Research Article

Pharmacokinetics and Tissue Disposition of Lenalidomide in Mice

Darlene M. Rozewski,^{1,2} Sarah E. M. Herman,² William H. Towns II,² Emilia Mahoney,²
Matthew R. Stefanovski,² Jungook D. Shin,² Xiaoxia Yang,¹ Yue Gao,¹ Xiaobai Li,³ David Jarjoura,³
John C. Byrd,^{2,4} Amy J. Johnson,^{2,4,5} and Mitch A. Phelps^{1,5}

Received 30 December 2011; accepted 8 August 2012; published online 7 September 2012

Abstract. Lenalidomide is a synthetic derivative of thalidomide exhibiting multiple immunomodulatory activities beneficial in the treatment of several hematological malignancies. Murine pharmacokinetic characterization necessary for translational and further preclinical investigations has not been published. Studies herein define mouse plasma pharmacokinetics and tissue distribution after intravenous (IV) bolus administration and bioavailability after oral and intraperitoneal delivery. Range finding studies used lenalidomide concentrations up to 15 mg/kg IV, 22.5 mg/kg intraperitoneal injections (IP), and 45 mg/kg oral gavage (PO). Pharmacokinetic studies evaluated doses of 0.5, 1.5, 5, and 10 mg/kg IV and 0.5 and 10 mg/kg doses for IP and oral routes. Liquid chromatography–tandem mass spectrometry was used to quantify lenalidomide in plasma, brain, lung, liver, heart, kidney, spleen, and muscle. Pharmacokinetic parameters were estimated using noncompartmental and compartmental methods. Doses of 15 mg/kg IV, 22.5 mg/kg IP, and 45 mg/kg PO lenalidomide caused no observable toxicity up to 24 h postdose. We observed dose-dependent kinetics over the evaluated dosing range. Administration of 0.5 and 10 mg/kg resulted in systemic bioavailability ranges of 90–105% and 60–75% *via* IP and oral routes, respectively. Lenalidomide was detectable in the brain only after IV dosing of 5 and 10 mg/kg. Dose-dependent distribution was also observed in some tissues. High oral bioavailability of lenalidomide in mice is consistent with oral bioavailability in humans. Atypical lenalidomide tissue distribution was observed in spleen and brain. The observed dose-dependent pharmacokinetics should be taken into consideration in translational and preclinical mouse studies.

KEY WORDS: bioavailability; distribution; lenalidomide; mouse; pharmacokinetics.

INTRODUCTION

Lenalidomide is a synthetic immunomodulatory drug (IMiD) analog of the anti-angiogenic drug, thalidomide. Although initially selected as a lead compound for potent inhibition of tumor necrosis factor- α (1), lenalidomide exhibits further activities including anti-angiogenic properties (2,3), increased erythropoiesis (4), direct induction of tumor apoptosis (5), modulation of pro-survival and inflammatory cytokines (6), an increase in immune effector cell response (5,7,8), and synergistic effects in combination therapies (9,10). With this extensive activity profile, exploration of drug efficacy across multiple diseases has been underway since the drug's clinical release in the early 2000s (11). In the last

decade, lenalidomide has met Food and Drug Administration approval for multiple myeloma (MM) and myelodysplastic syndrome (MDS). It also demonstrates clinical activity in systemic amyloidosis (12,13), chronic lymphocytic leukemia (CLL) (14–16), mantle cell lymphoma (17), myelofibrosis with myeloid metaplasia (18), and non-Hodgkin's lymphoma (19,20) and continues to be investigated for treatment of other malignancies and hematological conditions. This widespread anticancer response is believed to result from a unique combination of the drug's many activities. However, the mechanism responsible for induction of these events remains unclear.

Lenalidomide is orally administered with rapid absorption and bioavailability greater than 80% in healthy individuals (21). It is a small molecule (molecular weight=259) with moderate solubility at physiological pH where it is not ionized. In acidic conditions, such as in the stomach and proximal intestine, lenalidomide becomes protonated and its solubility increases greater than tenfold (22). While passive diffusion likely plays a role in its absorption, the high bioavailability and absorption of protonated lenalidomide may also be facilitated by transporter-mediated pathways. Approximately 90% of lenalidomide clearance occurs primarily by renal excretion, and a portion of this is believed to be transporter mediated (21,23). Renal reabsorption has not

¹Division of Pharmaceutics, College of Pharmacy, 230 Parks Hall, 500W. 12th Avenue, Columbus, Ohio 43210, USA.

²Division of Hematology, Department of Internal Medicine, 455C OSU CCC Bldg. 410W. 12th Avenue, Columbus, Ohio 43210, USA.

³Center for Biostatistics, The Ohio State University, Columbus, Ohio, USA.

⁴Division of Medicinal Chemistry, College of Pharmacy, The Ohio State University, Columbus, Ohio, USA.

⁵To whom correspondence should be addressed. (e-mail: phelps.32@osu.edu; e-mail: amy.johnson@osumc.edu)

been directly studied for lenalidomide, and it is therefore unknown if this contributes to its overall disposition. Plasma protein binding in patients is between 35% and 45%, does not vary significantly in patients with renal impairment, and is constant over physiological concentrations (24). Hydroxylated and acetylated metabolites (21) are formed at a low abundance less than 5% of dose (21). Hydrolysis products of lenalidomide have also been observed (21,25); however, to our knowledge, their structures and activities have not been reported. The contributing effects of these properties on lenalidomide linearity and differential tissue distribution need to be further characterized.

Lenalidomide therapy is approved for daily oral administration. In adults, lenalidomide doses are not adjusted to body weight or body surface area, and there is no accumulation of drug after multiple, daily doses (11,22). In healthy subjects, lenalidomide has a 3–4 h half-life of elimination and typical maximum plasma concentration occurring 0.6–1.5 h postdose (24). Data are commonly described by a one-compartment model with mono-exponential decline of plasma concentrations (24,26). Pharmacokinetic analyses of orally administered lenalidomide mostly conclude plasma exposures are linear with increasing doses. One exception to this was a recent report of greater than proportional maximum observed plasma concentrations ($C_{\max}$) and concentration–time curve (AUC) in a phase 1 trial of lenalidomide dosed from 5 mg up to 40 mg daily in patients with refractory metastatic cancers (27). The authors evaluated a Michaelis–Menten function to describe this perceived nonlinearity, but ultimately utilized a dose-proportional model which fit the data equally well. In addition to humans (21,23,24,28), pharmacokinetic studies of lenalidomide have been reported in rats and monkeys (3,29).

