CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-332

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA.	22-332
Submission Date:	July 23, 2008
Generic Name	Tadalafil
Brand Name (proposed)	Adcirca™ Tablets
Applicant	Eli Lily and Company
Dosage Form, Strength	Tablet, 20mg
Indication	Pulmonary Arterial Hypertension
DCP	Clinical Pharmacology 1
OND	Cardiovascular and Renal Drug Products
Primary Reviewer	Islam R. Younis, Ph.D.
Team Leader (Acting)	Angelica Dorantes, Ph.D.
Pharmacometrics Reviewer	Kevin M. Krudys, Ph.D.
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1.0 Executive Summary

Background

Eli Lily and Company submitted NDA 22-332 for Adcirca™ (tadalafil) tablets on July 23, 2008. The applicant is seeking approval of a new indication for tadalafil, which is increasing exercise capacity in pulmonary arterial hypertension (PAH) patients. Tadalafil was approved in the US on November 21, 2003 for the treatment of male erectile dysfunction under NDA 21-368; Division of Urologic and Reproductive Products. Tadalafil is currently marketed in the United States as Cialis®. Cialis is available in 2.5, 5, 10, and 20 mg tablets.

The proposed dose for tadalafil in PAH patients is 40 mg once-daily, two tablets of 20 mg one-daily.

Clinical Pharmacology/Biopharmaceutics Findings

I. Exposure-Response

- The optimal dose of tadalafil to improve exercise capacity in PAH patients is 20 mg.
- The median 6-minute walk distance (6-MWD) change from baseline appears to be similar for doses 10, 20 and 40 mg.
- The maximum effect of tadalafil on 6-MWD change from baseline and pulmonary vascular resistance is attained at tadalafil doses of 20 and 40 mg.
- The most common dose dependent adverse events were headache, flushing, and myalgia.
- The median 6-MWD at Week 16 is not affected by concomitant bosentan administration, as long as the tadalafil dose is 20 mg or 40 mg.

II. Pharmacokinetics

- Tadalafil steady state is achieved in 5 days in healthy Japanese subjects following the administration of 40 mg tadalafil once-daily.
- Tadalafil systemic exposure (C_{max} and $AUC_{0-\infty}$) is not proportional over the dose range of 5 to 40 mg in both Japanese and Caucasian subjects following single oral dose.

III. Drug-Drug Interactions

- Bosentan at steady state significantly reduces tadalafil $AUC_{0-24,ss}$ by 42% and $C_{max,ss}$ by 27%. Tadalafil significantly increases $C_{max,ss}$ of bosentan by 20% and has no significant effect on bosentan $AUC_{0-24,ss}$.
- Tadalafil at steady state does not alter the steady state systemic exposure of digoxin.

- Tadalafil at steady state significantly increases ethinylestradiol $AUC_{0-24,ss}$ by 25% and $C_{max,ss}$ by 70%. Tadalafil at steady state significantly reduces ethinylestradiol-sulfate $AUC_{0-24,ss}$ by 71% and $C_{max,ss}$ by 63%.
- Tadalafil at steady state does not alter the steady state systemic exposure ($AUC_{0-24,ss}$ and $C_{max,ss}$) of levonorgestrel.

IV. Food Effect

- Food does not alter the systemic exposure of tadalafil following the administration of a 40 mg single dose

V. Ethnic Effect

- Tadalafil systemic exposure ($AUC_{0-\infty}$) in Japanese subjects was significantly lower than in Caucasian subjects by 23% at the 40 mg dose of tadalafil.

RECOMENDATION

The Office of Clinical Pharmacology I finds the information submitted to NDA 22-332 acceptable with the following comments:

1. The starting dose of tadalafil in patients with pulmonary arterial hypertension should be 20 mg. The dose can be increased to 40 mg if deemed necessary based on patient's and physician's assessment of effectiveness.
2. No dose adjustment of tadalafil is needed when administered with bosentan.
3. We recommend changes in the labeling language to the exposure-response relationship in section 12.2 and population pharmacokinetics in section 12.3. Details of proposed changes are provided in section 3 (page 16).
4. There are no Phase IV commitments

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3/10/2009
Date

Kevin Krudys
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03/10/2009
Date

FT signed by:
Angelica Dorantes, Ph.D. (Acting Team Leader),
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Cc: NDA 22-332, HFD 110, HFD-860 (Younis, Mehta, Uppoor)

Clinical Pharmacology Briefing: 03/10/2009
Attendants: Mehul Mehta, Brian Booth, Ting Ong, Pravin Jadhav, Julie Kircheiner, Sara Schrieber, Divya Menon-Andersen, Hyunjh Kim, Apparaju, Sandhya, Kellie Renoylds, Ramana Uppoor, Robert Kumi, Joga Gobburu, Gilbert Burckart, Joo-Yeon Lee, Shiew-Mei Huang, Larry lesko, Issam Zineh, Lei Zhang, Micheal Pacanowski, Sheetal Agarwal, Hae-young Ahn, Angelica Dorantes, Islam Younis, Kevin Krudys, Norman Stockbridge, Tom Marciniak, Dan Brum

2.0 Question Based Review

An abbreviated version of the QBR will be adapted for this clinical pharmacology review since key QBR elements have been addressed previously in NDA 21-368. The current NDA includes data to support the approval of a new indication for Tadalafil Tablets. The new indication is treatment of pulmonary arterial hypertension.

The proposed dose for tadalafil in PAH is 40 mg once-daily, two tablets of 20 mg. The applicant is planning on marketing tadalafil for PAH patients under the trade name Adcirca™. Adcirca™ 20 mg tablet differs from the Cialis® 20 mg tablet in debossment and film coating color only.

The current submission included 9 clinical studies [i.e., one pivotal clinical trial, which contained the exposure-response data in PAH patients, three drug-drug interaction studies, one food effect study, and 2 pharmacokinetics studies (single and multiple dose)].

2.1 General attribute of tadalafil products

Eli Lily and Company submitted NDA 22-332; for tadalafil tablets on July 23, 2008. The applicant is seeking approval for a new indication for tadalafil which is increasing exercise capacity in pulmonary arterial hypertension (PAH) patients.

Previously, tadalafil was approved in the US on November 21, 2003 for the treatment of male erectile dysfunction under NDA 21-368; Division of Urologic and Reproductive Products. Tadalafil is currently marketed in the United States as Cialis®. Cialis is available in 2.5, 5, 10, and 20 mg tablets. The 5, 10, and 20 mg tablets are for on-demand use, while 2.5 and 5 mg tablets are for once daily use.

2.1.1. What are the highlights of the formulation of the drug product?

Tadalafil drug product is an immediate release, almond shaped, film coated, debossed tablet. The formulation is included in the next table.

The tablets are supplied in bottle [redacted]. Tadalafil 20 mg tablets consist of the same core tablet shape and components and are manufactured by the same process as the approved Cialis 20 mg tablets. The tadalafil 20 mg tablet differs from the Cialis 20 mg tablet in debossment and film coating color. The tadalafil (PAH) 20 mg tablet debossment is the four digit item code "4467". The Cialis debossment is "C20". The tadalafil 20 mg tablet film coating is an orange color mixture of the same formulation as the Cialis 20 mg tablet yellow color mixture with the addition of a [redacted] quantity of [redacted]

b(4)

b(4)

Ingredient	% w/w of Core Tablet	Function
Lactose Monohydrate		
Lactose Monohydrate (		
Hydroxypropyl Cellulose (		
Croscarmellose Sodium		
Hydroxypropyl Cellulose		
Sodium Lauryl Sulfate		
Microcrystalline Cellulose		
Croscarmellose Sodium		
Magnesium Stearate		
Talc		

b(4)

Total tablet weight is 350 mg

2.1.2. What are the proposed mechanism of action and therapeutic indication of tadalafil?

Tadalafil is a potent and selective inhibitor of the phosphodiesterase type 5 (PDE5) enzyme. Tadalafil is indicated for pulmonary arterial hypertension to improve exercise capacity.

2.1.3. What is the proposed dosage and rout of administration?

The proposed dosing regimen is 40mg (2 x 20mg tablets) once daily and the intended route of administration is oral.

2.2 General clinical pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

Tadalafil clinical development program is primarily based on one randomized, double-blind, placebo-controlled, parallel design clinical study (protocol # LVGY) to evaluate the efficacy and safety of 2.5, 10, 20, and 40 mg tadalafil taken once daily in patients with pulmonary arterial hypertension.

The clinical pharmacology section of the submission contained the following studies:

1. Three drug-drug interaction studies of tadalafil with bosentan, digoxin, and oral contraceptives (Studies # H6D-EW-LVHM, # H6D-EW-LVHL, and # H6D-MC-LVGZ, respectively).
2. Food effect study (Study # H6D-EW-LVHO).
3. Multiple dose safety and PK study in Japanese subjects (Study # H6D-MC-LVHC).
4. Single dose PK and ethnic effect study of tadalafil pharmacokinetics between Japanese and Caucasian Subjects (Study # H6D-EQ-LVCS).

5. Exposure-response data in pulmonary arterial hypertension patients (Clinical study LVGY).

2.2.2 What is the basis for selecting the response endpoints and how are they measured in clinical pharmacology and clinical studies?

The primary efficacy endpoint was the change from baseline in 6-minute walk (6-MW) distance at Week 16. This was an appropriate measure and is well documented in the literature as a standard for PAH therapies. Improved performance in the 6-MW test from baseline to 16 weeks predicts morbidity as well as survival in patients with idiopathic PAH. and Note that the 6-MW test is the clinical standard assessment used to demonstrate the primary benefit of approved treatments in PAH patients.

2.2.3 Exposure-response

2.2.3.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy?

Tadalafil concentrations are correlated with an increase in the primary endpoint, 6-MWD change from baseline. The exposure-response relationship suggests the maximum effect on 6-MWD change from baseline is attained at tadalafil concentrations corresponding to the 20 and 40 mg doses (Figure 1). The effect of tadalafil on the secondary endpoint, pulmonary vascular resistance (PVR), also appears to be at its maximum at doses of 20 mg or greater (Figure 2).

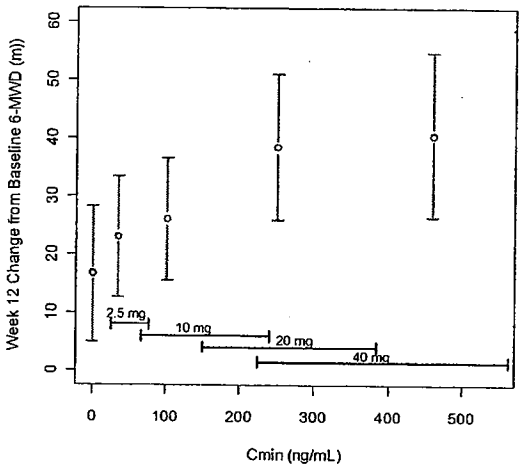


Figure 1. 6-MWD Mean (95% Confidence Interval) change from baseline (Week 16) by binned median C_{min}.

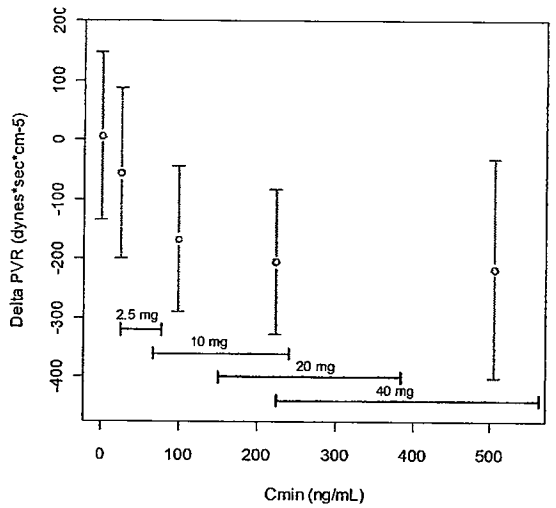


Figure 2. PVR Mean (95% Confidence Interval) change from baseline (Week 16) by binned median C_{min}.

2.2.3.2 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety?

The most common dose-dependent adverse events are listed in table below. No significant safety concerns arising from Study H6D-MC-LVGY were raised by the medical reviewer.

