Pharmacokinetic/Pharmacodynamic Modeling of Schedule-Dependent Interaction between Docetaxel and Cabozantinib in Human Prostate Cancer Xenograft Models[§]

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ABSTRACT

In this work, a semimechanistic pharmacokinetic/pharmacodynamic (PK/PD) model to quantitatively describe the antitumor activity of docetaxel (Doc) and cabozantinib (Cab) under monotherapy, concurrent therapy, interval therapy, and different sequential therapy in mouse xenograft models of castration-resistant prostate cancer was developed and evaluated. The pharmacokinetics (PK) of Doc and Cab when administered separately and simultaneously were investigated in nude mice, and PD study was conducted in tumor-bearing mice treated with different dosing schedules. The PK interaction between Doc and Cab was expressed by adding the effect of Cab on the clearance of Doc in the PK model. And the PD interaction between the two drugs was demonstrated by the developed PK/PD model through the combination index “ ϕ .” Our results showed that the concurrent therapy

and Doc followed by Cab (Doc ~ Cab) sequential therapy exhibited better tumor inhibitory efficacy than monotherapy. The Cab followed by Doc (Cab ~ Doc) sequential schedule was less effective than monotherapy, and the interval therapy did not enhance the antitumor efficacy compared with the concurrent therapy. The parameter ϕ estimated from the PK/PD model quantitatively characterized the action between Doc and Cab. There was no significant PD interaction between Doc and Cab in both the concurrent schedule and the interval schedule, whereas the effect of the two drugs in the “Doc ~ Cab” and “Cab ~ Doc” sequential schedule was synergistic and antagonistic, respectively. The proposed model properly described the antitumor effects of Doc and Cab under different treatment schedules and could be used for dose optimization through model-based simulation.

Introduction

Prostate cancer has the second highest incidence rate among males worldwide (Torre et al., 2016). Although advanced prostate cancer usually responds to antiandrogen therapies initially, resistance inevitably develops, leading to the emergence of castration-resistant prostate cancer (CRPC). Docetaxel (Doc) (Taxotere; Sanofi-Aventis, Paris, France) is a semisynthetic taxane microtubule inhibitor, and Doc plus prednisone has been the standard first-line chemotherapy in patients with CRPC (McKeage, 2012). Cabozantinib [Cab (also called XL184)] is an orally bioavailable multitarget tyrosine kinase inhibitor with activity primarily against MET (hepatocyte growth factor/scatter receptor) and vascular endothelial growth factor receptor 2 (VEGFR2) (Grüllich, 2014). The inhibition of MET and VEGFR2 subsequently downregulates a series of downstream signaling pathways,

which then inhibit cell proliferation and angiogenesis. It has been reported (Vaishampayan, 2014) that Cab demonstrated responses in soft tissue, visceral disease, and bone metastases in individuals with CRPC. Although two recently presented phase III trials (COMET-1 and COMET-2) evaluating Cab in CRPC did not meet their primary endpoints (Basch et al., 2015; Smith et al., 2015), this targeted strategy still seems to be a promising area of prostate cancer research (Modena et al., 2016).

Achieving better antitumor efficacy by drug combination is a mainstay in oncology. However, the combination of cytotoxic and antiangiogenic drugs might not be a simple synergy. For example, the pharmacological effect of combination therapy is not better than chemotherapy alone in four large randomized clinical trials in non-small cell lung cancer (Herbst et al., 2004, 2005; Manegold et al., 2005; Gatzemeier et al., 2007). To explore the clinical failure of erlotinib and gemcitabine combination, Li et al. (2013) found in preclinical research that the antitumor effect of erlotinib and gemcitabine in the interval group was significantly different from that in the concurrent group. In recent years, sequential therapy has drawn increasing attention in the process of optimizing the

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ABBREVIATIONS: ϕ , combination index; Cab, cabozantinib; CL, clearance; CRPC, castration-resistant prostate cancer; Doc, docetaxel; FBS, fetal bovine serum; KP, tumor-carrying capacity; LC, liquid chromatography; MS/MS, tandem mass spectrometry; NONMEM, nonlinear mixed effect models; OFV, objective function value; PD, pharmacodynamic; PK, pharmacokinetic; TGI, tumor growth inhibition; %TGI, percentage of tumor growth inhibition; TV, tumor volume; VEGFR2, vascular endothelial growth factor receptor 2; VPC, visual predictive check.

dosing regimen in cancer therapy. In preclinical studies, sequential treatment has been reported to augment the antitumor efficacy of monotherapy (Wang et al., 2015). Moreover, several clinical studies showed that sequential therapy had the advantage of improving survival (Moebus et al., 2010; Signorelli et al., 2015), reducing drug resistance (Buzdar and Hortobagyi, 2003), and exhibiting less toxicity compared with concurrent therapy (Fornier et al., 2001).

Pharmacokinetic/pharmacodynamic (PK/PD) modeling is a good way to explore the time course relationship between drug behaviors and effects and has become a key tool in the whole course of oncologic drug development (Manolis et al., 2013; Milligan et al., 2013). A couple of modeling approaches have been proposed to study the antitumor activity of drugs. These models not only can provide time-dependent quantitative estimates of the antitumor effect of a single compound (Simeoni et al., 2004), but also are capable of studying the interaction between coadministered drugs (Earp et al., 2004; Koch et al., 2009). They are also indicated as possible suitable tools for the translation from preclinical to clinical occasions (Rocchetti et al., 2007; Simeoni et al., 2013; Eigenmann et al., 2016). Among these models, a linear tumor growth inhibition (TGI) model proposed earlier by Simeoni et al. (2004) is the most popular one. However, despite its simplicity and flexibility, the linear TGI model based on a cell-killing hypothesis may not be able to capture the modes of action of angiogenesis inhibitors. Taking into account tumor angiogenesis, a semimechanistic model describing the effects of pazopanib was proposed by Ouerdani et al. (2015). The model supposed that the tumor, through proangiogenic factors such as VEGF, was capable of extending its carrying capacity [i.e., the maximal tumor volume (TV) or mass supported by the current level of tumor vascularization]. This model was developed using experimental data obtained from mice and then applied to the description of the effect of pazopanib in patients. More recently, in a study (Mollard et al., 2017) conducted to investigate the combination efficacy of bevacizumab and paclitaxel in the treatment of breast cancer in tumor-bearing mice, a mathematical model was developed to describe the antiangiogenic effect of bevacizumab on tumor vasculature, which was divided into two compartments: a stable one and an unstable one. Nevertheless, this model put forward higher requirements on data, although it explained more specific mechanisms.

The aim of this study was to develop a semimechanistic PK/PD model to quantitatively describe the antitumor effects of Doc and Cab under monotherapy, concurrent treatment schedule, interval treatment schedule, and different sequential treatment schedules, using 22Rv1 and PC3-derived nude mouse xenograft models, and thus to select the optimal regimen based on the results and simulations. This study may provide helpful suggestions for the combination use of Doc and Cab in the clinical treatment of CRPC.

Materials and Methods

Drugs and Reagents. Doc and Cab were purchased from Melone Pharmaceutical (Dalian, People's Republic of China). RPMI 1640 was bought from Magene Biotech Company, Ltd. (Beijing, People's Republic of China), and fetal bovine serum (FBS) was obtained from Gibco (Grand Island, NY). Matrigel was purchased from Becton Dickinson (Franklin Lakes, NJ). And chromatographic grade acetonitrile and methanol were bought from Sigma-Aldrich (St. Louis, MO).

Cell Culture and Animals. The human prostate cancer cell line 22Rv1 was purchased from Shanghai Institutes for Biologic Sciences of the Chinese Academy of Sciences (Shanghai, People's Republic of China) and cultured in RPMI 1640 medium containing 10% FBS and antibiotic (penicillin 100 IU/ml and streptomycin 100 μ g/ml). The human PC3 prostate cancer cell line was provided by Instructor Yan Song (School of Pharmaceutical Sciences, Peking University) and grown in RPMI 1640 medium containing 10% FBS. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

Male nu/nu nude mice (5 weeks old; weight, 17–20 g) were purchased from the Experimental Animal Center, Peking University Health Science Center (Beijing, People's Republic of China). The animals were housed in an environmentally controlled breeding room with specific pathogen-free conditions (21°C with 50%–60% relative humidity and a 12-hour light/dark cycle). All animal studies were approved by the Institutional Animal Care and Use Committee.