Disease-specific toxicity profiles and potentially life-threatening adverse events associated with lenalidomide treatment are responsible for considerable diversity in starting doses. In the majority of indications, neutropenia and thrombocytopenia are the most frequent dose-limiting toxicities (11,30,31). However, increased severity of these adverse events limits the dose to 10 mg in MDS compared to the standard 25 mg dose in MM (11). In contrast to lower-grade myelosuppression, patients with MM are at greater risk for thromboembolytic events (32,33) as compared to MDS patients. The conventional 25 mg starting dose in clinical trials is also poorly tolerated in other hematological malignancies such as systemic amyloidosis (12) and CLL (34). Tumor flare reaction appears to be exclusive to CLL and remains poorly understood mechanistically. Typically associated with a cytokine release syndrome, the extreme severity of lenalidomide's response in CLL is accountable for the significant limitations in starting dose for current clinical trials. In contrast, the maximum tolerable dose in patients with relapsed acute leukemias was found to be 50 mg (35) and in pediatric patients with primary tumors of the central nervous system (CNS) or with refractory solid tumors or MDS, maximum tolerated dose was not reached at normalized doses up to 70 or 116 mg/m² (36). The variable and unknown mechanism of action and ill-defined sources of adverse effects in these patient populations reflect an unmet need for further translational research. Use of mouse models to evaluate lenalidomide efficacy and mechanisms underlying

its activity and toxicity is highlighted in the recent literature (9,37–42). As the plasma concentration achieved in mice after lenalidomide dosing is unreported, it is unclear if the doses used in these previous studies produce systemic concentrations that are relevant to those observed in the clinic. In support of ongoing preclinical and translational evaluation of lenalidomide, we report a comprehensive pharmacokinetic characterization of lenalidomide in plasma and tissues of mice.

MATERIALS AND METHODS

Chemicals and Solvents

Stock lenalidomide powder was purified from commercial capsules in a method previously reported (34) and stored at room temperature with minimum light exposure. Dosing solutions were prepared by adding lenalidomide to the appropriate volume of sterile phosphate-buffered saline (PBS) containing 1% hydrochloric acid (HCl). Following complete drug dissolution, the pH of this preparation was adjusted to 7.0–7.6 using sodium hydroxide and sterile filtered using a 0.22 μ m Steriflip filter (Millipore, Billerica, MA, USA) prior to dosing. Genistein (>98% HPLC grade) was obtained from Sigma (St. Louis, MO, USA). All other materials were purchased as analytical or cell culture grade from commercial sources.

Mice

Imprinting control region (ICR) mice 8–10 weeks of age were obtained from Harlan Laboratories (Indianapolis, IN, USA) and acclimated to the animal care facilities for a minimum of 48 h before study initiation. To minimize exogenous exposure, mice were housed in micro-insulator cages. Room temperature was regulated between 70°F and 72°F, and automatic 12-h light/dark cycles were maintained. Mice received food and water *ad libitum*, with the exception of orally administered mice which had food removed on the evening prior to dosing and withheld until 3 h post dosing. All animal care and experiments were approved and performed in compliance with the Institutional Animal Care and Use Committee guidelines.

Dosing

Mice were administered sterile preparations of lenalidomide normalized to body weight. Intravenously (IV) dosed animals received drug by bolus tail vein injections, and extravascularly dosed mice received drug by bolus intraperitoneal injections (IP) or oral gavage (PO). Dosing solution, concentrations were adjusted so dose volumes ranged between approximately 100 and 150 μ L for IV injections and between approximately 150 and 250 μ L for IP and PO dosing in the pharmacokinetic study. However, for the range-finding study, increased dose volumes were used (up to 200 μ L IV, 300 μ L IP, and 600 μ L PO, per approved animal use protocol) to explore elevated lenalidomide doses. The bolus injection rates for all IV, IP, or PO injections were less than 5 s. Concentrations of dosing solutions were verified by liquid chromatography–mass spectrometry.

Range Finding Study

Lenalidomide was incompletely soluble at 3.5 mg/mL and above in PBS containing 1% HCl, as visible particulates remained after thorough mixing. We therefore selected 3 mg/mL as the maximum dosing solution concentration (with no visible particulates). Single, individual mice were initially dosed with 3, 10, or 15 mg/kg IV; 4.5, 15, or 22.5 mg/kg IP; and 9, 30, or 45 mg/kg PO. Additional mice ($n=4$) were then evaluated at the maximum dose achievable by volume and solubility of lenalidomide in the dosing solution. All mice were monitored closely for 1 h and re-evaluated for toxicities 3, 6, and 24 h postdose.

Pharmacokinetic Studies, Sampling, and Tissue Preparation

Mice were euthanized by carbon dioxide asphyxiation followed by exsanguination at time points 2 min to 8 h postdose ($n=4$ or 5 mice per time point). Blood was collected by cardiac puncture into lithium heparinized plasma separator tubes (BD Microtainer, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and immediately centrifuged at room temperature for 1.5 min at 4,200 RCF. Plasma was immediately removed and placed on dry ice prior to storage at -80°C until analysis. Liver, lung, heart, spleen, kidney, hind limb muscle, and brain were individually wrapped in foil and flash frozen in liquid nitrogen. Solid tissues were individually pulverized *via* mortar and pestle on dry ice, weighed in aliquots, and stored at -80°C until processing. Short-term stability of lenalidomide was not determined in the various sample types collected, although as IMiDs are reported relatively unstable in aqueous media (43–45), sample handling at room temperature was minimized to the 10 min collection period prior to freezing.

Sample Processing

Sample processing and quantitative analysis using liquid chromatography–tandem mass spectrometry (LC-MS/MS) is described elsewhere (manuscript submitted). Briefly, frozen samples were removed from storage and warmed to room temperature prior to processing. Standard curves were prepared in blank mouse plasma, neat solution (100% acetonitrile) or homogenized tissues. After brief vortex mixing and centrifugation, plasma aliquots were taken for processing and quantification. Solid tissue homogenates were incubated in an equal volume of water for 20 min prior to extraction. Drug was extracted from plasma and tissues *via* protein precipitation with 4°C acetonitrile containing 200 nM genistein as internal standard. Supernatant was removed and evaporated under vacuum for 6 h. Samples were reconstituted in HPLC grade water containing 0.1% formic acid and transferred to autosampler vials. Extracted impurities were separated by an Extended C-18 column (Agilent, 50×0.5 mm, $3\ \mu\text{m}$ particle size) using an ultra high performance Accela liquid chromatography system (Thermo, Waltham, MA, USA) and a flow gradient of water and acetonitrile both containing 0.1% formic acid. Analytes were ionized with atmospheric pressure chemical ionization, and fragment ions

were detected on a TSQ Quantum Discovery Max triple quadrupole mass spectrometer system (Thermo).