	Placebo (n = 82)	**2.5 mg (n = 82)**	**10 mg (n = 80)**	**20 mg (n = 82)**	**40 mg (n = 79)**
Headache	15%	18%	38%	32%	42%
Flushing	2.4%	3.4%	6.3%	6.1%	12.7%
Myalgia	3.7%	2.4%	3.8%	8.5%	13.9%

2.2.4 What are the PK characteristics of the drug?

Absolute bioavailability of tadalafil following oral dosing has not been determined. Tadalafil is highly distributed into tissues with mean apparent volume of distribution of 77 L. Tadalafil is highly bound to plasma proteins (94% bound). Tadalafil T_{max} is achieved between 2 and 8 hours (median time of 4 hours) after single oral-dose administration. The mean oral clearance for tadalafil is 3.4 L/hr and the mean terminal half-life is 15 hours in healthy subjects. Tadalafil is excreted predominantly as metabolites, mainly in the feces (approximately 61% of the dose) and to a lesser extent in the urine (approximately 36% of the dose).

Tadalafil is predominantly metabolized by CYP3A4 to a catechol metabolite, which undergoes extensive methylation and glucuronidation to form the methylcatechol and methylcatechol glucuronide conjugate, respectively. The major circulating metabolite is the methylcatechol glucuronide. Methylcatechol concentrations are less than 10% of glucuronide concentrations. *In vitro* data suggests that metabolites are not expected to be pharmacologically active at observed metabolite concentrations.

2.2.4.1 What are the single and multiple dose PK parameters?

Studies H6D-EW-LVCS and H6D-MC-LVHC evaluated the pharmacokinetics of Tadalafil in Japanese and Caucasian subjects after single and multiple doses, respectively.

Single Dose: The pharmacokinetics of tadalafil following single oral administration of 5, 10, 20, and 40 mg were assessed in healthy adult male Japanese and Caucasian subjects. Tadalafil's pharmacokinetic parameters in Japanese and Caucasian subjects are presented in the table below:

	Tadalafil Dose			
	5mg	10 mg	20 mg	40 mg
Japanese Subjects	N=23	N=23	N=24	N=23
$AUC_{0-\infty}$ (ng h/mL)	1784 (35.3)	3319 (32.5)	5825 (23.2)	10371 (32.3)
C_{max} (ng/mL)	95.6 (30.0)	174 (26.5)	292 (26.1)	446 (20.2)
t_{max} (h)*\	3.0 (0.5-4.0)	3.0 (0.5-4.0)	3.0 (1.0-4.0)	3.0 (0.5-4.0)
$t_{½}$ (h)	14.2 (19.9)	14.6 (20.9)	13.6 (17.1)	14.9 (20.0)
Caucasian Subjects	N=24	N=24	N=24	N=22
$AUC_{0-\infty}$ (ng h/mL)	1928 (36.5)	3701 (39.3)	7175 (40.3)	14015 (26.3)
C_{max} (ng/mL)	101 (31.4)	187 (29.0)	318 (29.9)	562 (26.6)
t_{max} (h)*	2.0 (1.0-8.0)	2.0 (0.5-4.0)	3.0 (1.0-4.0)	3.0 (0.5-4.0)
$t_{½}$ (h)	15.7 (35.1)	15.4 (32.2)	15.7 (30.1)	16.5 (26.9)

Values represent geometric mean (%CV)
*Values represent median (range)

Multiple Dose: Tadalafil pharmacokinetic parameters in healthy Japanese subjects following the administration of 40 mg tadalafil tablet once-daily for 10 days are listed in the table below:

	Day 1	Day 5	Day 10
N	18	15	15
AUC_{0-24} (ng h/mL)	7570 ± 24.5	10300 ± 23.8	9630 ± 20.5
C_{max} (ng/mL)	557 ± 19.0	732 ± 19.3	688 ± 16.1
T_{max} (h)[a]	3.00 (2.00 – 4.00)	3.00 (2.00 – 4.00)	3.00 (2.00 – 4.00)
$AUC_{(0-96)}$ (ng·h/mL)	-	-	14600 ± 24.5
$AUC_{(0-\infty)}$ (ng·h/mL)	-	-	14800 ± 24.9
CL_{ss}/F (L/h)	-		4.15 ± 20.5
Vz,ss/F (L)	-	-	85.7 ± 19.5
$t_{1/2}$ (h)	-	-	14.3 ± 12.1

Value represents geometric mean, ± %CV
[a] Median (range)

2.2.4.2 Based on PK parameters, what is the degree of linearity in the dose-concentration relationship?

Tadalafil systemic exposure (C_{max} and $AUC_{0-\infty}$) is not proportional over the dose range of 5 to 40 mg in both Japanese and Caucasian subjects following single oral dose, as shown in the table below:

	Estimate of Slope (90% CI)	Increase on Doubling Dose (90% CI)	Multiple of Dose Required for Doubling
Japanese Subjects			
$AUC_{0-\infty}$ (ng h/mL)	0.85 (0.81, 0.90)	1.81 (1.75, 1.86)	2.25
C_{max} (ng/L)	0.75 (0.71, 0.80)	1.68 (1.63, 1.74)	2.51
Caucasian Subjects			
$AUC_{0-\infty}$ (ng h/mL)	0.93 (0.89, 0.97)	1.91 (1.85, 1.96)	2.11
C_{max} (ng/mL)	0.80 (0.76, 0.85)	1.75 (1.69, 1.80)	2.37

2.2.4.3 How do the PK parameters change with time following chronic dosing?

Following chronic dosing of tadalafil, the pharmacokinetic parameters AUC and C_{max} increased due to accumulation. For example, in healthy Japanese subjects following chronic administration of 40 mg tadalafil tablet once-daily for 10 days, the steady state was attained by Day 5, and the mean accumulation ratio was 1.29.

2.3 Intrinsic Factors

2.3.1. What are the intrinsic factors that affect the exposure of tadalafil?

The intrinsic factors that affect the exposure of tadalafil were previously reviewed under NDA 21-368 for Cialis Tablets. Race was the only additional intrinsic factor evaluated in this review (i.e., Caucasians vs. Japanase).

Ethnic Factor: Study H6D-EW-LVCS compared the pharmacokinetics of tadalafil after single doses in Japanese and Caucasian subjects. The results showed that tadalafil systemic exposure ($AUC_{0-\infty}$) in Japanese subjects is significantly lower than in Caucasian subjects by 23% at 40 mg dose of tadalafil. The statistical comparison is shown in the table below.

PK Parameter	Tadalafil Dose (mg)	N	Ratio of LS means (Japanese/Caucasian)	90% CI Lower	90% CI Upper
$AUC_{0-\infty}$ (ng h/mL)	5	47	0.92	0.78	1.10
	10	47	0.90	0.76	1.06
	20	48	0.81	0.70	0.95
	40	45	0.74	0.64	0.86
C_{max} (ng/mL)	5	47	0.95	0.82	1.10
	10	47	0.93	0.81	1.06
	20	48	0.92	0.81	1.05
	40	45	0.79	0.71	0.89

2.4 Extrinsic Factors

2.4.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?

Tadalafil is a weak mechanism based inhibitor of CYP 3A4. Tadalafil inhibited CYP 3A4 *in vitro* in human liver microsomes in a time and concentration dependant manner, yielding k_{inact} and K_I values of 0.21 min^{-1} and 12 μM, respectively.

2.4.2 Is the drug an inhibitor and/or an inducer of PGP transport processes?

Tadalafil appeared to be P-gp inhibitor with estimated mean IC_{50} 11.5 ± 0.33 μM (n =3) in inside-out membrane vesicles prepared from stable over-expression of MDR1 in human embryonic kidney cells.

2.4.3 Are there other metabolic/transporter pathways that may be important?

In Madin-Darby canine kidney cells over-expressing human P-gp (MDCK-MDR1) tadalafil appeared to be a P-gp substrate.

2.4.4 What other co-medications are likely to be administered to the target population?

Studies H6D-MC-LVGZ, H6D-EW-LVHL, and H6D-EW-LVHM evaluated the pharmacokinetic interactions of tadalafil with bosentan, digoxin, and oral contraceptives, respectively. The results from these studies are summarized below.

Bosentan: The interaction between tadalafil (40 mg qd) and bosentan (125 mg qd) was evaluated in 14 healthy subjects. The results showed that bosentan at steady state significantly reduce tadalafil $AUC_{0-24,ss}$ by 42% and $C_{max,ss}$ by 27%.

Tadalafil	Geometric Means (%CV)		Ratio	90% CI	
	Tadalafil + Bosentan	Tadalafil		Lower	Upper
$AUC_{0-24,ss}$ (ng h/mL)	6950 (23.8)	11800(23.9)	0.56	0.55	0.62
$C_{max,ss}$ (ng/mL)	598 (20.2)	807 (19.8)	0.73	0.68	0.79

Tadalafil significantly increases $C_{max,ss}$ of bosentan by 20% and has no significant effect on bosentan $AUC_{0-24,ss}$.

Bosentan	Geometric Means (%CV)		Ratio	90% CI	
	Tadalafil + Bosentan	Tadalafil		Lower	Upper
$AUC_{0-24,ss}$ (ng h/mL)	5330(37.7)	4700 (37.2)	1.13	1.02	1.24
$C_{max,ss}$ (ng/mL)	1420 (43.5)	1190 (44.2)	1.20	1.05	1.36

Digoxin: The interaction of tadalafil (40 mg qd) with digoxin (0.25 mg qd) was evaluated in 20 healthy female subjects. The results showed that tadalafil at steady state do not alter the steady state systemic exposure of digoxin.

Digoxin	Geometric Means (%CV)		Ratio	90% CI	
	Digoxin Alone	Digoxin & Tadalafil		Lower	Upper
$AUC_{0-24,ss}$ (ng h/mL)	16.5 (23.8)	15.1 (20.3)	0.90	0.86	0.96
$C_{max,ss}$ (ng/mL)	1.48 (26.1)	1.4 (20.4)	0.95	0.86	1.04
$C_{min,ss}$ (ng/mL)	0.51 (25.7)	0.45 (23.0)	0.86	0.81	0.91

Oral Contraceptives: The interaction of tadalafil (40 mg qd) with an oral contraceptive containing 150 µg levonogestrel and 30 µg ethinylestradiol (one tablet qd) was evaluated in 28 healthy female subjects. The results showed that tadalafil at steady state significantly increases ethinylestradiol $AUC_{0-24,ss}$ by 25% and $C_{max,ss}$ by 70%.

Ethinylestradiol	Geometric Means (%CV)		Ratio	90% CI	
	Tadalafil + OC	Placebo + OC		Lower	Upper
$AUC_{0-24,ss}$ (pg h/mL)	940 (28.9)	739 (22.5)	1.25	1.17	1.33
$C_{max,ss}$ (pg/mL)	115 (33.0)	65.9 (36.0)	1.70	1.58	1.41

OC donates oral contraceptive

Tadalafil at steady state significantly reduces ethinylestradiol-sulfate $AUC_{0-24,ss}$ by 71% and $C_{max,ss}$ by 63%.

Ethinylestradiol-sulfate	Geometric Means (%CV)		Ratio	90% CI	
	Tadalafil + OC	Placebo + OC		Lower	Upper
$AUC_{0-24,ss}$ (pg h/mL)	3063 (85.9)	10565 (61.1)	0.29	0.24	0.33
$C_{max,ss}$ (pg/mL)	466 (53.8)	1276 (48.2)	0.37	0.33	0.41

OC donates oral contraceptive

Tadalafil at steady state does not alter the steady state systemic exposure ($AUC_{0-24,ss}$ and $C_{max,ss}$) of levonorgestrel.

Levonorgestrel	Geometric Means (%CV)		Ratio	90% CI	
	Tadalafil + OC	Placebo + OC		Lower	Upper
$AUC_{0-24,ss}$ (pg h/mL)	80479 (43.2)	77890 (49.2)	1.02	0.96	1.08
$C_{max,ss}$ (pg/mL)	6245 (33.7)	6047 (35.1)	1.02	0.97	1.08

OC donates oral contraceptive

2.5 General Biopharmaceutics

2.5.1 What is the effect of food on the bioavailability of the drug from the dosage form?

Study # H6D-EW-LVHO evaluated the effect that food has on the bioavailability of tadalafil. The results showed that food does not alter the systemic exposure of tadalafil following the administration of a 40 mg single dose.