In Vitro Cell Inhibition Assay. The sulforhodamine B colorimetric assay (Vichai and Kirtikara, 2006) was used to evaluate the growth-inhibitory activity of drugs at various concentrations. Exponentially growing 22Rv1 and PC3 cells were seeded in 96-well plastic plates at a density of 2×10^4 cells/well and 4×10^3 cells/well, respectively. Cells were incubated for 24 hours to allow sufficient cell adhesion and were treated with serial dilutions of Doc or Cab alone for 48 hours, respectively, in six replicated wells for each drug concentration. The IC₅₀ value represents the concentration resulting in 50% cell growth inhibition after a 48-hour exposure to the drug compared with untreated control cells, and it was calculated using GraphPad Prism versions 5.0 software (GraphPad Software, San Diego, CA). Then, the concentrations of Doc and Cab near the IC₃₀ values were used for combination treatment study considering the balance between efficacy and toxicity (Oliveras-Ferreros et al., 2008). The treatment schedules are shown in Fig. 1A.

PK Study. The mice were divided into three groups for the Doc short-term PK study, the Cab long-term PK study, and the PK study of Doc and Cab for long-term combination. 1) For the Doc short-term PK study, mice were injected via tail vein at a single dose of 20 mg/kg Doc [dissolved in a 90:5:5 (v/v/v) ratio, 0.9% sodium chloride/polysorbate 80/dehydrated alcohol], and blood samples were collected at 5, 15, and 30 minutes, and 1, 2, 4, 8, and 12 hours after administration. 2) The mice for the Cab long-term PK study were given Cab (formulated in water) at a dose of 10 mg/kg per day by oral gavage, and blood samples were collected at 5, 15, and 30 minutes, and 1, 2, 4, 8, 12, 72, 168, and 288 hours after the first dose. 3) The mice for the Doc and Cab combination PK study were administered Doc at a dose of 20 mg/kg per week and Cab at a dose of 10 mg/kg per day. The experiment lasted for 12 days, and at day 1 and day 8 Doc and Cab were administered simultaneously. Blood samples were collected at 5, 15, and 30 minutes, and 1, 2, 4, 8, 12, 72, 168, and 288 hours after the first dose. All the blood samples were centrifuged at 4000 rpm for 10 minutes, then the upper plasma was collected and stored at –80°C before analysis. The animals were euthanized after sampling.

The quantification of Doc and Cab was monitored using an API4000 QTRAP Mass Spectrometer (Applied Biosystems, Foster City, CA) equipped with an electrospray ionization source system, and controlled by Analyst TF Version 1.6 software (Applied Biosystems, Foster City, CA). Chromatographic separation was performed on a Dionex Ultimate 3000 UHPLC [ultra-high-performance liquid chromatography (LC)] System (Thermo Fisher Scientific, Waltham, MA). Doc was separated by an Ultimate C-18 column (3.0 $\times$ 50 mm i.d., 3 μ m particle size; Waters, Milford, MA) using an isocratic mobile phase (pure water containing 1 mM ammonium formate/acetonitrile = 45:55, v/v) at room temperature. The ion transitions monitored were from m/z 808.5 $\rightarrow$ 527.4, the ionspray voltage was kept at 5 kV, and the temperature was 300°C. The flow rate was 0.4 ml/min, and the overall run time was 4 minutes, with a quantitative range of 5–1500 ng/ml. The high-performance LC-tandem mass spectrometry (MS/MS) method was validated in accordance with the Guidance for Industry, Bioanalytical Method Validation, as specified by the Food and Drug

Fig. 1. In vitro cell inhibition effects of different treatment schedules on 22Rv1 and PC3 cells. (A) Cell combination treatment schedules. PBS, phosphate-buffered saline. (B) Cell inhibition effects of different treatment schedules on 22Rv1 and PC3 cells.

Administration (<https://www.fda.gov/downloads/drugs/guidanceregulatoryinformation/guidances/ucm368107.pdf>). The concentration of Cab was determined by a LC-MS/MS method previously developed by our group (Su et al., 2015). The separation of Cab was achieved on a reversed phase C18 column (50 × 2 mm, 5 μm) at ambient temperature using isocratic elution with acetonitrile/water (45:55, v/v) containing 5 mM ammonium formate buffer (finally adjusted to an apparent pH = 5.0 with formic acid). The flow rate was 0.4 ml/min. The calibration curve was linear ($r > 0.99$) in a concentration range of 0.5–1000 ng/ml.

In Vivo PD Study. 22Rv1 and PC3 tumor-bearing mice were used for the PD study. Both xenograft models were used to investigate and compare the anticancer efficacy of monotherapy, concurrent therapy, interval therapy, and sequential therapy with the two drugs. The various treatment groups are shown in Table 1, and the schematic diagram of treatment schedules is represented in Supplemental Fig. 1.

First, we established the 22Rv1-inoculated xenograft model. Briefly, 5×10^6 22Rv1 cells were suspended in a 100-μl mixed solution of RPMI 1640 free of FBS and matrigel (v/v = 1/1) and injected s.c. into the right flank of the mouse. Tumor diameter was measured by vernier

TABLE 1
Treatment schedules in vivo

Group No.	Treatment group	Treatment description	Group category
22Rv1 xenograft mice			
(1)	Control	Control	Control
(2)	Doc 5	Doc 5 mg/kg per week	Monotherapy
(3)	Doc 10	Doc 10 mg/kg per week	Monotherapy
(4)	Cab 10	Cab 10 mg/kg per day	Monotherapy
(5)	Doc 5 + Cab 10	Doc 5 mg/kg per week and Cab 10 mg/kg per day, simultaneous administration	Concurrent schedule
(6)	Doc 10 + Cab 10	Doc 10 mg/kg per week and Cab 10 mg/kg per day, simultaneous administration	Concurrent schedule
(7)	Doc 5 6h Cab 10	Doc 5 mg/kg per week and Cab 10 mg/kg per day, with 6-hour interval	Interval schedule
(8)	Doc 10 6h Cab 10	Doc 10 mg/kg per week and Cab 10 mg/kg per day, with 6-hour interval	Interval schedule
(9)	Doc 10 ~ Cab 10	Doc 10 mg/kg per week for the first week, followed by Cab 10 mg/kg per day for the second week, and alternated weekly	Sequential schedule
PC3 xenograft mice			
(1)	Control	Control	Control
(2)	Doc 5	Doc 5 mg/kg per week	Monotherapy
(3)	Cab 10	Cab 10 mg/kg per day	Monotherapy
(4)	Doc 5 + Cab 10	Doc 5 mg/kg per week and Cab 10 mg/kg per day, simultaneous administration	Concurrent schedule
(5)	Doc 5 6h Cab 10	Doc 5 mg/kg per week and Cab 10 mg/kg per day, with 6-hour interval	Interval schedule
(6)	Doc 5 – Cab 10	Doc 5 mg/kg per week for the first week, followed by Cab 10 mg/kg per day for the second week, and alternated weekly	Sequential schedule
(7)	Cab 10 ~ Doc 5	Cab 10 mg/kg per day for the first week, followed by Doc 5 mg/kg per week for the second week, and alternated weekly	Sequential schedule

All drug administration was conducted at 8:00 AM except the interval groups. For mice on a concurrent schedule, Doc and Cab were administered simultaneously (8:00 AM) on the first day of every week. For mice on an interval schedule, Cab was administered 6 hours after the Doc injection (i.e., Doc was injected at 8:00 AM and Cab was given by gavage at 2:00 PM).