Pharmacokinetic Analysis

Pharmacokinetic (PK) parameter estimates from each dose/route were generated from quantifiable plasma and tissue data using WinNonlin Professional version 5.2.1 (Pharsight Corporation, Mountain View, CA, USA) and NONMEM, v. 7.1.2 (Icon, Hanover, MD, USA). Concentration measurements below the lower limit of quantification (*e.g.*, 0.3 nM in plasma) were excluded from analysis. Non-compartmental analysis was performed for each dose level in WinNonlin using the mean time and concentration data at each nominal time point. A linear up/log down approach was used for AUC calculation, and uniform weighting was used for terminal phase regression. Compartmental model fitting of data from each dose/route was completed in WinNonlin by evaluating one-, two- and three-compartment models with uniform, $1/Y$, $1/Y^2$, $1/Y_{\text{hat}}$, and $1/Y_{\text{hat}}^2$ weighting of naïve pooled data. Final model selection was based on visual fit, weighted residuals, individual parameter coefficients of variation, and Akaike's information criterion. Simultaneous fitting of IV and PO plasma data for the 0.5 and 10 mg/kg doses was performed using two- and three-compartment models, respectively, in NONMEM and WinNonlin. The NONMEM data file was constructed with unique individual identification numbers assigned to each plasma concentration–time observation. Base simultaneous models with a dosing compartment, a central compartment, and either one or two peripheral compartments were coded with differential equations using the ADVAN 13 subroutine in NONMEM. For these single-response population data, between-subject variability was fixed to zero, and residual unexplained variability (RUV) parameters were estimated for both IV and PO data using proportional error models.

To assess pharmacokinetic dose proportionality, IV and PO data was combined, and the effect of dose was evaluated as a continuous covariate in NONMEM. This mixed effects approach was chosen instead of the standard comparison of PK parameter and standard error estimates between dose groups because it allowed for evaluation of objective function value as well as reduction in residual error to determine if dose had a significant impact on model fits and PK parameter estimates. The dose covariate was evaluated on each model parameter using the relationship in equation 1, where the dose impact on CL is shown as an example,

$$\text{CL}_i = \text{TVCL} \times (\text{dose}_i)^{\theta_1} \quad (1)$$

where CL_i is lenalidomide clearance for individual mouse i , TVCL is the typical or population value of clearance, Dose_i is the dose for individual i , and θ_1 is the estimated exponential representing the influence of dose on the PK parameter, CL. Base and covariate models were evaluated by the log-likelihood ratio test, visual inspection of fit and residual plots, standard errors of estimate, and reduction in RUV. A minimum reduction in the objective function value (OFV) of 3.84 was used as a cutoff to indicate the dose covariate had a significant impact on PK with $\alpha=0.05$.

RESULTS

Range-Finding Study

Doses administered to mice were limited by solubility of lenalidomide in the PBS dosing solution. Maximum achievable doses of up to 15, 22.5, and 45 mg/kg ($n=4$ each) were given through IV, IP, and PO routes, respectively. IV administration was fatal in one mouse immediately following the 15 mg/kg bolus injection. Cause of death was not determined, and no other fatalities or observable toxicities were noted at maximum doses. Due to solubility limitations which prevented further dose escalation, a maximum tolerated dose was not established.

Plasma Pharmacokinetics

IV Dosing

As the range finding study resulted in mortality in one mouse at the 15 mg/kg maximum dose in IV dose toxicity studies, upper-limit doses of 10 mg/kg were utilized in pharmacokinetic studies regardless of administration route. Dose proportionality was assessed following IV bolus doses of 0.5, 1.5, 5 and 10 mg/kg body weight. Observed plasma concentration *versus* time plots are

shown in Fig. 1, and noncompartmental observed $C_{\max}$ and AUCs are listed in Table I. $C_{\max}$ at 2 min postdose ranged from 0.28 to 17.75 $\mu\text{g/mL}$ across the four evaluated doses, and noncompartmental AUCs ranged from 7.8 to 226 $\mu\text{g}\times\text{min}\times\text{mL}^{-1}$ over the chosen dose range.

For data modeling, all final compartmental best fit IV models employed a $1/Y_{\text{hat}}$ weighting scheme (Fig. 1). Following IV doses of 1.5 mg/kg and greater, lenalidomide concentrations decreased triexponentially, while the 0.5 mg/kg concentration–time profile produced a biphasic decline. Table I lists compartmental estimated PK parameters. Plasma concentrations and AUC appeared to saturate at the higher doses. The data indicate an approximate 17-fold increase in AUC while the dose increased threefold from 0.5 to 1.5 mg/kg. Following doses of 1.5 mg/kg and greater, the increase in plasma AUC is less than proportional, with an average fold increase of 0.51 per unit fold increase in dose. It should be noted that, while the three-compartment models provided the overall best fits to the IV data, uncertainty in parameter estimation is significant (Table I). Three-compartment α -phase kinetics (and γ -phase kinetics for the 1.5 mg/kg dose group) was estimated with sparse data. Given the high uncertainty in α -phase kinetics across the dose groups, IV $C_{\max}$ estimates should be interpreted with caution.

Fig. 1. Plasma pharmacokinetics for intravenous (IV) doses. Concentration *vs.* time plots display observed points (filled circles). Lines are fits to the naïve pooled data using two-compartment (0.5 mg/kg) or three-compartment models (all other doses). Data was weighted as $1/Y_{\text{hat}}$ for all fitting

Table I. WinNonlin Plasma Compartmental and Noncompartmental Parameter Estimates for IV, IP, and PO Dosing Routes

Dose (mg/kg)	Rt	n	Cmt	Compartmental							Non-Compartmental							
				AUC (min×ug/mL)	C _{max} (ug/ml)	K _e (min ⁻¹)	K _a (min ⁻¹)	CL (mL/min/kg)	CL2 (mL/min/kg)	CL3 (mL/min/kg)	V1 (mL/kg)	V2 (mL/kg)	V3 (mL/kg)	F (%)	AUC (min×ug/mL)	C _{max} (ug/ml)	CL (mL/min/kg)	F (%)
10	IV	45 (45)	3	284 (20)	158 (68.2)	0.554 (48.7)	NA	35.2 (20.0)	31.9 (56.3)	3.70 (73.4)	63.5 (68.1)	325 (44.9)	354 (67.0)	NA	226	17.8	44.2	NA
5	IV	45 (45)	3	237 (25.4)	72 (153)	0.304 (131)	NA	21.1 (25.4)	45.4 (60.9)	3.77 (77.4)	69.4 (154)	178 (32.9)	429 (119)	NA	192	14.0	26.0	NA
1.5	IV	39 (40)	3	106 (142)	52.1 (571)	0.494 (430)	NA	14.2 (142)	15.9 (413)	0.723 (251)	28.8 (571)	196 (256)	85.4 (242)	NA	128	9.92	11.7	NA
0.5	IV	40 (40)	2	8.9 (19.8)	0.525 (22.3)	0.059 (28.6)	NA	55.9 (19.8)	54.5 (50.1)	NA	951 (22.3)	1.751 (62.7)	NA	NA	7.8	0.278	64.1	NA
10	PO	38 (45)	1	214 (11.0)	2.03 (8.5)	0.077 (31.4)	0.014 (20.0)	35.2 (11.0)	NA	NA	2,550 (16.1)	NA	NA	75	216.6	2.45	45.2	96
0.5	PO	26 (40)	1	6.2 (9.7)	0.086 (8.7)	0.040 (202)	0.035 (193)	55.9 (9.7)	NA	NA	1,577 (191)	NA	NA	69	5.7	0.092	64.1	74
10	IP	40 (45)	2	300 (4.1)	8.48 (4.7)	0.221 (31.5)	0.044 (11.7)	35.2 (4.1)	5.19 (40.6)	NA	806 (11.8)	401 (47.6)	NA	105	287.1	8.34	44.8	127
0.5	IP	37 (40)	2	8.1 (9.9)	0.232 (19.1)	0.292 (80.4)	0.041 (18.1)	55.9 (10.0)	11.4 (24.5)	NA	1,356 (21.4)	2,139 (61.4)	NA	90	10	0.246	63.7	129