Tadalafil	Geometric Mean (%CV)		Ratio	90% CI	
	Fed	Fast	Fed/Fast	Lower	Upper
AUC_{0-96} (ng h/mL)	16184(34.6)	14589(31.4)	1.14	1.05	1.22
$AUC_{0-\infty}$ (ng h/mL)	17386(41.9)	15404(36.8)	1.11	1.04	1.19
C_{max} (ng /mL)	586(18.0)	553(22.3)	1.07	0.99	1.15

2.6 Analytical Section

A brief summary of the different bioanalytical methods used is shown in the table below. Accepted validation indicates that method met the FDA guidance "Bioanalytical Method Validation" recommendations. Accepted study samples performance indicates that the quality control samples accuracy and precision met the guidance recommendations. Please refer to the individual studies review for more details. Note that HPLC-MS/MS was used in all the analytical methods described in the next table.

Study #	Analyte(s)	Matrix	Linear Range	Method Validation	Study Sample Performance
H6D-EW-LVCS	Tadalafil	Plasma	0.5 -500 ng/mL	Acceptable	Acceptable
H6D-MC-LVHC	Tadalafil	Plasma	0.5 -500 ng/mL	Acceptable	Acceptable
	Total IC710	Plasma	1.0 – 500 ng/mL	Acceptable	Acceptable
H6D-MC-LVGZ	Tadalafil	Plasma	0.5 -500 ng/mL	Acceptable	Acceptable
	Total IC710	Plasma	1.0 – 500 ng/mL	Acceptable	Acceptable
H6D-EW-LVHL	Digoxin	Plasma	0.1 – 20.0 ng/mL	Not Available	Acceptable
	Digoxin	urine	5.0 – 1000 ng/mL	Not Available	Acceptable
H6D-EW-LVHM	Ethinylestradiol	Plasma	2.0 - 250 pg/mL	Not Available	Acceptable
	Ethinylestradiol-sulfate	Plasma	50 – 10,000 pg/mL	Not Available	Acceptable
	Levonorgestrel	Plasma	50 – 12,500 pg/mL	Not Available	Acceptable
	Tadalafil	Plasma	0.5 -500 ng/mL	Acceptable	Acceptable
	Total IC710	Plasma	1.0 – 500 ng/mL	Acceptable	Acceptable

1 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

X § 552(b)(4) Draft Labeling

_____ § 552(b)(5) Deliberative Process

Withheld Track Number: Clin Pharm/Bio-1

4. Appendices

18 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

X § 552(b)(4) Draft Labeling

_____ § 552(b)(5) Deliberative Process

Withheld Track Number: Clin Pharm/Bio- 2

4.2. Appendix 2: Individual Studies Review

Study # H6D-MC-LVHC: Pharmacokinetics – Multiple Dose

Report #	H6D-MC-LVHC
Investigator	[redacted]
Study Site	[redacted] b(4)
Study Period	07/26/2006- 08/30/2006

Title

A study to examine the safety and pharmacokinetics of tadalafil (40 mg or placebo) in healthy adult Japanese subjects during multiple oral administration.

Objectives

Primary objective: Examine the safety of tadalafil in healthy adult Japanese subjects following 10 days multiple dose administration (once daily) with either 40 mg tadalafil or placebo.

Secondary objective: Assess the multiple dose pharmacokinetics of tadalafil and total methylcatechol glucuronide (Total IC710) metabolite following 40 mg tadalafil for 10 days.

Study Rationale (Per Applicant)

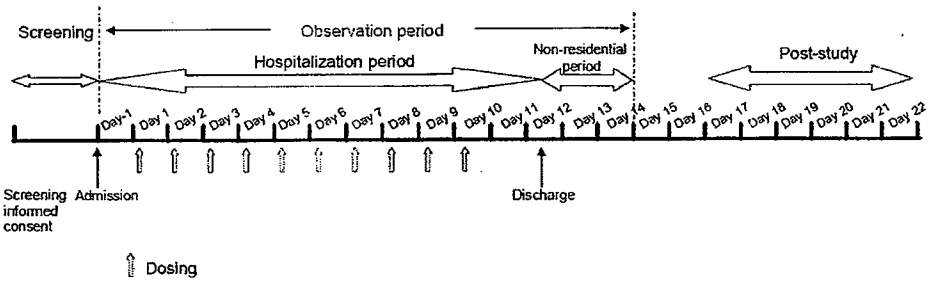
The highest proposed dose of tadalafil for patients with pulmonary arterial hypertension is 40 mg once-daily. The tolerability of multiple doses of tadalafil 40 mg once daily or more has not yet been studied in Japanese subjects. On the other hand, the tolerability of multiple doses of tadalafil up to 100 mg has been studied in Caucasian subjects.

Test Drug

Cialis®, 20 mg tadalafil tablets (lot #. CT526959)

Study Design

This was a single center, double-blind, randomized placebo controlled study in healthy Japanese subjects. Eighteen subjects were randomly assigned to the 40 mg tadalafil group and six subjects were assigned to the placebo group. The study design is illustrated in the schema below:



Pharmacokinetics sampling times

Pharmacokinetic sampling times following 40 mg administration of tadalafil is illustrated in the schema below:

Time (h)	0	0.5	1	2	3	4	8	12	16	24	48	72	96
Day1													
Day 5													
Day 7													
Day 8													
Day 10													

Assay Method

Two LC-MS/MS method were used to quantify tadalafil (Method 99VKJV01_LY_R4) and Total IC710 (Method 00183VKJV_LI_R1) in human plasma. The performance of the bioanalytical method during study sample analysis is displayed in the table below:

Analyte	Tadalafil	Total IC710
Accuracy %RE	-0.9-10.7	-9.1 – 2.0
Precision %CV	≤ 7.0	≤ 3.2

Details of methods validation are provided in the table below:

Method #	99VKJV01_LY_R4	00183VKJV_LI_R1
Type	HPLC-MS/MS	HPLC-MS/MS
Analyte	Tadalafil (IC351)	Total IC710
Calibration Range	0.5 – 500 ng/mL, weighted ($1/x^2$) quadratic fit	1.0 – 500 ng/mL, weighted ($1/x^2$) quadratic fit
LLOQ	0.5 ng/mL	1.0 ng/mL
Specificity	Analysis of blank and spiked plasma confirms method selectivity for tadalafil. Chromatograms were provided	Analysis of blank and spiked plasma confirms method selectivity for total IC710. Chromatograms were provided
Precision (intra-day) %CV	≤ 7.1	≤ 4.5
Precision (inter-day) %CV	≤ 10.5	≤ 5.5
Accuracy (inter-day) %RE	-1.5 – 1.3	2.3 – 6.8
Accuracy (intra-day) %RE	-9.8 – 9.2	-2.7 – 7.6
Recovery %	47.1 – 52.8	47.1 -65.5
Dilution	10-fold dilution of plasma samples is acceptable	10-fold dilution of plasma samples is acceptable
Stability	- Extracted plasma samples were stable for 41 hours - Plasma samples are stable up to 4 freeze/thaw cycles at ~ -70 °C - Plasma samples are stable for at least -425 days at -70 °C	- Extracted plasma samples were stable for 23 hours

Pharmacokinetic Analysis

Tadalafil and Total IC710 (methylcatechol glucuronide) pharmacokinetic parameters (AUC_{0-24}, $AUC_{0-tlast}$, $AUC_{0-\infty}$, C_{max}, T_{max}, $t_{1/2}$, CL_{ss}/F, $Vz_{,ss}/F$) on Days 1, 5, and 10 were computed by standard non-compartmental methods of analysis using WinNonlin.

Results

Twenty one subjects out of the enrolled 24 subjects completed the study. Three subjects were withdrawn due to adverse events mainly headache. Mean age was 22.4 ± 2.4 years for treatment group, and 21 ± 1.3 years for placebo group.

Tadalafil appeared to decline monoexponentially following the administration of once daily dose. Tadalafil and it major metabolite IC710 plasma pharmacokinetic parameters are displayed in the table below.

Tadalafil and it major metabolite IC710 plasma concentration vs. time profiles are displayed in Figures 1 and 2.

Steady state was achieved in 5 days as shown in Figure 3.

In general , mean plasma concentrations on Day 5 appear slightly higher than those on Day 10; however, any differences are generally <10% and within the variability of the measurements.

Total IC710 increased up to 24 hours post-dose on Day 1, reflecting a formation-dependent process. On Days 5 and 10, Total IC710 plasma concentrations were maintained throughout the dosing interval of 24 hours without appreciable elimination (Figure 4). On Day 10 after 24 hours, Total IC710 concentrations demonstrated monoexponential decay (Figure 2).

Tadalafil	Day 1	Day 5	Day 10
N	18	15	15
AUC_{τ} (ng h/mL)	7570 ± 24.5	10300 ± 23.8	9630 ± 20.5
C_{max} (ng/mL)	557 ± 19.0	732 ± 19.3	688 ± 16.1
T_{max} (h)[a]	3.00 (2.00 – 4.00)	3.00 (2.00 – 4.00)	3.00 (2.00 – 4.00)
$AUC_{(0\text{-}tlast)}$ (ng·h/mL)	-	-	14600 ± 24.5
$AUC_{(0\text{-}\infty)}$ (ng·h/mL)	-	-	14800 ± 24.9
RA1[b]	-	1.34 ± 11.2	1.25 ± 15.4
RA3[c]	-	1.39 ± 11.9	1.29 ± 14.0
CL_{ss}/F (L/h)	-		4.15 ± 20.5
Vz,ss/F (L)	-	-	85.7 ± 19.5
$t_{1/2}$ (h)	-	-	14.3 ± 12.1
Total IC710			
N	18	15	15
AUC_{τ} (ng h/mL)	4940 ± 25.4	13100 ± 32.9	13300 ± 31.2
C_{max} (ng/mL)	297 ± 28.5	615 ± 31.9	624 ± 30.9
T_{max} (h)[a]	23.8 (12.0 – 23.8)	8.0 (2.0 – 12.0)	4.00 (0.5 – 12.0)
$AUC_{(0\text{-}tlast)}$ (ng·h/mL)	-	-	29500 ± 36.0
$AUC_{(0\text{-}\infty)}$ (ng·h/mL)	-	-	31800 ± 37.8
RA1[b]	-	2.17 ± 16.1	2.20 ± 13.3
RA3[c]	-	2.76 ± 21.4	2.80 ± 21.0
CL_{ss}/F (L/h)	-		4.15 ± 20.5
Vz,ss/F (L)	-	-	85.7 ± 19.5
$t_{1/2}$ (h)	-	-	14.3 ± 12.1

Value represents geometric mean, ± %CV.

[a] Median (range)

[b] RA1 = $C_{max,ss}$ Day 5 or Day 10/ C_{max} Day1

[c] RA3 = $AUC_{\tau,ss}$ Day 5 or Day10/ AUC_{τ} Day 1

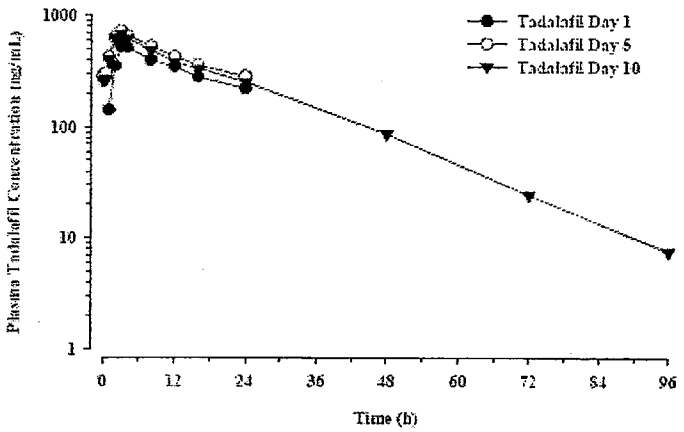


Figure 1. Tadalafil plasma concentration time profile following once daily administration of 40 mg tadalafil. Each point represents the arithmetic mean.