Fig. 2. Schematic of the integrated PK/PD model. Vc, Vp and GI represent the central, peripheral and absorption compartments, respectively.

caliper and converted to TV using the following formula: TV (cubic millimeters) = length $\times$ width² $\times$ 0.5. When TVs reached 100–200 mm³, mice were randomly assigned to nine different treatment groups ($n = 5$ –6 in each group), as follows: 1) vehicle control; 2) Doc 5; 3) Doc 10; 4) Cab 10; 5) Doc 5 + Cab 10; 6) Doc 10 + Cab 10; 7) Doc 5 6h Cab 10; 8) Doc 10 6h Cab 10; and 9) Doc 10 ~ Cab 10. Treatments lasted for 3 weeks, and the tumor size and body weight in various groups were recorded every 2 days. The percentage of TGI (%TGI) was determined as the average change in vehicle-treated tumors (Δ Vehicle) minus the average change in test agents treated tumors (Δ Treated) divided by Δ Vehicle and expressed as a percentage (Huck et al., 2014) (eq. 1). Because %TGI is a time-dependent efficacy endpoint, it is calculated at a fixed time point. In our study, %TGI was calculated at the end of the dosing period:

$$\%TGI = \frac{(Control_{t_{day}} - Control_{0day}) - (Treated_{t_{day}} - Treated_{0day})}{Control_{t_{day}} - Control_{0day}} \times 100\% \quad (1)$$

We then established the PC3 xenograft model. Similarly, 5×10^6 PC3 cells were suspended in a 100- μ l mixed solution of RPMI 1640 free of FBS and injected s.c. into the right flank of the mouse. Mice were randomly assigned to the following seven different treatment groups ($n = 5$ –6 each group): 1) vehicle control; 2) Doc 5; 3) Cab 10; 4) Doc 5 + Cab 10; 5) Doc 5 6h Cab 10; 6) Doc 5 ~ Cab 10; and 7) Cab 10 ~ Doc 5. Treatments were conducted for 4 weeks, and the tumor size and body weight were recorded as mentioned above.

PK/PD Models. The experimental data were modeled using the first-order conditional estimation with interaction method with

NONMEM version 7.2 software (ICON Development Solutions, Ellicott City, MD). The block diagram of the entire model is shown in Fig. 2.

PK Model. The Doc concentration-time curve was estimated using a two-compartment model, whereas the PK profile of Cab was described by a one-compartment model with first-order absorption. In our study, since Doc was injected once a week whereas Cab was administered by oral gavage every day, we assumed that Doc had no effect on Cab PK during the PK/PD modeling process. At the same time, we used the log-likelihood ratio test to evaluate the effect of Cab on the PK profile of Doc. As a result, for mice taking both Doc and Cab, Doc clearance (CL) was adjusted by the influence of Cab plasma concentration (eq. 2). In this equation, CL_c and CL_s represent the Doc CL of mice taking both drugs and single Doc, respectively; $IC50_{Cab}$ represents the concentration of Cab causing half of the maximum impact on Doc CL; and C_{Cab} is the concentration of Cab in mouse plasma, as follows:

$$CL_c = CL_s \cdot \left(1 - \frac{C_{Cab}}{IC50_{Cab} + C_{Cab}}\right) \quad (2)$$

PK/PD Model. A logistic tumor growth model was used as the base model for the tumor natural growth dynamics of 22Rv1 xenografts (Yamazaki et al., 2011). The natural growth of the tumor can be described by an exponential phase with a self-limitation, eventually reaching a plateau. The growth rate of the tumor can be described by eq. 3, as follows:

$$\frac{dX(t)}{dt} = k_{ng} \cdot X(t) \cdot \left(1 - \frac{X(t)}{KP}\right), \quad X(0) = V(0) \quad (3)$$

Fig. 3. Plasma concentration-time profiles of Doc and Cab in nude mice. (A) Concentration-time profiles of Doc after a single dose (20 mg/kg, i.v.) of Doc alone or Doc in combination with Cab (10 mg/kg, p.o.) (B). Concentration-time profiles of Cab after administering Cab at a dose of 10 mg/kg per day by oral gavage alone or Cab in combination with Doc 20 mg/kg per week, i.v. (mean $\pm$ S.D., $n = 3$).

TABLE 2

Estimated PK parameters of Doc and Cab

Parameter	Definition	Estimate (RSE%)	IIV (CV%)
Doc			
CL (l/kg per hour)	CL of central compartment	3.47 (11)	
IC50 _{Cab} (μg/l)	The concentration of Cab causing half of maximum impact on Doc CL	5270 (27)	
V _c (l/kg)	Distribution volume of central compartment	0.977 (32)	
Q (l/kg per hour)	CL of distribution between central and peripheral compartment	3.21 (27)	
V _p (l/kg)	Distribution volume of peripheral compartment	4.5 (15)	
Residual error			
σ ₁	Proportional error (CV%)	49.4 (10)	
σ ₂	Additive error (S.D., μg/l)		
Cab			
k _a (h ⁻¹)	The absorption rate constant of Cab	2.83 (22)	
CL/F (l/kg per hour)	Apparent CL of Cab	0.244 (9)	
V _c /F (l/kg)	Apparent distribution volume of central compartment	1.37 (11)	
Residual error			
σ ₁	Proportional error (CV%)	34.8 (14)	
σ ₂	Additive error (S.D., μg/l)	70.57 (25)	

IIV: interindividual variability, CV: coefficient of variation.

where $X(t)$ represents the volume of proliferating tumor cells at time t , $V(0)$ represents the initial TV at the time of randomization, k_{ng} is the constant net growth rate, and KP represents the tumor-carrying capacity (the maximum sustainable TV), which is assumed to be limited by the nutrition supplied through the vasculature as well as the growth space in the tumor (Yamazaki et al., 2011).

In Doc monotherapy groups, it is assumed that chemotherapy makes some cells nonproliferating and eventually brings them to death through a mortality chain (Simeoni et al., 2004). A transit compartment model has been used to characterize the time delay between drug exposure and drug effect; the portion of proliferating cells within the total TV is denoted as X_1 , and k_1 is the constant transit rate between nonproliferating compartments (X_2, X_3, X_4). $X(t)$ is the total volume of the cells in various stages. The rate from proliferating to nonproliferating cells is in proportion with the Doc concentration in plasma (C_{Doc}), with a coefficient of k_{Doc} , which describes its antitumor potency. The differential equations are as follows:

$$\frac{dX_1}{dt} = k_{ng} \cdot X_1 \cdot \left(1 - \frac{X(t)}{KP}\right) - k_{Doc} \cdot C_{Doc} \cdot X_1, \quad X_1(0) = V(0) \quad (4)$$

$$\frac{dX_2}{dt} = k_{Doc} \cdot C_{Doc} \cdot X_1 - k_1 \cdot X_2, \quad X_2(0) = 0 \quad (5)$$

$$\frac{dX_3}{dt} = k_1 \cdot (X_2 - X_3), \quad X_3(0) = 0 \quad (6)$$

$$\frac{dX_4}{dt} = k_1 \cdot (X_3 - X_4), \quad X_4(0) = 0 \quad (7)$$

$$X(t) = X_1 + X_2 + X_3 + X_4 \quad (8)$$

In the Cab treatment groups, because Cab is a receptor tyrosine kinase inhibitor with activity against MET and VEGFR2 (Grülllich, 2014), hence blocking the nutrition supply of the tumor, we accordingly assumed that Cab exerted an inhibitory effect on KP (Ouerdani et al., 2015) instead of damaging tumor cells (eq. 9). In this equation, the concentration of Cab causing half of maximum inhibition on KP is represented by $EC50_{Cab}$, as follows:

$$\frac{dX(t)}{dt} = k_{ng} \cdot X(t) \cdot \left(1 - \frac{X(t)}{KP \cdot \left(1 - \frac{C_{Cab}}{EC50_{Cab} + C_{Cab}}\right)}\right), \quad X(t) = V(0). \quad (9)$$