For extravascular doses, estimated CL/F and V/F were multiplied by F at each dose. Noncompartmental AUC, C_{max}, CL, and F are also presented for comparison of estimated vs. observed parameter values. Standard errors of estimate are indicated in parentheses as percent coefficients of variation. Note, WinNonlin estimates for absorption and elimination rate constants have been labeled K_e and K_a, respectively, for extravascular dosing routes to account for the observed absorption rate-limited or flip-flop kinetics.

Rt dose route, n number of concentration-time data points (number of mice dosed), AUC area under the concentration-time curve, C_{max} maximum observed (non-compartmental) or estimated (compartmental) concentration, K_e estimated elimination rate constant, K_a estimated absorption rate constant, CL clearance from central or peripheral (CL₂ and CL₃) compartments, V volume of distribution for central or peripheral (V₂ and V₃) compartments, F bioavailability calculated as $[100 \times (\text{AUC}_{\text{extravascular}} \times \text{dose}_{\text{IV}}) / (\text{AUC}_{\text{IV}} \times \text{dose}_{\text{extravascular}})]$

Extravascular dosing

Bioavailability was assessed for IP and PO administration routes following doses of 0.5 and 10 mg/kg. Concentration-time plots and resulting compartmental fit models are shown in Fig. 2. Maximum observed concentrations following 0.5 and 10 mg/kg were 0.246 and 8.343 $\mu\text{g/mL}$ for IP administration and 0.092 and 2.447 $\mu\text{g/mL}$ for PO administration (Table I). Observed time of maximum concentration (T_{max}) was 10 min for both IP doses and 20 and 40 min for 0.5 and 10 mg/kg oral doses, respectively. IP dosing achieved a C_{max} 2.7- to 4.2-fold greater than oral administration, whereas orally dosed mice sustained drug concentrations above 1.00 $\mu\text{g/mL}$ 1.5 h post a 10 mg/kg dose compared to 0.39 $\mu\text{g/mL}$ for mice receiving IP doses.

IP and oral compartmental data were weighted as 1/Y. Apparent absorption rate constants (K_a) were greater for IP than PO routes, with a fold difference of 7.3 and 2.9 for 0.5 and 10 mg/kg, respectively (Table I). Concentration-time data indicated monophasic and biphasic decline following PO and IP administration, respectively. Compartmental estimates for T_{max} were 10 and 27 min, respectively, regardless of dose. Systemic bioavailability determined from AUC estimates were 69% and 75% for PO doses versus 90% and 105% for IP doses of 0.5 and 10 mg/kg, respectively.

Simultaneous IV and Oral Fitting

To identify the oral K_a , IV and PO data were simultaneously fit to a multicompartiment model using NONMEM. A simultaneous, two-compartment base model was applied to data at the 0.5 mg/kg, while 10 mg/kg simultaneous data were modeled as three-compartment. Final model parameters and standard errors of estimate (expressed as CV%) are presented in Table II. Simultaneous fitting of IV and PO data indicated an absorption rate similar to the elimination rate estimate (K_e) at the 0.5 mg/kg dose. In contrast, K_a was much lower than K_e at the 10 mg/kg dose level in the evaluated models. Oral bioavailability estimates from these models were 65% and 87%, respectively. Notably, these population estimates are consistent with compartmental estimates determined with the naïve pooled approach in WinNonlin. Parameter estimates from these simultaneous models in NONMEM were confirmed using WinNonlin simultaneous user defined models (data not shown). Collectively, results from these analyses suggest lenalidomide exhibits absorption rate limited pharmacokinetic behavior when administered orally in mice. Therefore, the naïve pooled WinNonlin estimates for oral and intraperitoneal absorption and elimination rate constants are reported with $K_e > K_a$ in Table I.

Dose Proportionality

Dose linearity was assessed in NONMEM by combining IV and PO data to a two- and a three-compartment model. When dose was added as a covariate to each PK model parameter, OFV was decreased most significantly for dose as a covariate on Q2 (-7.51) in the two-compartment model and on Q3 (-37.245) in the three-compartment model. RUV also decreased 2% for the IV data in the two-compartment model and 1% and 7%, respectively, for the IV and PO data in the

Fig. 2. Plasma pharmacokinetics for oral (PO) and intraperitoneal (IP) doses. Concentration vs. time plots display observed data (filled circles). Lines are fits to the naïve pooled data using one-compartment (PO) or two-compartment models (IP). Data was weighted as 1/Y for all fitting

three-compartment model. Combined with the observed nonlinearity in drug exposure (Fig. 3a), results from these analyses provide further support that lenalidomide pharmacokinetic behavior is dose-dependent (or nonlinear) in ICR mice across the dose range evaluated.

Tissue Pharmacokinetics

Lenalidomide concentrations were quantified in multiple tissues collected from mice dosed with 0.5, 1.5, 5, and 10 mg/kg IV lenalidomide. The resulting concentration–time profiles from heart, lung, liver, muscle, spleen, brain, and kidney

tissues are displayed in Fig. 4. Initial concentrations expressed by 2 min time points in order from most prominent highest to lowest concentration were lung, liver, heart, and spleen. Following a dose of 10 mg/kg, the observed 2-min concentrations were 15, 5, 2.4, and 1.3 µg/g, respectively. At 45 min post the 10 mg/kg dose, the highest concentrations (268–612 ng/g) were observed in lung, liver, and spleen. Less than 15 ng/g remained in each tissue after 8 h.