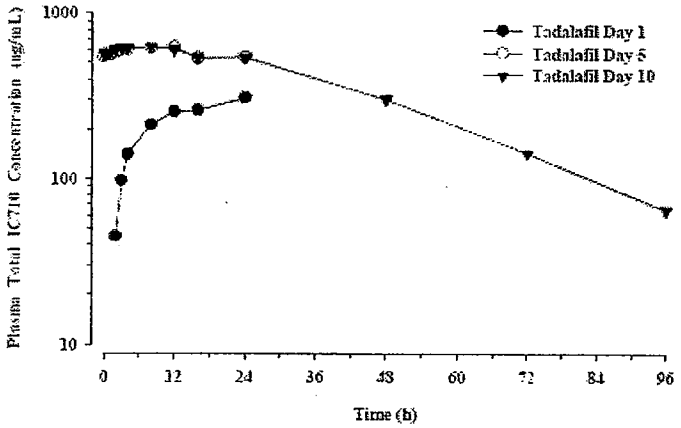


Figure 2. IC710 plasma concentration time profile following once daily administration of 40 mg tadalafil. Each point represents the arithmetic mean.

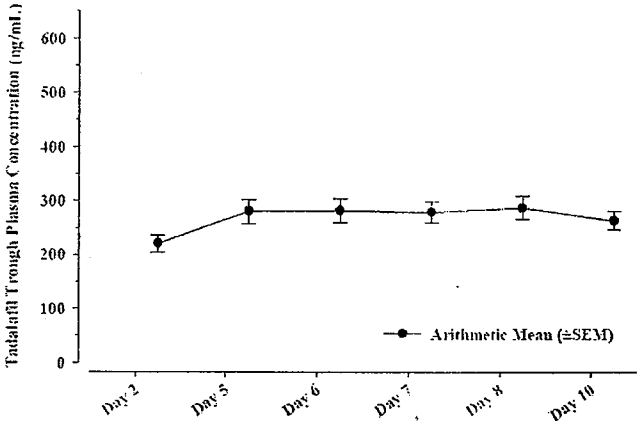


Figure 3. Tadalafil trough plasma concentrations on Days 2, 5, 6, 7, 8, and 10 following multiple once-daily doses of 40 mg tadalafil. Each point represents the arithmetic mean and error bars represent standard error of the mean.

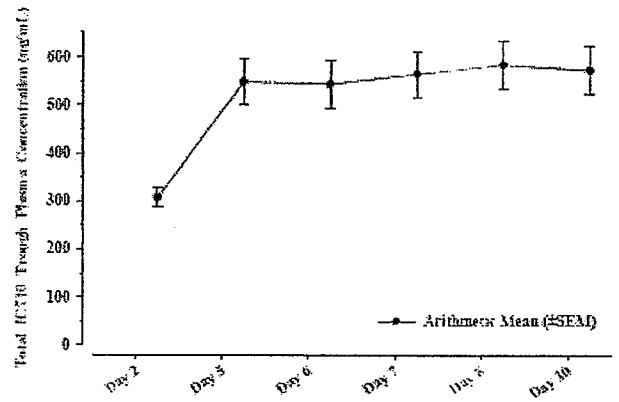


Figure 4. IC710 trough plasma concentrations on Days 2, 5, 6, 7, 8, and 10 following multiple once-daily doses of 40 mg tadalafil. Each point represents the arithmetic mean, and error bars represent standard error of the mean.

Safety (Per Applicant)

No death or serious adverse events were observed. The administration of 40 mg once-daily dose of tadalafil for 10 days was well tolerated in healthy Japanese subjects.

The most frequent drug-related adverse events reported by subjects receiving tadalafil were headache, pain in extremity, and back pain.

Three subjects were withdrawn due to headache, which was treated with acetaminophen. One subject had nausea and another had vomiting and back pain.

Conclusions

1. The steady-state of tadalafil is achieved by Day 5 for both tadalafil and its major metabolite IC710.
2. The frequency of tadalafil dosing (single or multiple once daily dose) does not affect the time to achieve maximum plasma concentrations.
3. The estimate of tadalafil accumulation is approximately 1.3-fold and for Total IC710 is 3- to 4-fold, following repeated once-daily dosing.
4. The administration of 40 mg of tadalafil once-daily is well tolerated in healthy Japanese subjects.

Study # H6D-EW-LVCS: Pharmacokinetics – Single Dose

Report #	H6D-EW-LVCS
Investigator	[redacted]
Study Sites	1. [redacted]
	2. [redacted]
Study Period	01/10/2001- 04/20/2001

b(4)

Title

A study to evaluate the safety and compare the pharmacokinetics of IC351 (tadalafil) in healthy adult male Japanese and healthy adult Caucasian subjects following single oral doses (5, 10, 20, and 40 mg and placebo).

Objectives

To assess the safety of IC351 (tadalafil) in healthy adult Japanese males and healthy adult Caucasian males during single oral administration at 5, 10, 20 and 40 mg and placebo.

A comparison in terms of both safety and the pharmacokinetics of tadalafil following single oral administration of IC351 at 5, 10, 20, and 40 mg between healthy adult Japanese males and healthy adult Caucasian males.

Study Rationale (Per Applicant)

Prior to this study there were no data for safety of tadalafil in healthy Japanese subjects. The study helps in comparing tadalafil pharmacokinetics in healthy adult Japanese and Caucasian subjects.

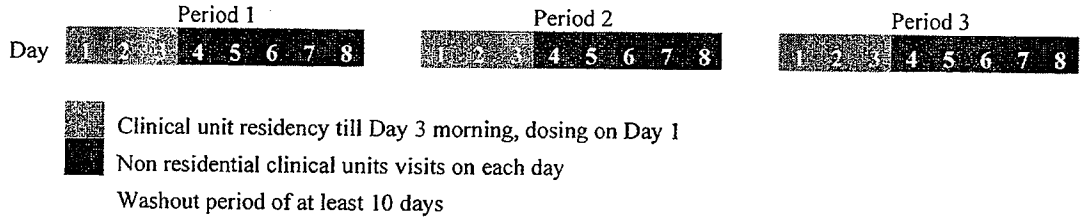
Test Drug

Product	Dose	Parent Lot No.	Japanese Lot No.	Caucasian Lot No.
IC351	5 mg	CT15001	CT18228	CT18298
IC351	10 mg	CT15185	CT18229	CT18299
IC351	20 mg	CT17988	CT18230	CT18300
Placebo	(5 mg)	CT15183	CT18225	CT18303
Placebo	(10 mg)	CT15181	CT18226	CT18304
Placebo	(20 mg)	CT18016	CT18227	CT18305

Study Design

This was a single-dose, double-blind, placebo-controlled, randomized three-period incomplete block design study in Japanese and Caucasian male subjects. Seventy two subjects (36 in each ethnic group) entered the study. Each subject participated in three treatment periods and received three of the five available study treatments (5, 10, 20 and

40 mg tadalafil and placebo). Tadalafil was administered as single daily oral doses of 5, 10, 20, and 40 mg in combination with sufficient placebo tablets to ensure that the same number of tablets (four) was given in each treatment period. The study design is illustrated in the schema below:



Pharmacokinetics sampling times

Blood samples for tadalafil pharmacokinetics were obtained at pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 8, 16, 24, 72, 96, 120 h post-dose.

Assay Method

An HPLC-MS/MS method was used to quantify tadalafil (Method 99VKJV01_LY_AM2) human plasma. During the unknown plasma study samples analysis the calibration range of tadalafil was 0.5 – 500.0 ng/mL (%CV ≤ 6.2 and RE (%) = -1.5 – 1.0). The precision of the quality control samples was ≤ 3.8 %, and the accuracy was between -3.5% and -1.4%. Details of methods validation are provided in the table below:

Method #	99VKJV01_LY_AM2
Type	HPLC-MS/MS
Analyte	Tadalafil (IC351)
Calibration Range	0.5 – 500 ng/mL, weighted ($1/x^2$) quadratic fit
LLOQ	0.5 ng/mL
Specificity	Analysis of blank and spiked plasma confirms method selectivity for tadalafil. Chromatograms were provided
Precision (intra-day) %CV	≤ 7.1
Precision (inter-day) %CV	≤ 10.5
Accuracy (inter-day) %RE	-1.5 – 1.3
Accuracy (intra-day) %RE	-9.8 – 9.2
Recovery %	47.1 – 52.8
Dilution	10-fold dilution of plasma samples is acceptable
Stability	- Extracted plasma samples were stable for 41 hours - Plasma samples are stable up to 4 freeze/thaw cycles at ~ -70 °C - Plasma samples are stable for at least -425 days at -70 °C

Pharmacokinetic Analysis

Tadalafil pharmacokinetic parameters ($AUC_{0-\infty}$, AUC_{0-120}, C_{max}, T_{max}, $t_{1/2}$, CL/F, and Vz/F) on Days 1, 5, and 10 were computed by standard non-compartmental methods of analysis using WinNonlin.

Statistical Method

Regression analysis was performed over dose ranges 5 to 20 mg and 5 to 40 mg to examine the relationship between pharmacokinetic parameters and dose. Dose-proportionality was declared for AUC and C_{max} by comparing the 90% confidence intervals of the regression line to equivalence limits (0.90, 1.11). Japanese to Caucasian ratios between regression line-slopes, intercepts and values at 20 and 40 mg were calculated for AUC and C_{max} along with 90% confidence intervals. Equivalence was declared by comparing the 90% confidence intervals to the equivalence limits (0.70, 1.43). Additional analyses were performed at each dose separately to compare least square means for AUC and C_{max} for the ethnic groups.

Results

Thirty four Japanese subjects and 33 Caucasian subjects completed the study. Mean age was 23 ± 2.1 (20 - 28) and 32 ± 6.6 (22 – 44) in Japanese and Caucasian subjects, respectively.

Tadalafil pharmacokinetic parameters in Japanese and Caucasian subjects are presented in the table below:

	Tadalafil Dose			
	5mg	10 mg	20 mg	40 mg
Japanese Subjects	N=23	N=23	N=24	N=23
$AUC_{0-\infty}$ (ng h/mL)	1784 (35.3)	3319 (32.5)	5825 (23.2)	10371 (32.3)
C_{max} (ng/mL)	95.6 (30.0)	174 (26.5)	292 (26.1)	446 (20.2)
t_{max} (h)*\	3.0 (0.5-4.0)	3.0 (0.5-4.0)	3.0 (1.0-4.0)	3.0 (0.5-4.0)
$t_{½}$ (h)	14.2 (19.9)	14.6 (20.9)	13.6 (17.1)	14.9 (20.0)
Caucasian Subjects	N=24	N=24	N=24	N=22
$AUC_{0-\infty}$ (ng h/mL)	1928 (36.5)	3701 (39.3)	7175 (40.3)	14015 (26.3)
C_{max} (ng/mL)	101 (31.4)	187 (29.0)	318 (29.9)	562 (26.6)
t_{max} (h)	2.0 (1.0-8.0)	2.0 (0.5-4.0)	3.0 (1.0-4.0)	3.0 (0.5-4.0)
$t_{½}$ (h)	15.7 (35.1)	15.4 (32.2)	15.7 (30.1)	16.5 (26.9)

Values represent geometric mean (%CV)

Over the full dose range of 5 to 40 mg, C_{max} and $AUC_{0-\infty}$ increased in a less than dose proportional manner for both ethnic groups, as shown in Figures 1 and 2 and the table below:

	Estimate of Slope (90% CI)	Increase on Doubling Dose (90% CI)	Multiple of Dose Required for Doubling
Japanese Subjects			
$AUC_{0-\infty}$ (ng h/mL)	0.85 (0.81, 0.90)	1.81 (1.75, 1.86)	2.25
C_{max} (ng/L)	0.75 (0.71, 0.80)	1.68 (1.63, 1.74)	2.51
Caucasian Subjects			
$AUC_{0-\infty}$ (ng h/mL)	0.93 (0.89, 0.97)	1.91 (1.85, 1.96)	2.11
C_{max} (ng/mL)	0.80 (0.76, 0.85)	1.75 (1.69, 1.80)	2.37