In the drug combination groups, the combination index “ ϕ ” was introduced to k_{Doc} to determine the effect of the interaction of Doc

and Cab on tumor growth. A ϕ value greater or less than 1 signifies the degree of increase or decrease in the antitumor effect. Therefore, parameter ϕ can indicate synergism or antagonism of the two drugs. The equation is shown as eq. 10, as follows:

$$\frac{dX_1}{dt} = k_{ng} \cdot X_1 \cdot \left(1 - \frac{X(t)}{KP \cdot \left(1 - \frac{C_{Cab}}{EC50_{Cab} + C_{Cab}}\right)}\right) - \phi \cdot k_{Doc} \cdot C_{Doc} \cdot X_1, \quad X_1(0) = V(0). \quad (10)$$

The Gompertz model (Laird, 1964) was used for fitting the tumor natural growth of PC3 xenografts (eq. 11), in which k_{ng} is the constant net growth rate and N_{ss} represents the maximum sustainable TV. Similarly, Doc exhibited a linear-killing effect on cell growth (eq. 12) and Cab affected N_{ss} (eq. 13). For combination groups, the ϕ values of the “Doc + Cab” group, the “Doc 6h Cab” group, the “Doc ~ Cab” group, and the “Cab ~ Doc” group were represented by ϕ_1 , ϕ_2 , ϕ_3 , and ϕ_4 , respectively (eq. 14).

$$\frac{dX(t)}{dt} = k_{ng} \cdot X(t) \cdot \ln\left(\frac{N_{ss}}{X(t)}\right), \quad X(t) = V(0) \quad (11)$$

$$\frac{dX_1}{dt} = k_{ng} \cdot X_1 \cdot \ln\left(\frac{N_{ss}}{X(t)}\right) - k_{Doc} \cdot C_{Doc} \cdot X_1, \quad X_1(0) = V(0) \quad (12)$$

$$\frac{dX(t)}{dt} = k_{ng} \cdot X(t) \cdot \ln\left(\frac{N_{ss} \cdot \left(1 - \frac{C_{Cab}}{EC50_{Cab} + C_{Cab}}\right)}{X(t)}\right), \quad X(t) = V(0) \quad (13)$$

$$\frac{dX_1}{dt} = k_{ng} \cdot X_1 \cdot \ln\left(\frac{N_{ss} \cdot \left(1 - \frac{C_{Cab}}{EC50_{Cab} + C_{Cab}}\right)}{X(t)}\right) - \phi \cdot k_{Doc} \cdot C_{Doc} \cdot X_1, \quad X_1(0) = V(0) \quad (14)$$

Model selection was based on the NONMEM objective function value (OFV), parameter estimates, relative S.E. (RSE) in the estimate, and exploratory analysis of the goodness-of-fit plots. Model validations were performed by visual predictive check (VPC) of the predictions with 1000 simulations using Perl speaks NONMEM (PsN; version 3.5.3).

Data Analysis and Model Simulations. The results of the PD study in vivo were presented as the mean $\pm$ S.D. using GraphPad Prism version 5.0 software. Student's t test was used to determine the significance among the groups, and difference at a level of $P < 0.05$ was considered to be statistically significant.

Fig. 4. Antitumor efficacy of different treatment schedules on 22Rv1 tumor-bearing mice. Data were presented as the mean $\pm$ S.D. ($n = 5$ or 6), *, $p < 0.05$ and ***, $p < 0.001$. (A) TV changes over time. (B) Tumor weight at the end of the treatment. (C) Picture of excised tumors at the end of the treatment.

The tumor growth profiles of mice bearing 22Rv1 and PC3 xenografts under concurrent and Doc ~ Cab sequential treatment with different dose combinations were simulated from day 0 to day 28. The model parameters obtained from the PK/PD model were fixed, and the initial TV was set to 160 mm³. The doses of Doc used for simulation were 0, 2, 4, 6, 8, 10, and 12 mg/kg per week on 22Rv1 tumor-bearing mice and 0, 2, 4, 6, 8, and 10 mg/kg per week on PC3 tumor-bearing mice. Accordingly, the doses of Cab for simulation were 0, 2, 4, 6, 8, 10, and 12 mg/kg per day on 22Rv1 tumor-bearing mice and 0, 2.5, 5, 7.5, 10, 12.5, 15, and 20 mg/kg per day on PC3 tumor-bearing mice. The doses of Doc and Cab for simulations were selected on the grounds that the %TGI was between 15% and 85% for monotherapy in each model. Furthermore, kinetic TV data were converted to %TGI using eq. 1, and %TGI of different combination schedules on day 28 was presented in the form of a response surface.

Results

In Vitro Cell Inhibition. The cell inhibition effects of Doc and Cab were found to be dose dependent in both 22Rv1 and PC3 cells according to our study. The IC₅₀ values of Doc were 9.86 and 4.95 nM for 22Rv1 and PC3 cells, respectively, and those of Cab were 13.54 and 12.80 μ M for 22Rv1 and PC3 cells, respectively. The concentrations of Doc and Cab used for combination study were 7 nM and 7.3 μ M, respectively, for 22Rv1 cells, and 2.5 nM and 9 μ M, respectively, for PC3 cells, which were approximately their IC₃₀ values. The results are shown in Fig. 1B. Concurrent treatment and Doc ~ Cab sequential treatment were more effective in inhibiting cancer cell growth than treatment with Doc or Cab alone in both cell

lines. In contrast, Cab ~ Doc sequential treatment was less effective than Doc ~ Cab sequential treatment.

PK Study. The plasma concentration-time curves of Doc on linear and logarithmic scales after injecting a single dose of 20 mg/kg Doc or administering 20 mg/kg Doc and 10 mg/kg Cab simultaneously in nude mice are shown in Fig. 3A. The mouse plasma concentration of Cab after giving 10 mg/kg per day Cab alone or 10 mg/kg per day Cab combined with 20 mg/kg per week Doc are shown in Fig. 3B. For a single dose of Doc, combination with Cab showed a discrepancy after 2 hours, which indicates a different terminal half-life. For long-term investigation of Cab PK shown in Fig. 3B, the combination with Doc once a week had no manifest influence on the concentration-time profile of Cab, nor did Cab have any self-induction or self-inhibition effect. Therefore, in the following PK/PD modeling, we only added the influence of Cab on Doc PK (eq. 2).

The concentration-time curve of Doc was fitted by a two-compartment model with correction on CL for concurrent treatment groups and interval treatment groups, whereas that of Cab was described by a one-compartment model with first-order absorption. The parameters estimated are summarized in Table 2.

In Vivo PD Study. Figure 4 shows the tumor inhibitory effects of various schedules on mice bearing 22Rv1 xenografts and the picture of excised tumors on day 21. The average % TGI values of the Doc 5, Doc 10 and Cab 10 groups at the end of treatment were 38.26%, 72.54%, and 71.49%, respectively. Compared with monotherapy, drug combination groups showed increased inhibition of tumor growth, with %TGI

Fig. 5. Antitumor efficacy of different treatment schedules on PC3 tumor-bearing mice. Data were presented as the mean $\pm$ S.D. ($n = 5$ or 6), **, $p < 0.01$. (A) TV changes over time. (B) Tumor weight at the end of the treatment. (C) Picture of excised tumors at the end of the treatment.

values of 88.50%, 84.93%, 100.96%, and 100.59% for the Doc 5 + Cab 10, Doc 5 6h Cab 10, Doc 10 + Cab 10, and Doc 10 6h Cab 10 groups, respectively. The interval therapy did not enhance the antitumor efficacy compared with the concurrent therapy. In addition, the %TGI value of Doc10 ~ Cab 10 sequential group was 90.07%, which was higher than those of monotherapy groups. As shown in Supplemental Fig. 2, A and B, the body weights of mice were stable during treatment, and organ coefficients [organ weight/(body weight - tumor weight) $\times$ 100%] showed no abnormality among various groups, indicating the safety of all the dosing regimens.