The proportional distribution of lenalidomide was varied across each tissue type. Additionally, the proportion of lenalidomide distributed to a single tissue was variable over the dose range evaluated. These apparent tissue nonlinearities

Table II. Model Parameter Estimates from Simultaneous IV and PO Data Fitting in NONMEM

Dose (mg/kg)	<i>n</i>	Cmt	<i>K_e</i> (min ^{−1})	<i>K_a</i> (min ^{−1})	CL1 (mL/min/kg)	CL2 (mL/min/kg)	CL3 (mL/min/kg)	V1 (mL/kg)	V2 (mL/kg)	V3 (mL/kg)	<i>F</i> (%)
10	83	3	0.127	0.013 (6.0)	47.3 (6.0)	4.0 (34.5)	42.0 (116.3)	372 (75.3)	529 (20.6)	512 (51.8)	87 (11.2)
0.5	77	2	0.033	0.047 (16.0)	52.3 (15.1)	4.2 (39.1)	–	1,570 (24.5)	1,130 (124.8)	–	65 (18.9)

All quantifiable data for IV and PO dosed mice (0.5 mg/kg, *n*=77; 10 mg/kg, *n*=83) were used to fit models comprising the PO dose depot compartment, a central compartment (1), and one or two peripheral compartments (2 and 3). Final base models were selected using OFV and other criteria as described in the text. Parameter estimates and standard errors (expressed in parentheses as percent coefficients of variation) are presented for two- and three-compartment base models
K_a first-order absorption rate constant, *CL* clearance from central or peripheral (CL2 and CL3) compartments, *V* volume of distribution for central or peripheral (V2 and V3) compartments, *K_e* elimination rate constant calculated as *K_e*=CL/*V*1, *F* bioavailability

Fig. 3. Noncompartmental tissue and plasma area under the concentration–time curve (AUC). **a** A line plot showing the plasma nonlinear pharmacokinetic behavior of IV lenalidomide within the dose range evaluated in this study. This pattern is qualitatively observed in some (*e.g.*, spleen) but not all (*e.g.*, lung and liver) tissues. **b** and **c** Plasma-normalized and absolute lenalidomide AUCs in tissues, respectively. **d** The same data normalized to tissue perfusion rates. Units for plasma are (minutes by microgram per milliliter)

are observed when tissue noncompartmental AUC's are plotted against a linear dosing scale and when lenalidomide distribution to tissues is expressed as the ratio of tissue exposure/plasma exposure (Fig. 3). The proportion of lenalidomide distributed to tissues was frequently lowest within the 1.5 mg/kg dose. Tissues with the greatest lenalidomide exposures were lung, spleen and liver. Lenalidomide had the highest affinity or retention in spleen, as displayed in Fig. 3d when AUC is normalized to organ perfusion rate (46,47). This is consistent with our observation of an absorption phase in spleen (Fig. 4). Lung, liver, and muscle also displayed high lenalidomide concentrations after normalization to perfusion rate. Of note, an absorption phase was also observed in muscle; however, only at the 10 mg/kg dose level (Fig. 4). Lenalidomide could not be detected in brain tissues following 0.5 and 1.5 mg/kg doses but was quantifiable at low concentrations following 5 and 10 mg/kg injections.

DISCUSSION AND CONCLUSIONS

Clinical efficacy of lenalidomide in multiple malignancies results from its many activities spanning from anti-angiogenic and hematological responses to enhanced erythropoiesis.

Extensive clinical evaluations are underway, but dose-dependent and disease-specific toxicities remain a challenge. Further translational research in animal models is required to enhance our understanding of lenalidomide mechanisms of action and toxicity. With limited published pharmacokinetic data in animals, we sought to provide a thorough characterization of lenalidomide pharmacokinetics in mice to guide ongoing and future lenalidomide investigations in murine disease models.

In the present study, we characterized lenalidomide pharmacokinetics in ICR mice. Prior to conducting the pharmacokinetic studies, we explored toxicity of lenalidomide doses up to 15, 22.5, and 45 mg/kg *via* IV, IP, and PO routes of administration. Limited by solubility in our PBS dosing vehicle, these maximum achievable lenalidomide doses were well tolerated with the exception of one mouse death (of four total dosed) at the 15 mg/kg IV dose. As cause of death was not determined, it is unclear if this was due to drug exposure, possible precipitation of lenalidomide in blood, or other drug-related causes. Notably, no other toxicities were observed in the study at IV doses of 15 mg/kg ($n=3$) or 10 mg/kg ($n=45$) or at any other dose level through IV, IP, and PO routes.

Low doses in mice result in similar plasma concentrations, high oral bioavailability, and monoexponential decline

Fig. 4. Tissue concentration vs. time data. Data points are means from $n=2$ to 5 animals, and error bars are $\pm$ SD

as seen in humans. With doses of 0.5–10 mg/kg, systemic bioavailability of lenalidomide ranged from 69% to 75% after oral administration and 90% to 105% after IP administration using compartmentally estimated AUC values. When comparing mouse pharmacokinetic data to that in humans, 1.5 and 5 mg/kg IV doses in mice produce noncompartmental AUCs of 2,130 and 3,200 $\text{h} \times \text{ng/mL}$ (128 and 192 $\text{min} \times \mu\text{g/mL}$), which are most similar to observed lenalidomide plasma exposures following doses of approximately 25 mg in adults (examples, mean AUCs of

2,154 $\text{h} \times \text{ng/mL}$ (11) or 2,415 $\text{h} \times \text{ng/mL}$ (35)) or 15–30 mg/m^2 in children (examples, mean AUCs ranging from 2,740 to 3,850 $\text{h} \times \text{ng/mL}$; 36,48). Interestingly, the 1.5 and 5 mg/kg doses in mice translate to human equivalent doses of approximately 7 and 23 mg in adults or 4.5 and 15 mg/m^2 in children, based on allometric scaling factors (49). Notably, these doses are low relative to the observed data in clinical reports. Future doses used for translational studies in mice should consider this data while choosing clinically relevant doses.

Our data suggests lenalidomide has absorption rate limited and dose-dependent (or nonlinear) kinetics in ICR mice over a dosing range of 0.5–10 mg/kg. This nonlinearity is observed as a greater than proportional increase in plasma AUC and $C_{\max}$ between the 0.5 and 1.5 mg/kg doses, followed by a less than proportional increase in AUC and $C_{\max}$ between the 1.5, 5, and 10 mg/kg doses. These data indicate at least two distinct mechanisms contribute to the observed nonlinear pharmacokinetic behavior. Greater than proportional increases in AUC are consistent with saturated clearance mechanisms or altered distribution between two dose levels. Consistent with previous suggestions that active transport contributes to renal clearance of lenalidomide (21,24), our group previously presented data suggesting P-glycoprotein is a potential mechanism for this apparent saturation in renal excretion (23). Although additional transporters of lenalidomide have not yet been identified, a second contributing mechanism may be a saturation of active renal reabsorption. The likelihood of saturated reabsorption is uncertain and would depend on abundance of specific transporters and their affinities for lenalidomide.