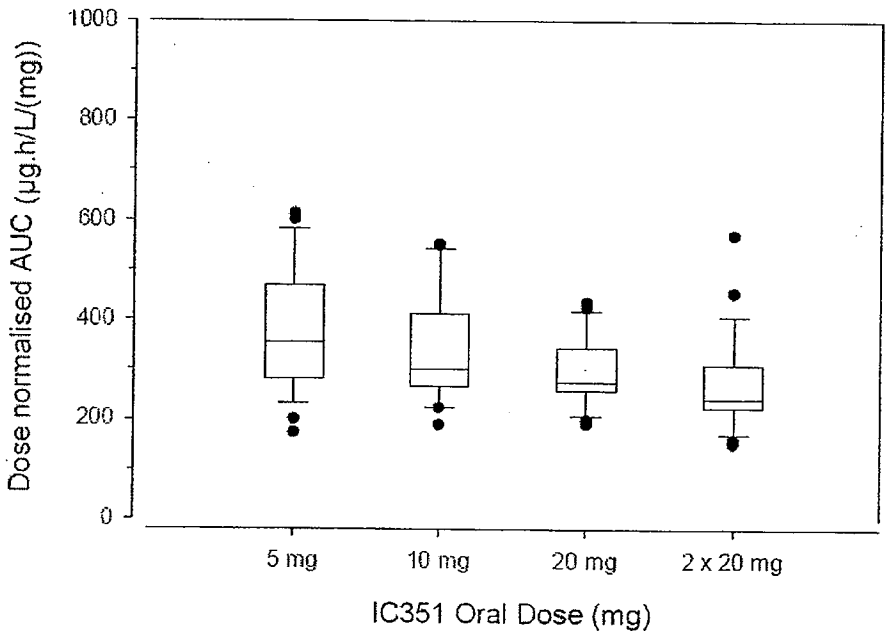


Figure 1. Box and Whisker plots for dose normalized $AUC_{0-\infty}$ following single oral administration of 5, 10, 20 or 40 mg (2 x 20 mg) of tadalafil in healthy male Japanese subjects.

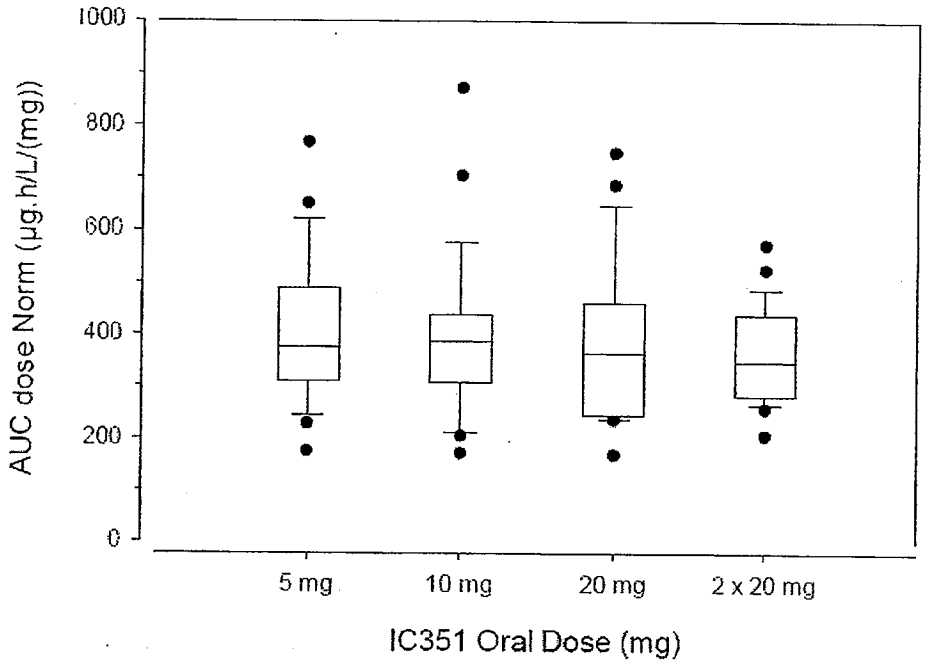


Figure 2. Box and Whisker plots for dose normalized $AUC_{0-\infty}$ following single oral administration of 5, 10, 20 or 40 mg (2 x 20 mg) of tadalafil in healthy male Caucasian subjects.

The statistical comparison of tadalafil pharmacokinetic parameters in Japanese and Caucasian is shown in the table below. Tadalafil systemic exposure ($AUC_{0-\infty}$) in Japanese subjects was significantly lower than in Caucasian subjects by 23% at 40 mg dose of tadalafil.

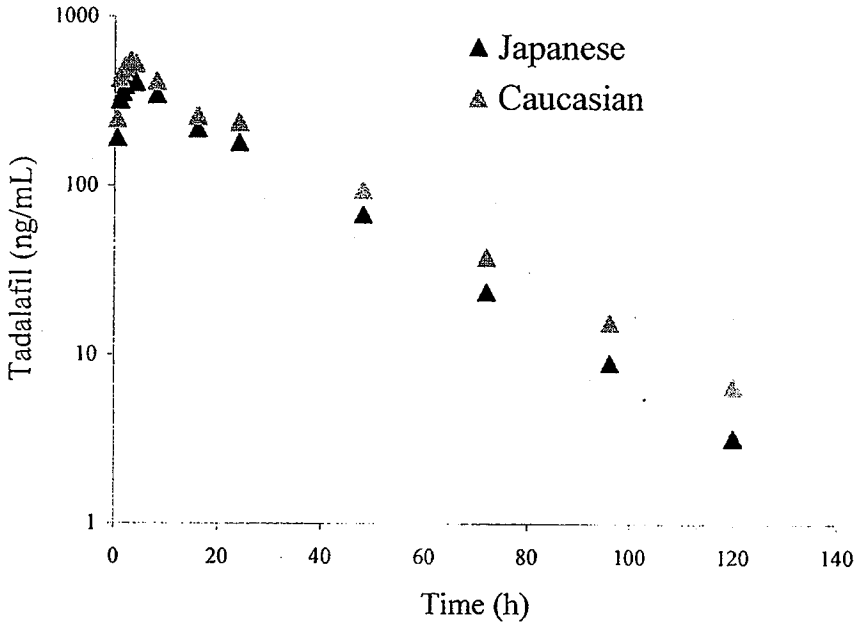
Parameter	Tadalafil Dose (mg)	Ratio of LS means (Japanese/Caucasian)	90% CI Lower	90% CI Upper	N
$AUC_{0-\infty}$ (ng h/mL)	5	0.92	0.78	1.10	47
	10	0.90	0.76	1.06	47
	20	0.81	0.70	0.95	48
	40	0.74	0.64	0.86	45
Cmax (ng/mL)	5	0.95	0.82	1.10	47
	10	0.93	0.81	1.06	47
	20	0.92	0.81	1.05	48
	40	0.79	0.71	0.89	45

Conclusions

1. Tadalafil systemic exposure ($AUC_{0-\infty}$ and C_{max}) is less than dose proportional in the dose range of 5.0 – 40 mg, in Japanese and Caucasian subjects.
2. Tadalafil AUC in Japanese subjects is significantly lower than Caucasian subjects.

Reviewer Comments:

The difference in tadalafil exposure (AUC) between Japanese and Caucasian subjects at 40 mg dose can not be attributed to weight. Per applicant adding the weight as a covariate in the analysis did not make AUC at 40 mg equivalent between ethnic groups. It also can not be attributed to drug elimination since the terminal phase of the plasma concentration vs. time profile are parallel but consistently lower in Japanese subjects as shown in the Figure below. The difference can be attributed to tadalafil absorption evident by the lower Cmax in Japanese compared to Caucasian subjects.



Study # 2033IV-DI001: In Vitro Metabolism

Report #	2003IV-DI001
Investigator	[redacted] b(4)
Study Site	Eli Lilly and Company, Lilly Corporate Center Indianapolis, IN 46285
Study Period	December 2003

Title

Kinetic examination of mechanism-based inhibition of cytochrome P450 3A4 by tadalafil

Objective

To examine the potential mechanism-based inhibition of CYP3A4 by tadalafil in a kinetically rigorous manner using CYP3A4 supersomes.

Study Rationale

Tadalafil (IC351; LY450190) was suspected as a mechanism-based inhibitor of CYP3A due to the presence of a methylenedioxy functional group. A previous in vitro study in human liver microsomes showed that inhibition of CYP3A activity occurred in a time- and concentration-dependent manner.

Study Design

Tadalafil (1.0 -20 μM) was first incubated with CYP3A4 supersomes, with or without NADPH (inactivation assay). At timed intervals up to 8 minutes, aliquots of the inactivation assay mixtures were added to an activity assay mixture containing the CYP3A4 substrate midazolam (100 μM) and NADPH (CYP3A4 activity assay).

Results

Tadalafil preincubation inhibited 1'-OH midazolam formation in a time- and concentration-dependent manner. Mechanism-based inhibition of CYP3A4 by tadalafil was observed, yielding k_{inact} and K_I values of 0.21 min^{-1} and 12 μM, respectively.

Conclusion

Tadalafil is a weak CYP3A4 mechanism based inhibitor compared to diltiazem, moderate CYP3A4 mechanism based inhibitor[1], and erthyromycin, as shown in the table below.

	Tadalafil	Eryhtromycin	Diltiazem
$Cl_{iact}(min^{-1}\ mM^{-1})$	17	59	327

$Cl_{inact} = K_{inact}/K_I$

[1] Br J Clin Pharmacol 2001; 51: 461-470

Study # 2006TP-Pgp02: In Vitro Transport

Report #	2006TP-Pgp02
Investigator	[redacted] b(4)
Study Site	Eli Lilly and Company, Lilly Corporate Center Indianapolis, IN 46285
Study Period	August 2006

Title

Use of in vitro models to assess the interaction of LY450190 with P-glycoprotein

Objective

To examines if tadalafil (LY450190) interacts with P-glycoprotein (P-gp) as a possible substrate and as an inhibitor.

Study Rationale

The interaction of a drug with P-gp has potentially significant clinical consequences. P-gp has been shown to play a role in drug-drug interaction and impact the pharmacokinetics of drugs by limiting oral absorption, modulating excretion, and restricting penetration into the central nervous system.

Study Design

Madin-Darby canine kidney cells over-expressing human P-gp (MDCK-MDR1) were utilized to examine ^{14}C-tadalafil as a P-gp substrate. Inside-out membrane vesicles prepared from stable over-expression of MDR1 in human embryonic kidney cells were used to determine if tadalafil was an inhibitor of human P-gp. ^{3}H-vinblastine was used as positive control. LSN335984, verapamil and quinidine were used as P-gp inhibitors.

Results

The bi-directional transport of tadalafil and vinblastine in the presence and absence of 5 µM LSN335984 (potent P-gp inhibitor), in two different experiments, is shown in the table below:

Exp	Substrate (5 µM)	Permeability ± SD (X 10^{-6}, cm/s)					
		B to A	A to B	B to A with inhibitor	A to B with inhibitor	Ratio without inhibitor	Ratio with inhibitor
1	Tadalafil	80.1 ± 3.92	17.5 ± 0.48	57.4 ± 3.22	52.7 ± 0.88	4.58	1.09
1	Vinblastine	47.6 ± 2.86	0.99 ± 0.08	15.3 ± 0.54	5.56 ± 0.36	48.5	2.75
2	Tadalafil	79.8 ± 3.68	17.2 ± 0.83	57.6 ± 3.62	49.3 ± 0.60	4.64	1.17
2	Vinblastine	65.5 ± 6.62	1.94 ± 0.11	23.9 ± 1.07	5.51 ± 0.19	33.8	4.34

The bi-directional transport of tadalafil was inhibited in the presence of three P-gp inhibitors as shown in the table below:

Inhibitor	Tadalafil Permeability ± SD (X 10^{-6}, cm/s)		Ratio
	B to A	A to B	
No Inhibitor	85.8 ± 5.55	14.3 ± 1.18	6.00
5μM LSN335984	57.9 ± 2.05	49.6 ± 2.73	1.17
50 μM verapamil	56.8 ± 2.73	41.9 ± 2.83	1.36
50 μM quinidine	58.9 ± 1.37	43.4 ± 2.31	1.36

Tadalafil was evaluated as P-gp inhibitor in the concentration range 0.5 – 100 μM. The estimated mean IC_{50} value of tadalafil in three different experiments was 11.5 ± 0.33 μM (95% CI 12.9 – 10.1). The estimated 95% CI of [I]/ IC_{50} ratio is 0.16 – 0.2. [I] was set to 2.07 μM and was calculated using C_{max} on Day 10 following ten oral daily doses of 40 mg tadalafil.