The TGI under different dosing regimens in mice bearing PC3 xenografts is shown in Fig. 5. The inhibition ratios compared with control groups were 62.10% and 33.87% in the Doc 5 and Cab 10 monotherapy groups, respectively, as well as 88.92%, 86.72%, 74.48%, and 30.76% in the Doc 5 + Cab 10, Doc 5 6h Cab 10, Doc 5 ~ Cab 10, and Cab 10 ~ Doc 5 groups, respectively. Being consistent with the results from 22Rv1 model, the concurrent group exerted lower tumor burden than all monotherapy groups, and the antitumor efficacy in the interval group was similar to that in the concurrent group. In addition, the TGI in two sequential

Fig. 6. VPC results of PK models. The black hollow dots are the observed data. The black solid and dashed lines represent the medians of prediction and observed data, respectively. The dark gray dashed lines showed the 2.5th and 97.5th percentiles, and the light gray areas depicted the 95% predictive confidence intervals. (A) Single dose of 20 mg/kg Doc. (B) Single dose of 20 mg/kg Doc in combination with 10 mg/kg Cab. (C) Multiple doses of 10 mg/kg per day Cab.

TABLE 3

PD parameters estimated from the 22Rv1 and PC3 xenografts PK/PD model

Parameter	Definition	22Rv1 Estimate (RSE%) (IIV, CV%)	PC3 Estimate (RSE%) (IIV, CV%)
V(0) (mm ³)	Initial TV (control group) ^a	195 (7) (16.9)	145 (3) (2.7)
k_{ng} (h ⁻¹)	Tumor growth rate	0.0072 (6) (2.9)	0.0025 (9) (21.8)
KP (mm ³)	Tumor carrying capacity of 22Rv1 model	4560 (19) (4)	
Nss (mm ³)	Tumor carrying capacity of PC3 model		2090 (16) (30)
k_{Doc} (l · μg ⁻¹ · h ⁻¹)	The antitumor effect coefficient of Doc	0.000206 (16) (38.9)	0.000289 (13) (30.3)
k_1 (h ⁻¹)	Transit-rate constant (Doc monotherapy) ^b	0.0218 (34) (27)	0.0179 (16) (26.8)
EC50 _{Cab} (μg/l)	The concentration of Cab causing half of maximum inhibition on KP (Nss)	399 (14) (31.9)	2210 (23) (62.1)
ϕ_1	ϕ Value of concurrent schedule	0.98 (11) (33.1)	1.25 (10) (21.8)
ϕ_2	ϕ Value of interval schedule	1.19 (11) (34.4)	1.34 (13) (30.8)
ϕ_3	ϕ Value of Doc ~ Cab sequential schedule	2.09 (18) (39.2)	3.63 (19) (42.4)
ϕ_4^c	ϕ Value of Cab ~ Doc sequential schedule		0.54 (21) (76.9)
σ_1 (CV%/S.D.)	Residual error of control group	9.2/15.9	—/34.1
σ_2 (CV%/S.D.)	Residual error of Doc monotherapy	10.7/—	5.5/6.9
σ_3 (CV%/S.D.)	Residual error of Cab monotherapy	7.2/—	6.1/8.4
σ_4 (CV%/S.D.)	Residual error of concurrent schedule	9.4/16	3.1/10.8
σ_5 (CV%/S.D.)	Residual error of interval schedule	8.5/10.6	4.3/18.0
σ_6 (CV%/S.D.)	Residual error of Doc ~ Cab	10.9/6.4	—/21.2
σ_7 (CV%/S.D.)	Residual error of Cab ~ Doc		5.6/18.3

^aV0 estimated values (RSE%) in the 22Rv1 model were 212 (9), 230 (8), 178 (7), 196 (6), and 169 (13) for the Doc, Cab, concurrent groups, interval groups, and Doc ~ Cab sequential group, respectively, and estimated values in the PC3 model were 122 (12), 112 (12), 97.8 (4), 105 (13), 132 (7), and 140 (15) for Doc, Cab, concurrent group, interval group, Doc ~ Cab, and Cab ~ Doc sequential group, respectively.

^b k_1 estimated values (RSE%) in the 22Rv1 model were 0.0251 (7), 0.0306 (6), and 0.0183 (12) for the concurrent groups, interval groups, and Doc ~ Cab sequential group, respectively, and estimated values in the PC3 model were 0.0061 (5), 0.0272 (10), 0, and 0.0271 (21) for the concurrent group, interval group, Doc ~ Cab, and Cab ~ Doc sequential groups, respectively.

^cA subject was removed during the modeling process since its ϕ approximated to 0.

groups exhibited obvious contrary patterns. Compared with all monotherapy groups, the tumor burden was lower in the Doc 5 ~ Cab 10 sequential group but was higher in the Cab 10 ~ Doc 5 group. The mouse body weight during treatment and the organ coefficients among various groups are shown in Supplemental Fig. 2, C and D.

PK/PD Model. The estimated PK model parameters are listed in Table 2. And the VPC results and goodness-of-fit plots of PK models are presented in Fig. 6 and Supplemental Fig. 3, respectively.

TV data were fitted using the sequential-fitting method to eliminate the confounding effects in the estimation of drug efficacy parameters and ϕ . Tumor growth data of control group were fitted at the first step, and then growth parameters obtained were fixed in the subsequent fitting process. The ϕ value was estimated by fixing the inherent drug efficacy parameters obtained from the modeling of monotherapy groups. The parameters estimated are summarized in Table 3, and the VPC results stratified by group are presented in Figs. 7 and 8 for 22Rv1 and PC3 xenograft models, respectively. The ϕ values obtained from PK/PD models are compared in Fig. 9.

Obtained from the 22Rv1 PK/PD model, the ϕ values of the concurrent group and the interval group were 0.98 (0.77–1.19) (95% confidence interval) and 1.19 (0.93–1.45) respectively, and 1 was within their 95% confidence interval, indicating that Doc and Cab had no significant PD interaction under both treatment schedules. However, the ϕ value obtained from Doc ~ Cab group was 2.09 (1.35–2.83), which was higher than 1 as well as that of the concurrent and interval schedule, indicating synergy of the two drugs in Doc ~ Cab sequential treatment.

For PC3 xenograft model, the ϕ values of the concurrent group, interval group, and Doc ~ Cab and Cab ~ Doc sequential groups were 1.25 (1.00–1.50), 1.34 (1.00–1.68), 3.63 (2.28–4.98), and 0.54 (0.32–0.76), respectively. These results suggested that Doc and Cab exhibited a similar effect

under the concurrent schedule and interval schedule, since most of their 95% confidence intervals were overlapped. Moreover, the Doc ~ Cab sequential schedule showed the synergy of Doc and Cab because the ϕ value was much greater than 1, which was consistent with the results from the 22Rv1 PK/PD model. However, the Cab ~ Doc sequential group exhibited antagonism indicated by the ϕ value, which was less than 1.

Model Simulations. Simulations were performed on the concurrent schedule and the Doc ~ Cab sequential schedule in this study since the experimental data demonstrated the enhanced antitumor effect in the concurrent groups and the PK/PD model parameter ϕ confirmed the highest synergy of the two drugs in the Doc ~ Cab sequential groups. In addition, the concurrent schedule is more convenient and acceptable than the interval schedule in medical practice, although there was no significant difference in the antitumor effect between them.

By fixing the parameters obtained from the 22Rv1 PK/PD model, the simulated response surfaces of %TGI on day 28 under different dose combinations are shown in Fig 10, A and B. For the Doc + Cab concurrent schedule, Doc combined with Cab showed enhanced tumor inhibition compared with Doc or Cab monotherapy. It should be noticed that under the concurrent treatment, a low dose of Cab could increase the total anticancer effect dramatically, which can be seen from the steepness of the surface along the y-axis. For example, the %TGI under treatment with 6 mg/kg per week Doc was 47.3%, whereas the %TGI of “Doc 6 + Cab 2” rose to 68.6%. However, for the Doc ~ Cab sequential schedule, a low dose of Doc (2 mg/kg per week) combined with Cab showed no benefit compared with Cab monotherapy, but when the dose of Doc was higher than 4 mg/kg per week, sequential treatment was obviously superior to monotherapy with either Doc or Cab.