Tissue distribution displayed in Fig. 3 demonstrates a collective trend for the tissue AUC/plasma AUC ratio which is high for the 0.5 mg/kg dose, decreases for the 1.5 mg/kg dose, then increases for the 5 and 10 mg/kg doses. This indicates a greater portion of the low dose is distributed into the tissue volume as compared to the higher doses. Conceivably tissue binding is saturated at the 1.5 mg/kg dose, and a greater portion of lenalidomide is retained in plasma than at the 0.5 mg/kg dose. However as dose increases further, plasma protein binding may also become saturated thus increasing clearance at higher doses. In addition, solubility cannot be overlooked as a potential cause for the observed nonlinearity at higher doses. A 3 mg/mL concentration was selected for the maximum IV dosing solutions, which was just below the 3.5 mg/mL concentration with visible, undissolved particulates. These concentrated dosing solutions may have partially precipitated before or immediately upon injection into mice (fatality in one of the four mice dosed with 15 mg/kg IV lenalidomide may be evidence for the later). Although concentrations of dosing solutions were verified using LC-MS/MS, this required dilution to enable measured concentrations to fall within linear ranges of our assay, and may therefore not accurately represent the more concentrated dosing solutions. Furthermore, our observed limitations in solubility prevented an assessment of linearity and drug exposures with doses of 50 mg/kg (9,50) and 100 mg/kg (51,52), which were used by other groups previously in preclinical murine studies.

Lenalidomide was distributed to all analyzed tissues, although levels were undetectable in brain following 0.5 and 1.5 mg/kg doses. Tissue-to-plasma ratios calculated at each dose further demonstrate the nonlinearity observed in plasma. As an example, quantification of lenalidomide in eight tissues from IV dosed mice shows dissimilar kinetics in spleen and brain (Figs. 3 and 4). Lenalidomide distribution to spleen includes an absorption phase with a $T_{\max}$ at 10 min, regardless of dose. Furthermore, spleen demonstrated the highest relative exposure when tissue AUCs were normalized to blood perfusion rate (Fig. 3). This apparent accumulation may be explained by the presence of uptake transporters or

high affinity binding to biomolecules expressed specifically in the spleen (either intra- or extra-cellular). To our knowledge, these possibilities have not been reported previously and should be further explored. This preferential distribution to spleen may be relevant for lenalidomide efficacy and toxicity mechanisms in lymphoid organs.

Lenalidomide distribution to brain was detectable only at doses of 5 mg/kg and above. With an assay lower limit of quantification of 0.3 ng/g in brain tissue, the data suggest relatively poor penetration into brain. This is in contrast to its analog, thalidomide ($\log P=0.3$; 53), which has considerable brain penetration (54,55). Although lenalidomide structure and lipophilicity ($\log P=-0.5$; 53) are similar to thalidomide, recent evidence indicates lenalidomide is a substrate of the efflux transporter, P-glycoprotein (23). This active transporter mediates xenobiotic removal from the brain as part of the blood brain barrier (56) which would reduce overall lenalidomide uptake in the CNS. A recent publication exploring distribution of lenalidomide in nonhuman primates determined 11% exposure in cerebrospinal fluid relative to plasma following a dose of 20 mg (29). Similarly, we report lenalidomide brain exposures following 5 and 10 mg/kg in mice are approximately 0.9% and 2.3% of that observed in plasma. These results may explain why clinical neurotoxicities are minimal and reported in few patients relative to thalidomide (57,58). While studies are being pursued to evaluate lenalidomide efficacy in cancers of the CNS and other neurological disorders, the relatively low lenalidomide CNS penetration may become an important factor.

This work provides data for plasma pharmacokinetics and tissue distribution of lenalidomide in mice at clinically relevant doses. Intraperitoneal and oral administration routes demonstrate good bioavailability in mice. These studies can be used to estimate concentrations achieved at relevant sites of drug action when lenalidomide efficacy studies are performed in murine models. As presented above, transporter-mediated disposition and potential intra- or extra-cellular preferential binding of lenalidomide offer possible explanations for the observed nonlinearity. Considerations of nonlinearity and selective tissue distribution reported herein may aid in selection of doses with greater clinical relevance to better enable interspecies extrapolation of results in continued clinical and translational development of lenalidomide.

ACKNOWLEDGMENTS

This work was supported by an Eli Lilly Graduate Fellowship (DMR), a Leukemia and Lymphoma Society SCOR grant (JCB, AJJ, and MP), the D. Warren Brown Foundation (JCB), and NIH grants 1 P50 CA140158 (JCB and AJJ), K12CA133250 (AJJ), and 5KL2RR025754.

REFERENCES

1. Muller GW, Chen R, Huang SY, Corral LG, Wong LM, Patterson RT, *et al.* Amino-substituted thalidomide analogs: potent inhibitors of TNF- α production. *Bioorg Med Chem Lett.* 1999;9(11):1625–30. Epub 1999/07/01.