Conclusions

1. Tadalafil appears to be P-gp substrate.
2. Tadalafil appears to be P-gp inhibitor.

Reviewer Comments:

A clinical study (H6D-EW-LVHL) demonstrated that tadalafil at steady states does not affect digoxin pharmacokinetics, indicating that tadalafil does not affect P-gp activity in vivo.

Study # H6D-MC-LVGZ: Tadalafil-Bosentan DDI

Report #	H6D-MC-LVGZ
Investigator	
Study Site	b(4)
Study Period	09/19/2005 - 05/12/2006

Title

A pharmacokinetic interaction study between tadalafil and bosentan in healthy male subjects

Objectives

Primary objective: To determine if there is a pharmacokinetic interaction between tadalafil and bosentan

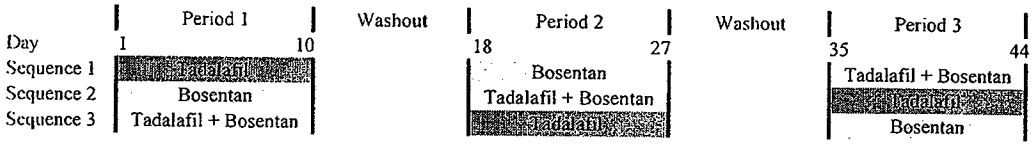
Secondary Objective: to assess the safety and tolerability of tadalafil and bosentan when administered alone and together; to assess the pharmacokinetics of tadalafil (40 mg once-daily) and bosentan (125 mg twice-daily) administered alone; and to assess the effect on vital signs of tadalafil (40 mg once-daily) and bosentan (125 mg twice-daily) administered alone and in combination.

Study Rationale

Bosentan is used for the chronic treatment of pulmonary arterial hypertension (PAH) and tadalafil is being developed for the treatment of PAH. Bosentan induces CYP 2C9 and 3A4 and tadalafil is a CYP3A4 substrate, therefore a pharmacokinetic interaction may occur.

Study Design

This was a randomized, open-label, three-period crossover pharmacokinetic drug-drug interaction study. Fifteen subjects were enrolled in the study. The study schema is illustrated below:



Tadalafil 40-mg QD
Bosentan 125 mg BID

Test drugs

Bosentan and tadalafil were administered orally:

1. Bosentan : Tracleer® 0.25 mg tablets (batch # BP042A0102)
2. Tadalafil : Cialis® 20 mg tablets (batch # A105967)

Pharmacokinetics Sampling Times

1. Tadalafil Treatment	
Day1	Pre-dose and at 0.5, 1, 2, 3, 4, 8, 12, 16 and 24 h post-dose
Day5	Pre-dose and at 0.5, 1, 2, 3, 4, 8, 12, 16 and 24 h post-dose
Day 7 and Day 8	Pre-dose
Day 10	Pre-dose and at 0.5, 1, 2, 3, 4, 8, 12, 16, 24, 48, 72 and 96 h post-dose
2. Bosentan Treatment	
Day 1	Pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, and 12 h post morning dose
Day 10	Pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, and 12 h post morning dose
3. Tadalafil + Bosentan Treatment	
Day 1	Tadalafil: Pre-dose and at 0.5, 1, 2, 3, 4, 8, 12, 16 and 24 h post-dose
Day 1	Bosentan: Pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, and 12 h post morning dose
Day 10	Tadalafil: Pre-dose and at 0.5, 1, 2, 3, 4, 8, 12, 16, 24, 48, 72 and 96 h post-dose
Day 10	Bosentan: Pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, and 12 h post morning dose

Assay Methods

1. An HPLC-MS/MS method was used to quantify tadalafil and its metabolite (IC710) in plasma (Method 00178VKJV_LI_R1). The calibration range of tadalafil was 0.5 – 500.0 ng/mL (%CV ≤ 7.7 and RE (%) = -1.6 – 0.9). The precision of the quality control samples was ≤ 9.6 %, and the accuracy was between -1.6% and 2.7%. The calibration range of IC710 was 1.0 – 500 ng/mL (%CV ≤ 5.9 and RE (%) = -2.1 – 3.0). The precision of the quality control samples was ≤ 6.1 %, and the accuracy was between -8.4% and 2.2%. Details of the method validation are provided in the table below:

Method #	99VKJV01_LY_R4	
Type	HPLC-MS/MS	
Analyte	Tadalafil (IC351)	Metabolite (IC710)
Calibration Range	0.5 – 500 ng/mL,	1.0 – 500 ng/mL
	weighted ($1/x^2$) quadratic fit	
LLOQ	0.5 ng/mL	1.0 ng/mL
Specificity	Analysis of blank and spiked plasma confirms method selectivity for the two analytes. Chromatograms were provided	
Precision (intra-day) %CV	≤ 6.9	≤ 5.4
Precision (inter-day) %CV	≤ 6.1	≤ 7.6
Accuracy (inter-day) %RE	4.5 – 8.3	-.5 – 2.8
Accuracy (intra-day) %RE	1.2 – 13.4	-6.3 – 7.0
Recovery %	56.3 – 67.5	49.5 – 64.7
Stability	- Extracted plasma samples were stable for 67 hours	

2. An HPLC-MS/MS method was used to quantify total IC710 in plasma (Method 00178VKJV_LI_R1). The calibration range of total IC710 was 1.0 – 500.0 ng/mL (CV ≤ 4.3% and RE (%) = -0.4 – 0.7). The precision of the quality control samples was ≤ 4.6 %, and the accuracy was between 0.1% and -4.4%. There were no interfering peaks in the submitted study subject's chromatograms. Details of the method validation are provided in the table below:

Method #	00183VKJV_LI_R1
Type	HPLC-MS/MS
Analyte	Total IC710
Calibration Range	1.0 – 500 ng/mL, weighted ($1/x^2$) quadratic fit
LLOQ	1.0 ng/mL
Specificity	Analysis of blank and spiked plasma confirms method selectivity for total IC710. Chromatograms were provided
Precision (intra-day) %CV	≤ 4.5
Precision (inter-day) %CV	≤ 5.5
Accuracy (inter-day) %RE	2.3 – 6.8
Accuracy (intra-day) %RE	-2.7 – 7.6
Recovery %	47.1 -65.5
Stability	Extracted plasma samples were stable for 23 hours

Reviewer Note: Bosentan and its metabolites analytical method validation and performance were not provided.

Statistical Analysis

Tadalafil and bosentan pharmacokinetic parameters were computed by standard non-compartmental methods of analysis

The following pharmacokinetic parameters were subject to statistical analysis for tadalafil, total IC710, bosentan, and bosentan metabolites (Ro 47-8634, Ro 48-5033, and Ro 64-1056) on Day 1 and Day 10: AUC_{0-24}, C_{max} and t_{max}. Log-transformed (base e) pharmacokinetic parameters AUC_{0-24} and C_{max} were analyzed using a mixed effects model allowing for the fixed effect of sequence, period and treatment, and the random effect of subject. Comparison of pharmacokinetic parameters was run between the following pairs of treatments:

a) Tadalafil (2 x 20 mg tablet marketed Cialis®) alone and its combination with bosentan (125 mg twice-daily).

b) Bosentan (125 mg twice-daily) alone and its combination with tadalafil (2 x 20 mg tablet marketed Cialis®).

This was achieved by estimating the difference and 90% confidence intervals of the difference on the log scale and then back transforming to produce their ratio and its 90% confidence interval on the natural scale.

A nonparametric analysis of t_{max} using Wilcoxon Signed Rank Test was conducted between the following pairs of treatments:

a) Tadalafil (2 x 20 mg tablet marketed Cialis®) alone and its combination with bosentan (125 mg twice-daily).

b) Bosentan (125 mg twice-daily) alone and its combination with tadalafil (2 x 20 mg tablet marketed Cialis®).

The median of the differences and the corresponding 90% confidence intervals between the treatment combinations were calculated

Results

Study Population

Fifteen healthy subjects, 9 males and 6 females, participated in the study with mean age of 29.4 ± 10.9 years (range 19 - 52). One subject withdrew due to backache and another was withdrawn due to hematuria.

Pharmacokinetics Analysis

As shown in Figure 1, steady state levels of tadalafil were obtained within 5 days of the initiation of tadalafil 40 mg daily dose. Also, tadalafil systemic exposure (AUC_{0-24} and C_{max}) was similar across Days 5 and 10, as shown in the table below:

	Tadalafil Pharmacokinetic Parameters Geometric Mean (%CV)		
	Day 1	Day 5	Day 10
N	14	13	13
AUC_{0-24} (ng h/mL)	8560 (22.8)	10900 (24.6)	11800 (23.9)
C_{max} (ng/mL)	614 (23.0)	763 (20.7)	807 (19.8)

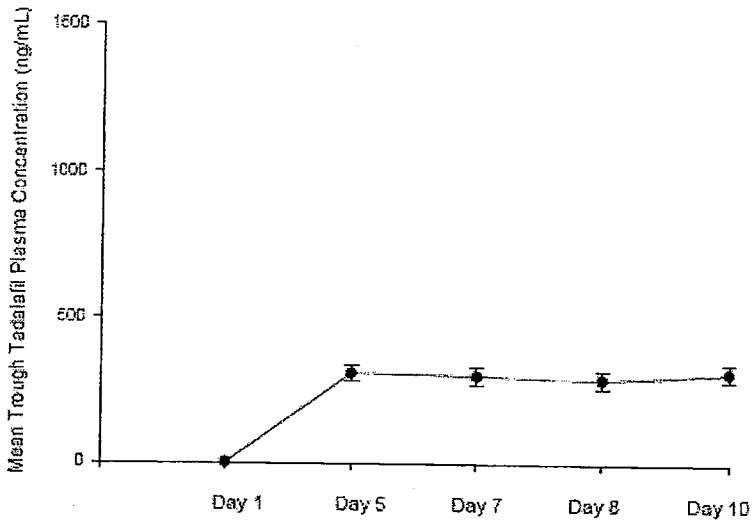


Figure 1. Tadalafil trough plasma concentrations on Days 1, 5, 7, 8, and 10 following multiple doses of 40 mg tadalafil once-daily for 10 days. Each point represent the arithmetic mean (n=13) and error bars represent standard error of the mean.

The co-administration of bosentan significantly reduced tadalafil $AUC_{0\text{-}24,ss}$ and $C_{max,ss}$ by 42% and 27%, respectively, as shown in the table below:

Dependent Variable	Geometric Means (%CV)		Ratio	90% CI	
	Tadalafil + Bosentan	Tadalafil		Lower	Upper
AUC_{0-24} (ng h/mL)					
Day 1	8640 (14.9)	8560 (22.8)	1.01	0.92	1.12
Day10	6950 (23.8)	11800(23.9)	0.56	0.55	0.62
C_{max}, (ng/mL)					
Day1	650 (16.2)	614 (23.0)	1.06	0.941	1.20
Day 10	598 (20.2)	807 (19.8)	0.73	0.68	0.79
T_{max}, (h)					
Day 1	4.0 (1.0 – 4.1)*	4.0 (3.0 – 4.1)*	-0.03**	-1.0	0.00
Day 10	3.0 (2.0- 4.1)*	4.0 (2.0 – 4.1)*	-0.03**	-1.0	0.00

* Median and range, ** Median difference

The co-administration of bosentan slightly but not significantly increased Total IC710 (tadalafil metabolite) as shown in the table below:

Dependent Variable	Geometric Means (%CV)		Ratio	90% CI	
	Tadalafil + Bosentan	Tadalafil		Lower	Upper
AUC_{0-24} (ng h/mL)					
Day 1	4110 (26.0)	3540 (17.3.8)	1.15	1.02	1.30
Day10	14700 (37.9)	12800 (32.5)	1.11	0.98	1.26
$C_{max,}$ (ng/mL)					
Day1	260 (30.3)	225 (23.1)	1.12	0.99	1.26
Day 10	702 (38.6)	616 (30.6)	1.09	0.96	1.25

When administered alone bosentan systemic exposure was lower on Day 10 compared to Day 1, as shown in the table below. This can be attributed to CYP3A induction by bosentan. No overt differences in bosentan metabolites Ro 47-8634, Ro 48-5033 or Ro 64-1056 pharmacokinetic parameters were discerned between Day 1 and Day 10.