Simulated %TGI values based on the PC3 PK/PD model on day 28 are shown in Fig. 10, C and D. Both the concurrent and

Fig. 7. Observed and model-simulated TV-time profiles of 22Rv1 tumor-bearing mice stratified by group. The black hollow dots are the observed data. The black solid lines represent the medians of prediction value, and the light gray areas depicted the 95% predictive confidence intervals. The black dashed lines showed the 2.5th, 50th, and 97.5th percentiles, respectively.

sequential treatment exhibited an obvious advantage over monotherapy. For both the concurrent and Doc ~ Cab sequential schedule, Doc combined with a low dose of Cab could increase the total anticancer effect dramatically. Subsequently, the %TGI value increased modestly with the increasing dose of Cab, given the same dose of Doc. For instance, the %TGI was 59.3% for treatment with a 6 mg/kg per week dose of Doc and 14.0% for monotherapy with a 2.5 mg/kg per day dose of Cab, whereas the %TGI of “Doc 6 + Cab 2.5” and “Doc 6 ~ Cab 2.5” were 80.0% and 79.5%, respectively. However, when the dose of Cab increased to 5 mg/kg per day, the %TGI rose only to 87.3% and 80.9% for Doc 6 + Cab 5 and Doc 6 ~ Cab 5, respectively.

Discussion

In this study on male nude mice bearing 22Rv1 and PC3 xenografts, a semimechanistic PK/PD model with PK interaction, which well characterized the quantitative relationship between plasma concentration and tumor progression in various therapeutic regimens of Doc combined with Cab, was

developed for the first time. Simulations-based evaluation showed that the model performed well in all treatment schedules.

For Doc, a cytotoxic agent, the responses to treatment were consistent in vitro and in vivo in both xenograft models, which can be seen from the IC_{50} values (9.86 and 4.95 nM for 22Rv1 and PC3 cells, respectively) in vitro and the antitumor effect coefficient k_{Doc} in two xenograft models (0.000206 for 22Rv1 and 0.000289 for PC3 xenografts). The IC_{50} values of Cab in the two cell lines were approximate (13.54 and 12.80 μ M for 22Rv1 and PC3 cells, respectively), suggesting that its cytotoxic effect on both cells is similar and weak. However, a more than 5-fold difference in the EC_{50} value estimated from the PK/PD model in two xenograft models (399 μ g/l for PC3 cells vs. 2210 μ g/l for 22Rv1 cells) was noticed. As a multi-targeted tyrosine kinase inhibitor, Cab exhibits strong inhibition of phosphorylation of the tyrosine kinase receptor and corresponding downstream signaling pathways. The difference in EC_{50} values means Cab exhibits stronger inhibition of the phosphorylation of the tyrosine kinase receptor in the 22Rv1 xenograft model than that in the PC3 xenograft model.

Fig. 8. Observed and model-simulated TV-time profiles of PC3 tumor-bearing mice stratified by group. The black hollow dots are the observed data. The black solid lines represent the medians of prediction value, and the light gray areas depicted the 95% predictive confidence intervals. The black dashed lines showed the 2.5th, 50th, and 97.5th percentiles, respectively.

This may be due to different origins and cell type-specific characteristics (Wu et al., 2013) as well as the different tumor microenvironments in the two xenograft models. In addition, recent observation indicated that the PC3 cell line is more characteristic of prostatic small cell neuroendocrine carcinoma rather than adenocarcinoma (Tai et al., 2011), so the results of the PC3 PK/PD model may have more reference value in small cell carcinoma patients.

The Cab ~ Doc sequential schedule was less effective than the Doc ~ Cab sequential schedule both in vitro and in vivo, which may be explained by the different effects of the two drugs on cell cycle arrest (Pan et al., 2011). As an antimicrotubule agent, Doc stabilizes microtubules during cell division and causes cell cycle arrest in the G2/M phase (Bissery, 1995). However, Cab has been reported to affect the G0/G1 phase of cell cycle (Lu et al., 2016). When Cab was given first, cells were arrested in the G0/G1 phase, leading to reduction in the proportion of cells in the G2/M phase, and thus may affect the effect of Doc subsequently. Therefore, the Cab ~ Doc sequential regimen was less effective. Similar findings of

schedule-dependent interactions have been reported by several teams (Ricotti et al., 2003; Pan et al., 2011; Tamatani et al., 2012; Wang et al., 2012; Jiang et al., 2014).

Our in vivo experimental results showed that the antitumor effect of Doc + Cab concurrent therapy was better than that of

Fig. 9. The ϕ value of each combination schedule obtained from PK/PD models (mean with 95% confidence interval).

Fig. 10. Surface response plots relating Doc and Cab doses to %TGI values generated from simulation based on PK/PD models on day 28. Effect of a concurrent schedule for Doc + Cab administration on the 22Rv1 xenograft model (A), of a sequential schedule for Doc ~ Cab administration on the 22Rv1 xenograft model (B), of a concurrent schedule for Doc + Cab administration on the PC3 xenograft model (C), and of a sequential schedule for Doc ~ Cab administration on the PC3 xenograft model (D).

Doc ~ Cab sequential therapy, although the values of ϕ in the latter treatment groups were much higher than that in the former groups (2.09 vs. 0.98, and 3.63 vs. 1.25 in 22Rv1 and PC3 xenograft models, respectively). This is because the total doses of the two schedules were different. For a duration of 2 weeks, the mice in the Doc 5 ~ Cab 10 group were administered 5 mg/kg Doc and 10×7 mg/kg Cab, whereas the mice in the Doc 5 + Cab 10 group were administered 5×2 mg/kg Doc and 10×14 mg/kg Cab. The total dose in the concurrent group was twice that in the sequential group in 2 weeks, leading to smaller tumor sizes in the Doc 5 + Cab 10 group. Because a higher ϕ value means greater synergy of PD interactions, the ϕ value estimated from the concurrent schedule showed no significant PD interaction of the two drugs since the value of ϕ was close to 1, whereas that of the Doc ~ Cab sequential therapy showed the synergy of the two drugs. These results suggest that Doc ~ Cab sequential treatment could be a promising therapeutic strategy for prostate cancer treatment considering long-term drug resistance or toxicity.

Doc and Cab are both substrates of CYP3A4 enzyme (Engels et al., 2005; Nguyen et al., 2015); therefore, it is necessary to investigate the PK interaction between them when they are

used in combination. Based on the data from our PK studies, for mice administered Doc and Cab simultaneously, the area under the Doc concentration-time curve for 0–12 hours increased by 30% compared with that when Doc was administered alone, suggesting the likelihood of a PK interaction between Doc and Cab. We further investigated this possible PK interaction using a population approach. We considered monotherapy versus combination treatment as a binary covariate (E_{com} , 0 for monotherapy, otherwise to be estimated for combination treatment) and used the log-likelihood ratio test to evaluate the significance of E_{com} . The inclusion of E_{com} on Doc CL caused a significant decrease of OFV (-22.4 ; $P < 0.001$). Similarly, we investigated the effect of Doc on Cab PK, and the results showed that the inclusion of E_{com} on the CL or V of Cab did not significantly decrease OFV. These results were in agreement with our speculation. Because Cab was administered every day whereas Doc was given once a week, the effect of Doc on Cab PK could be neglected during the modeling process. Thus, we added the PK interaction only for Doc in the PK/PD model and finally used eq. 2 to describe the effect of Cab on Doc CL. We have compared the parameters estimated with or without PK interaction (Supplemental Tables. S1 and S2). The

values of ϕ estimated from the model without PK interaction were higher than those with PK interaction in the concurrent and interval schedules, indicating that part of the PD interaction of the two drugs was interpreted by PK interaction, especially in the concurrent schedule.