2. Dredge K, Marriott JB, Macdonald CD, Man HW, Chen R, Muller GW, *et al.* Novel thalidomide analogues display anti-angiogenic activity independently of immunomodulatory effects. *Br J Cancer*. 2002;87(10):1166–72. Epub 2002/10/29.
3. Dredge K, Horsfall R, Robinson SP, Zhang LH, Lu L, Tang Y, *et al.* Orally administered lenalidomide (CC-5013) is anti-angiogenic *in vivo* and inhibits endothelial cell migration and Akt phosphorylation *in vitro*. *Microvasc Res*. 2005;69(1-2):56–63. Epub 2005/03/31.
4. Moutouh-de Parseval LA, Verhelle D, Glezer E, Jensen-Pergakes K, Ferguson GD, Corral LG, *et al.* Pomalidomide and lenalidomide regulate erythropoiesis and fetal hemoglobin production in human CD34+ cells. *J Clin Invest*. 2008;118(1):248–58. Epub 2007/12/08.
5. Davies FE, Raju N, Hideshima T, Lentzsch S, Young G, Tai YT, *et al.* Thalidomide and immunomodulatory derivatives augment natural killer cell cytotoxicity in multiple myeloma. *Blood*. 2001;98(1):210–6. Epub 2001/06/22.
6. Corral LG, Haslett PA, Muller GW, Chen R, Wong LM, Ocampo CJ, *et al.* Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF- α . *J Immunol*. 1999;163(1):380–6. Epub 1999/06/29.
7. Wu L, Adams M, Carter T, Chen R, Muller G, Stirling D, *et al.* lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CD20+ tumor cells. *Clinical cancer research*. *Off J Am Assoc Cancer Res*. 2008;14(14):4650–7. Epub 2008/07/17.
8. Zhu D, Corral LG, Fleming YW, Stein B. Immunomodulatory drugs Revlimid (lenalidomide) and CC-4047 induce apoptosis of both hematological and solid tumor cells through NK cell activation. *Cancer Immunol Immunother: CII*. 2008;57(12):1849–59. Epub 2008/04/09.
9. Lu L, Payvandi F, Wu L, Zhang LH, Hariri RJ, Man HW, *et al.* The anti-cancer drug lenalidomide inhibits angiogenesis and metastasis *via* multiple inhibitory effects on endothelial cell function in normoxic and hypoxic conditions. *Microvasc Res*. 2009;77(2):78–86.
10. Gandhi AK, Kang J, Capone L, Parton A, Wu L, Zhang LH, *et al.* Dexamethasone synergizes with lenalidomide to inhibit multiple myeloma tumor growth, but reduces lenalidomide-induced immunomodulation of T and NK cell function. *Curr Cancer Drug Targets*. 2010;10(2):155–67. Epub 2010/01/22.
11. Richardson PG, Schlossman RL, Weller E, Hideshima T, Mitsiades C, Davies F, *et al.* Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma. *Blood*. 2002;100(9):3063–7. Epub 2002/10/18.
12. Sanchawalala V, Wright DG, Rosenzweig M, Finn KT, Fennessey S, Zeldis JB, *et al.* Lenalidomide and dexamethasone in the treatment of AL amyloidosis: results of a phase 2 trial. *Blood*. 2007;109(2):492–6. Epub 2006/09/09.
13. Dispenzieri A, Lacy MQ, Zeldenrust SR, Hayman SR, Kumar SK, Geyer SM, *et al.* The activity of lenalidomide with or without dexamethasone in patients with primary systemic amyloidosis. *Blood*. 2007;109(2):465–70. Epub 2006/09/30.
14. Chanan-Khan A, Miller KC, Musial L, Lawrence D, Padmanabhan S, Takeshita K, *et al.* Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study. *J Clin Oncol Off J Am Soc Clin Oncol*. 2006;24(34):5343–9. Epub 2006/11/08.
15. Chanan-Khan AA, Cheson BD. Lenalidomide for the treatment of B-cell malignancies. *J Clin Oncol Off J Am Soc Clin Oncol*. 2008;26(9):1544–52. Epub 2008/02/21.
16. Ferrajoli A, Lee BN, Schlette EJ, O'Brien SM, Gao H, Wen S, *et al.* Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. *Blood*. 2008;111(11):5291–7.
17. Habermann TM, Lossos IS, Justice G, Vose JM, Wiernik PH, McBride K, *et al.* Lenalidomide oral monotherapy produces a high response rate in patients with relapsed or refractory mantle cell lymphoma. *Br J Haematol*. 2009;145(3):344–9. Epub 2009/02/28.
18. Tefferi A, Cortes J, Verstovsek S, Mesa RA, Thomas D, Lasho TL, *et al.* Lenalidomide therapy in myelofibrosis with myeloid metaplasia. *Blood*. 2006;108(4):1158–64. Epub 2006/04/13.
19. Witzig TE, Wiernik PH, Moore T, Reeder C, Cole C, Justice G, *et al.* Lenalidomide oral monotherapy produces durable responses in relapsed or refractory indolent non-Hodgkin's Lymphoma. *J Clin Oncol Off J Am Soc Clin Oncol*. 2009;27(32):5404–9. Epub 2009/10/07.
20. Wiernik PH, Lossos IS, Tusciano JM, Justice G, Vose JM, Cole CE, *et al.* Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma. *J Clin Oncol Off J Am Soc Clin Oncol*. 2008;26(30):4952–7. Epub 2008/07/09.
21. Chen N, Wen L, Lau H, Surapaneni S, Kumar G. Pharmacokinetics, metabolism and excretion of [(14)C]-lenalidomide following oral administration in healthy male subjects. *Cancer Chemotherapy and Pharmacology*. 2012;69(3):789–97.
22. Revlimid Label: REVLIMID® (lenalidomide) 5 mg, 10 mg, 15 mg and 25 mg capsules. 2009.
23. Hofmeister CC, Yang X, Pichiorri F, Chen P, Rozewski DM, Johnson AJ, *et al.* Phase I trial of lenalidomide and CCI-779 in patients with relapsed multiple myeloma: evidence for lenalidomide-CCI-779 interaction *via* P-glycoprotein. *J Clin Oncol Off J Am Soc Clin Oncol*. 2011;29(25):3427–34. Epub 2011/08/10.
24. Chen N, Lau H, Kong L, Kumar G, Zeldis JB, Knight R, *et al.* Pharmacokinetics of lenalidomide in subjects with various degrees of renal impairment and in subjects on hemodialysis. *J Clin Pharmacol*. 2007;47(12):1466–75.
25. Chen N, Lau H, Choudhury S, Wang X, Assaf M, Laskin OL. Distribution of lenalidomide into semen of healthy men after multiple oral doses. *J Clin Pharmacol*. 2010;50(7):767–74. Epub 2010/02/18.
26. Fine HA, Kim L, Albert PS, Duic JP, Ma H, Zhang W, *et al.* A phase I trial of lenalidomide in patients with recurrent primary central nervous system tumors. *Clin Cancer Res*. 2007;13(23):7101–6. Epub 2007/12/07.
27. Dahut WL, Aragon-Ching JB, Woo S, Tohnya TM, Gulley JL, Arlen PM, *et al.* Phase I study of oral lenalidomide in patients with refractory metastatic cancer. *J Clin Pharmacol*. 2009;49(6):650–60. Epub 2009/05/20.
28. Iida S, Chou T, Okamoto S, Nagai H, Hatake K, Murakami H, *et al.* Lenalidomide plus dexamethasone treatment in Japanese patients with relapsed/refractory multiple myeloma. *Int J Hematol*. 2010;92(1):118–26. Epub 2010/06/19.
29. Muscal JA, Sun Y, Nuchtern JG, Dauser RC, McGuffey LH, Gibson BW, *et al.* Plasma and cerebrospinal fluid pharmacokinetics of thalidomide and lenalidomide in nonhuman primates. *Cancer Chemother Pharmacol*. 2011. Epub 2011/11/24.
30. Richardson PG, Blood E, Mitsiades CS, Jagannath S, Zeldenrust SR, Alsina M, *et al.* A randomized phase 2 study of lenalidomide therapy for patients with relapsed or relapsed and refractory multiple myeloma. *Blood*. 2006;108(10):3458–64. Epub 2006/07/15.
31. Rajkumar SV, Hayman SR, Lacy MQ, Dispenzieri A, Geyer SM, Kabat B, *et al.* Combination therapy with lenalidomide plus dexamethasone (Rev/Dex) for newly diagnosed myeloma. *Blood*. 2005;106(13):4050–3. Epub 2005/08/25.
32. Zonder JA, Barlogie B, Durie BG, McCoy J, Crowley J, Hussein MA. Thrombotic complications in patients with newly diagnosed multiple myeloma treated with lenalidomide and dexamethasone: benefit of aspirin prophylaxis. *Blood*. 2006;108(1):403. author reply 4. Epub 2006/06/23.
33. Palumbo A, Rus C, Zeldis JB, Rodeghiero F, Boccadoro M. Enoxaparin or aspirin for the prevention of recurrent thromboembolism in newly diagnosed myeloma patients treated with melphalan and prednisone plus thalidomide or lenalidomide. *J Thromb Haemost*. 2006;4(8):1842–5. Epub 2006/08/02.
34. Andritsos LA, Johnson AJ, Lozanski G, Blum W, Kefauver C, Awan F, *et al.* Higher doses of lenalidomide are associated with unacceptable toxicity including life-threatening tumor flare in patients with chronic lymphocytic leukemia. *J Clin Oncol*. 2008;26(15):2519–25. Epub 2008/04/23.
35. Blum W, Klisovic RB, Becker H, Yang X, Rozewski DM, Phelps MA, *et al.* Dose escalation of lenalidomide in relapsed or refractory acute leukemias. *J Clin Oncol*. 2010;28(33):4919–25. Epub 2010/10/20.
36. Warren KE, Goldman S, Pollack IF, Fangusaro J, Schaiquevich P, Stewart CF, *et al.* Phase I trial of lenalidomide in pediatric patients with recurrent, refractory, or progressive primary CNS

