Japan Society for the Promotion of Science.

Conflict of Interest Kazutaka Saito, Yasuhisa Fujii, and Kazunori Kihara received a research grant from Pfizer Japan Inc. All other authors have no conflict of interest.

REFERENCES

17) Friberg LE, Henningsson A, Maas H, Nguyen L, Karlsson MO.