Since Cab is an angiogenesis inhibitor rather than a cytotoxic agent, our model took into account the role of tumor vasculature in tumor growth and shrinkage. In the modeling process, the model with Cab having both cytotoxic and antiangiogenic effects has been tested. We found that the plasma concentration of Cab had almost no impact on cytotoxicity (k_{Cab} was initially estimated at 0.0000001), whereas the impact on angiogenesis was significant (EC_{50Cab} , estimated at 399 and 2210 $\mu\text{g/l}$ for the 22Rv1 and PC3 models, respectively). Therefore, in our model structure, the effect of Cab was assumed on the KP (or Nss) only, although both the cytotoxicity and antiangiogenesis of Cab had been reported by Ouerdani et al. (2015). It may be due to the relatively low dose of Cab in our study (Yakes et al., 2011; Bentzien et al., 2013), which cannot cause a significant cell-killing effect directly.

To determine the dosing regimens of both drugs, we referenced those in clinic treatment. The standard dose of Doc for patients with CRPC is 75 mg/m^2 per 3 weeks, and the equivalent dose in mice is about 18 mg/kg per 3 weeks according to body surface area, which means 6 mg/kg per week in mice equivalently. The simulated %TGI for both 22Rv1 and PC3 tumor-bearing mice on day 28 under the treatment of 6 mg/kg per week Doc could be augmented notably when combined with a low dose of Cab (2 or 2.5 mg/kg per day, equivalent to 15–20 mg/d in humans), especially in the PC3 xenograft model. It can be seen from Fig. 10, C and D that the %TGI was 59.3% for Doc 6 mg/kg per week monotherapy, whereas the %TGI values of Doc 6 + Cab 2.5 and Doc 6 ~ Cab 2.5 were 80% and 79.5%, respectively. Notably, it has been reported that, compared with the 140 mg daily dose of Cab used in thyroid cancer, lower doses of 60 and 40 mg daily in prostate cancer phase II studies demonstrated lower toxicity without compromising efficacy (Lee et al., 2013; Vaishampayan, 2014). Therefore, a small dose of Cab may enhance efficacy when combined with Doc in the treatment of CRPC.

In summary, the proposed PK/PD model quantitatively described the relationship between the plasma concentration and the anticancer efficacy of Doc and Cab in different schedules for the treatment of CRPC in mice. However, the developed model was not able to make simulations apart from the experimental schedules since the parameter ϕ was empirical and varied among schedules. The current model needs to be further optimized after we obtain more mechanistic data in the future. In our study, concurrent treatment with Doc and Cab showed better tumor inhibitory efficacy than monotherapy, the Doc ~ Cab sequential schedule was superior to monotherapy, whereas the Cab ~ Doc sequential schedule was less effective. PK/PD modeling results indicated that the two drugs exhibited optimal synergy under the Doc ~ Cab sequential schedule. The developed PK/PD model may provide reference for the rational use of chemotherapeutic drugs in combination with antiangiogenic agents.

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Authorship Contributions

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References

- Basch EM, Scholz MC, De Bono JS, Vogelzang NJ, De Souza PL, Marx GM, Vaishampayan UN, George S, Schwarz JK, and Antonarakis ES (2015) Final analysis of COMET-2: cabozantinib (Cabo) versus mitoxantrone/prednisone (MP) in metastatic castration-resistant prostate cancer (mCRPC) patients (pts) with moderate to severe pain who were previously treated with docetaxel (D) and abiraterone (A) and/or enzalutamide (E). *J Clin Oncol* **33** (7 Suppl):141.
- Bentzien F, Zuzow M, Heald N, Gibson A, Shi Y, Goon L, Yu P, Engst S, Zhang W, Huang D, et al. (2013) In vitro and in vivo activity of cabozantinib (XL184), an inhibitor of RET, MET, and VEGFR2, in a model of medullary thyroid cancer. *Thyroid* **23**(12):1569–1577.
- Bissery MC (1995) Preclinical pharmacology of docetaxel. *Eur J Cancer* **31A** (Suppl 4):S1–S6.
- Buzdar AU and Hortobagyi GN (2003) Sequential paclitaxel reduces recurrence and mortality in women with early breast cancer. *Cancer Treat Rev* **29**:449–452.
- Earp J, Krzyzanski W, Chakraborty A, Zamacona MK, and Jusko WJ (2004) Assessment of drug interactions relevant to pharmacodynamic indirect response models. *J Pharmacokinetic Pharmacodyn* **31**:345–380.
- Eigenmann MJ, Frances N, Hoffmann G, Lavé T, and Walz AC (2016) Combining nonclinical experiments with translational PKPD modeling to differentiate erlotinib and gefitinib. *Mol Cancer Ther* **15**:3110–3119.
- Engels FK, Sparreboom A, Mathot RA, and Verweij J (2005) Potential for improvement of docetaxel-based chemotherapy: a pharmacological review. *Br J Cancer* **93**:173–177.
- Fornier MN, Seidman AD, Theodoulou M, Moynahan ME, Currie V, Moasser M, Sklarin N, Gilewski T, D'Andrea G, Salvaggio R, et al. (2001) Doxorubicin followed by sequential paclitaxel and cyclophosphamide versus concurrent paclitaxel and cyclophosphamide: 5-year results of a phase II randomized trial of adjuvant dose-dense chemotherapy for women with node-positive breast carcinoma. *Clin Cancer Res* **7**:3934–3941.
- Gatzemeier U, Pluzanska A, Szczesna A, Kaukel E, Roubec J, De Rosa F, Milanowski J, Karnicka-Mlodkowski H, Pesek M, Serwatowski P, et al. (2007) Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non-small-cell lung cancer: the Tarceva Lung Cancer Investigation Trial. *J Clin Oncol* **25**: 1545–1552.
- Grülich C (2014) Cabozantinib: a MET, RET, and VEGFR2 tyrosine kinase inhibitor. *Small Molecules in Oncology*, pp 207–214. Springer, Berlin, Heidelberg.
- Herbst RS, Giaccone G, Schiller JH, Natale RB, Miller V, Manegold C, Scagliotti G, Rosell R, Oliff I, Reeves JA, et al. (2004) Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 2. *J Clin Oncol* **22**:785–794.
- Herbst RS, Prager D, Hermann R, Fehrenbacher L, Johnson BE, Sandler A, Kris MG, Tran HT, Klein P, Li X, et al.; TRIBUTE Investigator Group (2005) TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol* **23**: 5892–5899.
- Huck JJ, Zhang M, Mettetal J, Chakravarty A, Venkatakrishnan K, Zhou X, Kleinfeld R, Hyer ML, Kannan K, Shinde V, et al. (2014) Translational exposure-efficacy modeling to optimize the dose and schedule of taxanes combined with the investigational Aurora A kinase inhibitor MLN8237 (alisertib). *Mol Cancer Ther* **13**:2170–2183.
- Jiang Y, Yuan Q, and Fang Q (2014) Schedule-dependent synergistic interaction between docetaxel and gefitinib in NSCLC cell lines regardless of the mutation status of EGFR and KRAS and its molecular mechanisms. *J Cancer Res Clin Oncol* **140**:1087–1095.
- Koch G, Walz A, Lahu G, and Schropp J (2009) Modeling of tumor growth and anticancer effects of combination therapy. *J Pharmacokinetic Pharmacodyn* **36**:179–197.
- Laird AK (1964) Dynamics of tumour growth. *Br J Cancer* **13**:490–502.
- Lee RJ, Saylor PJ, Michaelson MD, Rothenberg SM, Smas ME, Miyamoto DT, Gurski CA, Xie W, Maheswaran S, Haber DA, et al. (2013) A dose-ranging study of cabozantinib in men with castration-resistant prostate cancer and bone metastases. *Clin Cancer Res* **19**(11):3088–3094.
- Li M, Li H, Cheng X, Wang X, Li L, Zhou T, and Lu W (2013) Preclinical pharmacokinetic/pharmacodynamic models to predict schedule-dependent interaction between erlotinib and gemcitabine. *Pharm Res* **30**:1400–1408.
- Lu JW, Wang AN, Liao HA, Chen CY, Hou HA, Hu CY, Tien HF, Ou DL, and Lin LI (2016) Cabozantinib is selectively cytotoxic in acute myeloid leukemia cells with FLT3-internal tandem duplication (FLT3-ITD). *Cancer Lett* **376**:218–225.
- Manegold C, Pilz LR, Koschel G, Romer KS, Mezger J, Hruska D, Dornof W, Gosse H, and Gatzemeier U (2005) Randomized multicenter phase II study of gemcitabine versus docetaxel as first-line therapy with second-line crossover in advanced-stage non-small-cell lung cancer. *Clin Lung Cancer* **7**:208–214.
- Manolis E, Rohou S, Hemmings R, Salmonson T, Karlsson M, and Milligan PA (2013) The role of modeling and simulation in development and registration of medicinal products: output from the EFPIA/EMA modeling and simulation workshop. *CPT Pharmacometrics Syst Pharmacol* **2**:e31.
- McKeage K (2012) Docetaxel: a review of its use for the first-line treatment of advanced castration-resistant prostate cancer. *Drugs* **72**:1559–1577.
- Milligan PA, Brown MJ, Marchant B, Martin SW, van der Graaf PH, Benson N, Nucci G, Nichols DJ, Boyd RA, Mandema JW, et al. (2013) Model-based drug development: a rational approach to efficiently accelerate drug development. *Clin Pharmacol Ther* **93**:502–514.