- tumors: Pediatric Brain Tumor Consortium study PBTC-018. *J Clin Oncol.* 2011;29(3):324–9. Epub 2010/12/15.
37. Qian Z, Zhang L, Cai Z, Sun L, Wang H, Yi Q, *et al.* Lenalidomide synergizes with dexamethasone to induce growth arrest and apoptosis of mantle cell lymphoma cells *in vitro* and *in vivo*. *Leuk Res.* 2011;35(3):380–6. Epub 2010/11/05.
 38. Santo L, Hideshima T, Cirstea D, Bandi M, Nelson EA, Gorgun G, *et al.* Antimyeloma activity of a multitargeted kinase inhibitor, AT9283, *via* potent Aurora kinase and STAT3 inhibition either alone or in combination with lenalidomide. *Clin Cancer Res: Off J Am Assoc Cancer Res.* 2011;17(10):3259–71. Epub 2011/03/25.
 39. Schmidt M, Kim Y, Gast SM, Endo T, Lu D, Carson D, *et al.* Increased *in vivo* efficacy of lenalidomide and thalidomide by addition of ethacrynic acid. *In vivo.* 2011;25(3):325–33.
 40. Benson Jr DM, Bakan CE, Zhang S, Collins SM, Liang J, Srivastava S, *et al.* IPH2101, a novel anti-inhibitory KIR antibody, and lenalidomide combine to enhance the natural killer cell *versus* multiple myeloma effect. *Blood.* 2011;118(24):6387–91. Epub 2011/10/28.
 41. Chauhan D, Singh AV, Ciccarelli B, Richardson PG, Palladino MA, Anderson KC. Combination of novel proteasome inhibitor NPI-0052 and lenalidomide trigger *in vitro* and *in vivo* synergistic cytotoxicity in multiple myeloma. *Blood.* 2010;115(4):834–45. Epub 2009/12/08.
 42. Galustian C, Meyer B, Labarthe MC, Dredge K, Klaschka D, Henry J, *et al.* The anti-cancer agents lenalidomide and pomalidomide inhibit the proliferation and function of T regulatory cells. *Cancer Immunol Immunother: CII.* 2009;58(7):1033–45. Epub 2008/11/15.
 43. Yang XX, Hu ZP, Xu AL, Duan W, Zhu YZ, Huang M, *et al.* A mechanistic study on reduced toxicity of irinotecan by coadministered thalidomide, a tumor necrosis factor-alpha inhibitor. *J Pharmacol Exp Ther.* 2006;319(1):82–104. Epub 2006/07/04.
 44. Kenyon BM, Browne F, D'Amato RJ. Effects of thalidomide and related metabolites in a mouse corneal model of neovascularization. *Exp Eye Res.* 1997;64(6):971–8. Epub 1997/06/01.
 45. Meyring M, Muhlbacher J, Messer K, Kastner-Pustet N, Bringmann G, Mannschreck A, *et al.* *In vitro* biotransformation of (R)- and (S)-thalidomide: application of circular dichroism spectroscopy to the stereochemical characterization of the hydroxylated metabolites. *Anal Chem.* 2002;74(15):3726–35. Epub 2002/08/15.
 46. Physiological Parameter Values for PBPK Models. [database on the Internet]. International Life Sciences Institute and Risk Science Institute. 1994.
 47. Gjedde SB, Gjeode A. Organ blood flow rates and cardiac output of the BALB/c mouse. *Comp Biochem Physiol Part A: Physiol.* 1980;67(4):671–4.
 48. Berg SL, Cairo MS, Russell H, Ayello J, Ingle AM, Lau H, *et al.* Safety, pharmacokinetics, and immunomodulatory effects of lenalidomide in children and adolescents with relapsed/refractory solid tumors or myelodysplastic syndrome: a Children's Oncology Group Phase I Consortium report. *J Clin Oncol: Off J Am Soc Clin Oncol.* 2011;29(3):316–23. Epub 2010/12/15.
 49. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J: Off Publ Fed Am Soc Exp Biol.* 2008;22(3):659–61. Epub 2007/10/19.
 50. Liu WM, Laux H, Henry JY, Bolton TB, Dagleish AG, Galustian C. A microarray study of altered gene expression in colorectal cancer cells after treatment with immunomodulatory drugs: differences in action *in vivo* and *in vitro*. *Mol Biol Rep.* 2010;37(4):1801–14. Epub 2009/07/15.
 51. Kiaei M, Petri S, Kipiani K, Gardian G, Choi DK, Chen J, *et al.* Thalidomide and lenalidomide extend survival in a transgenic mouse model of amyotrophic lateral sclerosis. *J Neurosci: Off J Soc Neurosci.* 2006;26(9):2467–73. Epub 2006/03/03.
 52. Neymotin A, Petri S, Calingasan NY, Wille E, Schafer P, Stewart C, *et al.* Lenalidomide (Revlimid) administration at symptom onset is neuroprotective in a mouse model of amyotrophic lateral sclerosis. *Exp Neurol.* 2009;220(1):191–7. Epub 2009/09/08.
 53. NCBI. PubChem Compound. NCBI; 2012 [cited 2012 March 21].
 54. Baird R, van Zyl-Smit RN, Iveson A, Duddy J, Rassam SM. Thalidomide is highly effective in a patient with meningeal acute myeloid leukaemia. *Leuk Lymphoma.* 2004;45(1):179–81. Epub 2004/04/06.
 55. Hwu WJ, Raizer J, Panageas KS, Lis E. Treatment of metastatic melanoma in the brain with temozolomide and thalidomide. *Lancet Oncol.* 2001;2(10):634–5. Epub 2002/03/21.
 56. Loscher W, Potschka H. Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. *Neuro Rx.* 2005;2(1):86–98. Epub 2005/02/18.
 57. Sharma RA, Steward WP, Daines CA, Knight RD, O'Byrne KJ, Dagleish AG. Toxicity profile of the immunomodulatory thalidomide analogue, lenalidomide: phase I clinical trial of three dosing schedules in patients with solid malignancies. *Eur J Cancer.* 2006;42(14):2318–25. Epub 2006/08/11.
 58. Rao KV. Lenalidomide in the treatment of multiple myeloma. *Am J Health Syst Pharm.* 2007;64(17):1799–807.