	Bosentan Pharmacokinetic Parameters Geometric Mean (%CV)	
	Day 1	Day 10
N	14	14
AUC_{0-24} (ng·h/mL)	7540 (33.3)	4700 (37.2)
C_{max} (ng/mL)	1890 (36.9)	1190 (44.2)
t_{max} (h)*	3.50 (2.00 - 6.05)	4.02 (2.00 - 5.00)

* Median and range

The co-administration of tadalafil significantly increased bosentan $C_{max,ss}$ by 20%. Bosentan It also increased bosentan $AUC_{0-24,ss}$ by 13%, though this increase was not statistically significant, as shown in the table below. Tadalafil did not have statistically significant effect on the pharmacokinetics of bosentan metabolites Ro 47-8634, Ro 48-5033, and Ro 64-1056.

Dependent Variable	Geometric Means (%CV)		Ratio	90% CI	
	Tadalafil + Bosentan	Tadalafil		Lower	Upper
AUC_{0-24} (ng h/mL)					
Day 1	8200 (46.1)	7540 (33.3)	1.08	0.92	1.13
Day10	5330(37.7)	4700 (37.2)	1.13	1.02	1.24
$C_{max,}$ (ng/mL)					
Day1	2020 (40.0)	1890 (36.9)	1.08	0.93	1.25
Day 10	1420 (43.5)	1190 (44.2)	1.20	1.05	**1.36**
$T_{max,}$ (h)					
Day 1	3.0 (1.0 – 5.1)*	3.5 (2.0 – 6.1)*	0.0**	-1.0	0.00
Day 10	3.0 (1.1- 5.1)*	4.0 (2.0 – 5.0)*	-0.99**	-1.03	0.00

Safety Results (Per Applicant)

No death or serious adverse events occurred during this study.

Once-daily doses of 40 mg tadalafil were generally well tolerated when either administered alone for 10 days or co-administered with 125 mg bosentan for 10 days. The incidence of adverse events and number of subjects reporting adverse events were similar when tadalafil was administered alone and in combination with bosentan. All adverse events reported during the study were mild or moderate in severity, with the exception of one severe back pain that occurred in a subject during tadalafil treatment.

The most frequently reported drug-related adverse events during tadalafil treatment were headache, nasal congestion, nausea, myalgia, back pain, ocular hyperemia and flushing.

Reviewer Note: The evaluation of the adverse events might be bias due to the fact that the study was conducted as an open-label.

Conclusions

1. Bosentan significantly reduces tadalafil $AUC_{0-24,ss}$ by 42% and $C_{max,ss}$ by 27%.
2. Tadalafil significantly increases $C_{max,ss}$ of bosentan by 20%.
3. Tadalafil has no significant effect on bosentan $AUC_{0-24,ss}$.

Study # H6D-EW-LVHL: Tadalafil-Digoxin DDI

Report #	H6D-EW-LVHL
Investigator	
Study Site	
Study Period	12/28/2006 - 03/02/2007

b(4)

Title

A study to investigate the effect of tadalafil on the steady-state pharmacokinetics of digoxin in healthy subjects.

Objectives

Primary objective: To determine the effects of multiple-dose tadalafil (40 mg) on the steady state pharmacokinetics of digoxin

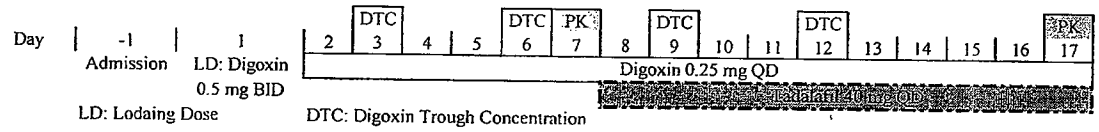
Secondary Objective: To assess the safety and tolerability of co-administration of tadalafil and digoxin in healthy subjects.

Study Rationale

Due to the likelihood for concomitant use of digoxin and tadalafil in clinical practice, and the narrow therapeutic index of digoxin, changes in steady-state serum concentrations of digoxin may result in toxicity or loss of efficacy.

Study Design

This was a single center, open-label, single-sequence drug-drug interaction study. The Study schema is displayed below:



Test drugs

Digoxin and tadalafil were administered orally:
Digoxin : Lanoxin® 0.25 mg tablets, (batch #. B2675L), 1 tablet qd.
Tadalafil : Cialis® 20 mg tablets (batch #. A226547), 2 tablets qd.

Pharmacokinetics Sampling Times

Blood samples for digoxin pharmacokinetics on Day 7 and Day 17 were obtained at per-dose and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 18.0, and 24.0 hours post dose. Urine output was collected at per-dose and at 0 to 6, 6 to 12, and 12 to 24 hours post digoxin administration on Day 7 and Day 17.

Assay Methods

1. The quantification of digoxin in serum was performed using an LC-MS/MS method (Method 04265VBTH_AD_R3).

   During the study sample analysis the calibration range of digoxin was 0.1 – 20.0 ng/mL (CV ≤ 4.8% and RE (%) = -2.4 – 2.0%). The precision of the quality control samples was ≤ 11.0 %, and the accuracy was between -2.6% and 3.0%. There were no interfering peaks in the submitted study subject's chromatograms.

2. The quantification of digoxin in urine samples was performed using a validated LC-MS/MS method (Method 05002VBTH_AIN_R2).

   During the study sample analysis the calibration range of digoxin was 5.0 – 1000.0 ng/mL (CV ≤ 2.3% and RE (%) = -4.8 – 2.5%). The precision of the quality control samples was ≤ 12.5 %, and the accuracy was between -2.9% and 1.4%. There were no interfering peaks in the submitted study subject's chromatograms.

Reviewer Note: Validation report for both analytical methods was not provided by the sponsor.

Statistical Analysis

Digoxin pharmacokinetic parameters ($AUC_{\tau,ss}$, $C_{max,ss}$, $t_{max,ss}$ and $C_{min,ss}$) were computed by standard non-compartmental methods of analysis

The following pharmacokinetic parameters for digoxin were subject to statistical analysis on Day 7 and Day 17: $AUC_{\tau,ss}$, $C_{max,ss}$, $t_{max,ss}$ and $C_{min,ss}$. Log-transformed (base e) $AUC_{\tau,ss}$, $C_{max.ss}$ and $C_{min,ss}$ were analyzed using a mixed effect model allowing for the fixed effect of treatment (digoxin alone and digoxin + tadalafil), and the random effect of subject. For these parameters, least squares (LS) means were calculated for each treatment. Mean differences between digoxin given in combination with tadalafil (Day 17) and digoxin given alone (Day 7) were calculated. The residual variance from the mixed model was used to calculate 90% confidence intervals (CI) for the differences. These values were back transformed to give geometric LS means, a point estimate and 90% CI for the ratio of digoxin given in combination with tadalafil (Day 17) relative to digoxin given alone (Day 7).

The parameter $t_{max,ss}$ was analyzed non-parametrically. The median difference and 90%

CI between digoxin given in combination with tadalafil (Day 17) and digoxin given alone (Day 7) was calculated.

Results

Study Population

Twenty healthy subjects, 14 males and 6 females, participated in the study with mean age of 40 ± 13.4 years (range 22 - 53). One subject was withdrawn from the study due to alcohol consumption.

Pharmacokinetics Analysis

The co-administration of tadalafil reduced digoxin $AUC_{\tau,ss}$, $C_{max,ss}$, and $C_{min,ss}$ by 10%, 5%, and 14%, respectively. However this reduction was not statistically significant as shown in the table below. As shown in Figure 1, the average digoxin concentration was reduced on all time points upon the co-administration of tadalafil.

Dependent Variable	Geometric Means (%CV)		Ratio	90% CI	
	Digoxin Alone	Digoxin & Tadalafil		Lower	Upper
$AUC_{\tau,ss}$ (ng h/mL)	16.5 (23.8)	15.1 (20.3)	0.90	0.86	0.96
$C_{max,ss}$ (ng/mL)	1.48 (26.1)	1.4 (20.4)	0.95	0.86	1.04
$C_{min,ss}$ (ng/mL)	0.51 (25.7)	0.45 (23.0)	0.86	0.81	0.91
$T_{max,ss}$ (h)	1.30 (0.5 – 4.0)*	1.50 (0.5 – 3.0)*	0.2**	-0.25	0.45

* Median and range, ** Median difference

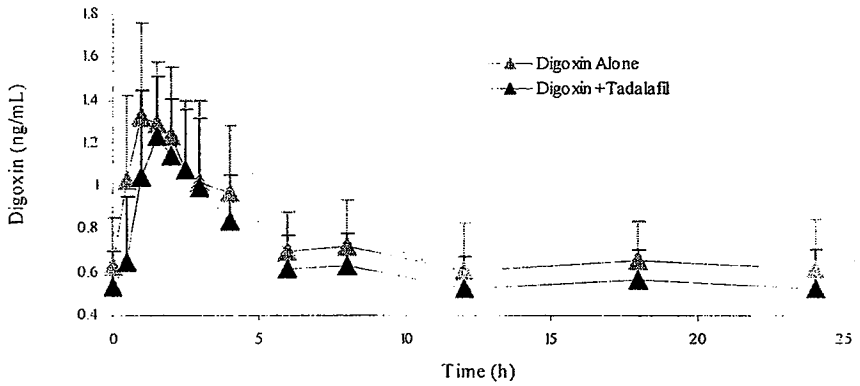


Figure 1. Digoxin serum concentration-time profiles following oral administration of digoxin alone, and following co-administration with steady-state concentrations of tadalafil. Each point represents the mean (n =19), and error bars represent the standard deviation.

The renal clearance of digoxin was not altered by the co-administration of tadalafil. The renal clearance of digoxin was 136 mL/min following digoxin alone administration and 130 mL/min following the co-administration of digoxin and tadalafil.

Safety Results (Per Applicant)

No death or serious adverse events occurred during this study.

All adverse events were either mild or moderate in severity, with the exception of a severe headache reported by one subject following co-administration of tadalafil and digoxin that was considered possibly related to tadalafil.

The incidence of adverse events was highest over the first three days of tadalafil co-administration with digoxin. The most frequently reported drug-related adverse events following co-administration of 40 mg tadalafil and digoxin were headache, myalgia, nausea, and back pain.

Conclusions

1. Based on $AUC_{\tau,ss}$, $C_{max,ss}$, and $C_{min,ss}$, multiple dose of tadalafil does not alter the systemic exposure to digoxin at steady state.
2. In general, tadalafil lowers digoxin pharmacokinetic parameters. However, this reduction is not statistically significant.

Study # H6D-EW-LVHM: Tadalafil-Oral Contraceptive DDI

Report #	H6D-EW-LVHM
Investigator	
Study Site	b(4)
Study Period	01/08/2007 - 08/10/2007

Title

A study to investigate the effect of tadalafil on oral contraceptive pharmacokinetics in healthy female subjects

Objectives

Primary objective: To assess the effect of tadalafil (40 mg) and placebo on the first (Day 1) and multiple-dose (Day 21) pharmacokinetics of a combination oral contraceptive (ethinylestradiol/levonorgestrel)

Secondary Objective: To assess the safety and tolerability of co-administration of tadalafil and placebo taken together with an oral contraceptive in healthy female subjects, and to assess the pharmacokinetics of tadalafil and its major metabolite, total IC710, after single dose and multiple oral dose administration (21 days) with combination oral contraceptive.

Study Rationale

A statistically significant increase in the steady-state systemic exposure of ethinylestradiol was observed upon the co-administration of oral contraceptive and 10 mg tadalafil. Also, given that the prevalence of pulmonary arterial hypertension tends to be higher in women than in men, co-administration of tadalafil with oral contraceptives is possible, thus this study was conducted to assess whether a 40-mg dose of tadalafil results in a clinically significant pharmacokinetic drug-drug interaction.