- Modena A, Massari F, Ciccarese C, Brunelli M, Santoni M, Montironi R, Martignoni G, and Tortora G (2016) Targeting met and VEGFR axis in metastatic castration-resistant prostate cancer: "game over"? *Target Oncol* **11**:431–446.
- Moebus V, Jackisch C, Lueck HJ, du Bois A, Thomssen C, Kurbacher C, Kuhn W, Nitz U, Schneeweiss A, Huober J, et al. (2010) Intense dose-dense sequential chemotherapy with epirubicin, paclitaxel, and cyclophosphamide compared with conventionally scheduled chemotherapy in high-risk primary breast cancer: mature results of an AGO phase III study. *J Clin Oncol* **28**:2874–2880.
- Mollard S, Ciccolini J, Imbs DC, El Cheikh R, Barbolosi D, and Benzekry S (2017) Model driven optimization of antiangiogenics + cytotoxics combination: application to breast cancer mice treated with bevacizumab + paclitaxel doublet leads to reduced tumor growth and fewer metastasis. *Oncotarget* **8**:23087–23098.
- Nguyen L, Holland J, Miles D, Engel C, Benrimoh N, O'Reilly T, and Lacy S (2015) Pharmacokinetic (PK) drug interaction studies of cabozantinib: effect of CYP3A inducer rifampin and inhibitor ketoconazole on cabozantinib plasma PK and effect of cabozantinib on CYP2C8 probe substrate rosiglitazone plasma PK. *J Clin Pharmacol* **55**:1012–1023.
- Oliveras-Ferraros C, Vazquez-Martin A, Colomer R, De Llorens R, Brunet J, and Menendez JA (2008) Sequence-dependent synergism and antagonism between paclitaxel and gemcitabine in breast cancer cells: the importance of scheduling. *Int J Oncol* **32**:113–120.
- Ouerdani A, Struemper H, Suttle AB, Ouellet D, and Ribba B (2015) Preclinical modeling of tumor growth and angiogenesis inhibition to describe pazopanib clinical effects in renal cell carcinoma. *CPT Pharmacometrics Syst Pharmacol* **4**:660–668.
- Pan F, Tian J, Zhang X, Zhang Y, and Pan Y (2011) Synergistic interaction between sunitinib and docetaxel is sequence dependent in human non-small lung cancer with EGFR TKIs-resistant mutation. *J Cancer Res Clin Oncol* **137**:1397–1408.
- Ricotti L, Tesei A, De Paola F, Ulivi P, Frassinetti GL, Milandri C, Amadori D, and Zoli W (2003) In vitro schedule-dependent interaction between docetaxel and gemcitabine in human gastric cancer cell lines. *Clin Cancer Res* **9**(2):900–905.
- Rocchetti M, Simeoni M, Pesenti E, De Nicolao G, and Poggesi I (2007) Predicting the active doses in humans from animal studies: a novel approach in oncology. *Eur J Cancer* **43**:1862–1868.
- Signorelli M, Lissoni AA, De Ponti E, Grassi T, Ponti S, and Fruscio R (2015) Adjuvant sequential chemo and radiotherapy improves the oncological outcome in high risk endometrial cancer. *J Gynecol Oncol* **26**:284–292.
- Simeoni M, Magni P, Cammia C, De Nicolao G, Croci V, Pesenti E, Germani M, Poggesi I, and Rocchetti M (2004) Predictive pharmacokinetic-pharmacodynamic modeling of tumor growth kinetics in xenograft models after administration of anticancer agents. *Cancer Res* **64**:1094–1101.
- Simeoni M, De Nicolao G, Magni P, Rocchetti M, and Poggesi I (2013) Modeling of human tumor xenografts and dose rationale in oncology. *Drug Discov Today Technol* **10**:e365–e372.
- Smith MR, De Bono JS, Sternberg CN, Le Moulec S, Oudard S, De Giorgi U, Krainer M, Bergman AM, Hoelzer W, and De Wit R (2015) Final analysis of COMET-1: cabozantinib (Cabo) versus prednisone (Pred) in metastatic castration-resistant prostate cancer (mCRPC) patients (pts) previously treated with docetaxel (D) and abiraterone (A) and/or enzalutamide (E). *J Clin Oncol* **33** (7 Suppl):139.
- Su Q, Li J, Ji X, Li J, Zhou T, Lu W, and Li L (2015) An LC-MS/MS method for the quantitation of cabozantinib in rat plasma: application to a pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* **985**:119–123.
- Tai S, Sun Y, Squires JM, Zhang H, Oh WK, Liang CZ, and Huang J (2011) PC3 is a cell line characteristic of prostatic small cell carcinoma. *Prostate* **71**:1668–1679.
- Tamatani T, Ferdous T, Takamaru N, Hara K, Kinouchi M, Kuribayashi N, Ohe G, Uchida D, Nagai H, Fujisawa K, et al. (2012) Antitumor efficacy of sequential treatment with docetaxel and 5-fluorouracil against human oral cancer cells. *Int J Oncol* **41**:1148–1156.
- Torre LA, Siegel RL, Ward EM, and Jemal A (2016) Global cancer incidence and mortality rates and trends—an update. *Cancer Epidemiol Biomarkers Prev* **25**: 16–27.
- Vaishampayan UN (2014) Development of cabozantinib for the treatment of prostate cancer. *Core Evid* **9**:61–67.
- Vichai V and Kirtikara K (2006) Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat Protoc* **1**:1112–1116.
- Wang D, Jiang Z, and Zhang L (2012) Concurrent and sequential administration of sunitinib malate and docetaxel in human non-small cell lung cancer cells and xenografts. *Med Oncol* **29**:600–606.
- Wang Z, Zhao Z, Wu T, Song L, and Zhang Y (2015) Sorafenib-irinotecan sequential therapy augmented the anti-tumor efficacy of monotherapy in hepatocellular carcinoma cells HepG2. *Neoplasia* **62**:172–179.
- Wu X, Gong S, Roy-Burman P, Lee P, and Culig Z (2013) Current mouse and cell models in prostate cancer research. *Endocr Relat Cancer* **20**:R155–R170.
- Yakes FM, Chen J, Tan J, Yamaguchi K, Shi Y, Yu P, Qian F, Chu F, Bentzien F, Cancilla B, et al. (2011) Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. *Mol Cancer Ther* **10**:2298–2308.
- Yamazaki S, Nguyen L, Vekich S, Shen Z, Yin MJ, Mehta PP, Kung PP, and Vicini P (2011) Pharmacokinetic-pharmacodynamic modeling of biomarker response and tumor growth inhibition to an orally available heat shock protein 90 inhibitor in a human tumor xenograft mouse model. *J Pharmacol Exp Ther* **338**:964–973.

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