Study Design

This was a randomized, double-blind, placebo-controlled, three periods, two-sequence, cross-over study in healthy female subjects. Thirty subjects were enrolled in the study. The study schema is provided below. Note that period 2 was included to allow adequate washout of tadalafil.

	Period 1 Day 1 - Day 21	7 Days Washout	Period 2 Day 1 - Day 21	7 Days Washout	Period 3 Day 1 - Day 21	7 Days Washout
Sequnce 1	[OC + Tadalafil 40 mg] QD		OC alone QD		[OC + Placebo] QD	
Sequence 2	[OC + Placebo] QD		OC alone QD		[OC + Tadalafil 40 mg] QD	
	PK on Days 1, 2, 21, and 22				PK on Days 1, 2, 21, and 22	

OC = Oral Contraceptive

Test drugs

Oral contraceptive and tadalafil were administered orally:

- ***Oral contraceptive:*** Microgynon 30® (batch # WEB9JC) containing 150 µg levonogestrel and 30 µg ethinylestradiol, one tablet qd.
- ***Tadalafil:*** Cialis® 20 mg tablets (lot # CT528000), two tablets qd.
- Placebo tadalafil: lot # CT528001

Pharmacokinetics Sampling Times

Blood samples for ethinylestradiol and levonogestrel pharmacokinetics were obtained at per-dose and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10, 12.0, 16.0, and 24.0 hours post-dose.

Blood samples for tadalafil and its metabolite (Total IC710), and ethyinylestradiol-sulfate pharmacokinetics were obtained at per-dose and at 0.5, 1.0, 2.0, 3.0, 4.0, 8.0, 12.0, 16.0, and 24.0 hours post dose.

Analytical Methods

1. An HPLC-MS/MS method was used to quantify ethinylestradiol, ethinylestradiol-sulfate, and levonorgestrel in plasma. The table below shows the performance of these bioanalytical methods during the analysis of the study unknown plasma samples:

Analyte	Ethinylestradiol	Ethinylestradiol-sulfate	Levonorgestrel
Calibration Range	2.0 - 250 pg/mL	50 – 10,000 pg/mL	50 – 12,500 pg/mL
%CV	≤ 7.1	≤ 7.7	≤ 8.9
% RE	-1.8 -2.8	-2.6 -5.1	-0.9 – 1.0
Precision %CV	≤ 8.5	≤ 12.6	≤ 10.1
Accuracy %RE	-2.0 – 1.0	-10.5 – -4.3	-1.0 – 4.1

Reviewer Note: Validation report for the above three bioanalytical methods was not submitted.

2. An HPLC-MS/MS method was used to quantify tadalafil and total IC710 in plasma. The table below shows the performance of these bioanalytical methods during the analysis of the study's unknown plasma samples:

Analyte	Tadalafil	Total IC710
Calibration Range	0.5 - 500 ng/mL	1.0 – 500 ng/mL
%CV	≤ 6.6	≤ 7.2
% RE	-0.9 - 0.8	-8.2 – 2.5
Precision %CV	≤ 7.5	≤ 6.4
Accuracy %RE	-3.6 – 0.8	-2.5 – 1.8

Statistical Analysis

Pharmacokinetic parameters (AUC_{0-24}, C_{max}, C_{min}, and t_{max}) were computed by standard non-compartmental methods of analysis

Log-transformed AUC_{0-24}, C_{max}, and C_{min} of ethinylestradiol, ethinylestradiol-sulfate, and levonogestrel were analyzed using a mixed effect model. Geometric least squares (LS) mean ratios (median differences for t_{max}) and 90% confidence intervals (CI) for comparisons between the test and reference treatments were determined. A comparison of t_{max} values between the treatments was performed using the Wilcoxon sign rank test. In these analyses, the test treatment was oral contraceptive + tadalafil, and the reference treatment was oral contraceptive + placebo.

Results

Study Population

Thirty healthy female subjects participated in the study with mean age of 27 ± 6.5 (range 21 - 43 years). Four subjects were withdrawn from the study, one of which was due to adverse events. Two subjects were replaced and total of 28 subjects completed the study.

Pharmacokinetics Analysis

The co-administration of tadalafil increased ethinylestradiol AUC_{0-24} and C_{max}. This increase was statistically significant and was lower at steady state (Day 21 vs. Day1), as shown in the table below:

Dependent Variable	Geometric Means (%CV)		Ratio	90% CI	
	Tadalafil + Oral Contraceptive	Placebo + Oral Contraceptive		Lower	Upper
AUC_{0-24} (pg h/mL)					
Day 1	786 (22.5)	518 (32.1)	1.53	1.43	1.64
Day21	940 (28.9)	739 (22.5)	1.25	1.17	1.33
C_{max} (pg/mL)					
Day1	98.0 (35.2)	52.3 (41.0)	1.90	1.71	1.82
Day 21	115 (33.0)	65.9 (36.0)	1.70	1.58	1.41
C_{min} (pg/mL)					
Day1	16.9 (30.5)	13.2 (38.8)	1.29	1.18	1.41
Day 21	19.7 (42.2)	17.4 (40.5)	1.07	1.00	1.15
T_{max} (h)					
Day 1	2.5 (1.0 – 5.1)*	3.0 (0.5 – 5.0)*	0**	-0.5	0.53
Day 21	2.6 (1.0 – 4.0)*	3.0 (0.5- 5.0)*	-0.5**	-1.0	-0.05

* Median and range, ** Median difference

The co-administration of tadalafil significantly reduced ethinylestradiol-sulfate AUC_{0-24} and C_{max}. This reduction was lower at steady state (Day 21 vs. Day1), as shown in the table below. The metabolic ratio (AUC ethinylestradiol-sulfate/ AUC ethinylestradiol) was 3.8 and 3.15 following the co-administration of tadalafil and oral contraceptive on Day 1 and Day 21, respectively. The metabolic ratio was higher following the co-administration of placebo and oral contraceptive, 15.0 on Day 1 and 14.3 on Day 21.

Dependent Variable	Geometric Means (%CV)		Ratio	90% CI	
	Tadalafil + Oral Contraceptive	Placebo + Oral Contraceptive		Lower	Upper
AUC_{0-24} (pg h/mL)					
Day 1	3088 (82.1)	7874 (43.6)	0.38	0.33	0.45
Day21	3063 (85.9)	10565 (61.1)	0.29	0.24	0.33
C_{max} (pg/mL)					
Day1	513 (45.3)	1143 (35.4)	0.45	0.39	0.51
Day 21	466 (53.8)	1276 (48.2)	0.37	0.33	0.41
C_{min} (pg/mL)					
Day1	NC	NC			
Day 21	NC	228 (71.1)			
T_{max} (h)					
Day 1	1.99 (0.5 – 5.1)*	2.0 (0.5 – 4.0)*	-0.48**	-0.99	0.03
Day 21	2.0 (0.5 – 4.0)*	2.0 (0.5- 4.0)*	-0.5**	-1.0	0.0

Median and range, ** Median difference
NC: Not calculated

The co-administration of tadalafil did not have a significant effect on levonorgestrel AUC_{0-24}, C_{max}, and C_{min} as shown in the table below:

Dependent Variable	Geometric Means (%CV)		Ratio	90% CI	
	Tadalafil + Oral Contraceptive	Placebo + Oral Contraceptive		Lower	Upper
AUC_{0-24} (pg h/mL)					
Day 1	28420 (36.3)	30630 (85.9)	0.89	0.82	0.97
Day21	80479 (43.2)	77890 (49.2)	1.02	0.96	1.08
C_{max} (pg/mL)					
Day1	3085 (39.5)	3800 (32.2)	0.83	**0.75**	0.91
Day 21	6245 (33.7)	6047 (35.1)	1.02	0.97	1.08
C_{min} (pg/mL)					
Day1	689 (40.2)	771 (51.3)	0.92	0.82	1.02
Day 21	2306 (50.9)	2279 (58.4)	0.99	0.92	1.07
T_{max} (h)					
Day 1	1.6 (1.0 – 5.0)*	1.5 (1.0 – 4.0)*	-0.25**	-0.99	0.03
Day 21	1.5 (0.5 – 3.0)*	2.0 (0.5- 4.0)*	-0.008**	-1.0	0.0

Median and range, ** Median difference and associated 90% CI for $t_{max,ss}$

The accumulation ratio of tadalafil was approximately 1.3 fold for both AUC_{0-24} and C_{max}, which is consistent with that calculated based on terminal $t_{1/2}$ of ~ 16.0 hours. The metabolic ratio (AUC_{0-24} Total IC710/ AUC_{0-24} Tadalafil) was 1.33.

Safety Results (Per Applicant)

No death or serious adverse events were reported. Multiple oral doses of 40 mg tadalafil were reasonably well tolerated when co-administered with oral contraceptive.

The number and severity of adverse events reported following co-administration of oral contraceptive with tadalafil was 4-fold higher than following placebo. There was a high incidence of headache, nausea and back pain that were considered to be related to the study drug.

Conclusions

1. Tadalafil significantly increases ethinylestradiol $AUC_{0-24,ss}$ by 25% and $C_{max,ss}$ by 70%.
2. Tadalafil significantly reduces ethinylestradiol-sulfate $AUC_{0-24,ss}$ by 71% and $C_{max,ss}$ by 63%.
3. Tadalafil does not alter the steady state systemic exposure (AUC and C_{max}) of levonorgestrel.
4. Tadalafil significantly reduce levonorgestrel C_{max} following single oral dose by 17%, but does not affect AUC_{0-24}.

5. Tadalafil (40 mg) was safe and moderately well tolerated following multiple oral dose administration with oral contraceptive over 21 days in healthy female subjects.

Reviewer Comments

1. The decrease in ethinylestradiol (EE) systemic exposure and the increase in EE-sulfate systemic exposure upon the co-administration of tadalafil can be attributed to the inhibition of sulfotransferases (SULTs) in the gastrointestinal tract. In a similar manner, acetaminophen increased EE's AUC by 22% and decreased EE-sulfate's AUC by 66%[2]. Acetaminophen competes with EE for sulfation in the gut wall[3]. It should be noted that tadalafil is not reported to be a substrate for SULTs. On the other hand, EE-tadalafil interaction can not be attributed to CYP3A4 induction or inhibition, although tadalafil produces mechanism-based inhibition of CYP3A and induction of CYP3A proteins expression in cultured human hepatocytes. Tadalafil did not alter the systemic exposure of levonorgestrel which is mainly metabolized by CYP3A4.
2. Based on the above results: it will be recommended to avoid the co-administration of tadalafil with contraceptive drug that undergoes SULTs mediated sulfation.

[2] Clin Pharmacokinet 2007; 46(2): 133-157
[3] Drug Saf 1993; 9(1): 21-37

Study # H6D-EW-LVHO: Food Effect

Report #	H6D-EW-LVHO
Investigator	
Study Site	C D
Study Period	01/08/2007-02/16/2007

b(4)

Title

A study in healthy subjects to assess the effect of food on the pharmacokinetics of tadalafil administered as a single oral dose of 40 mg.

Objectives

Primary objective: To assess the effect of food on the pharmacokinetics of tadalafil when administered as a single oral dose of 40 mg.

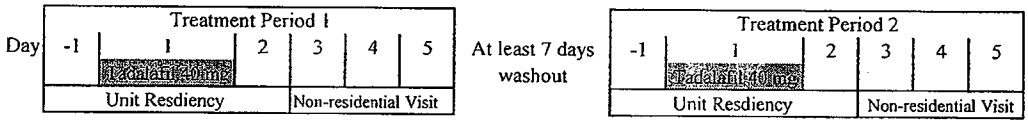
Secondary objective: To further assess the safety and tolerability of single oral doses of 40 mg tadalafil

Study Rationale

The anticipated highest dose of tadalafil in clinical trials is 40 mg. Therefore, the current study evaluates the effect of food on the potential highest clinical dose of tadalafil.

Study Design

This was a single-dose, single-center, open-label, randomized, two-period crossover study. The study was conducted in healthy volunteers. The study design is illustrated in the schema below:



Study Drug

Tadalafil (Cialis® 20 mg tablets, batch number A226547). Tadalafil was administered as single 40 mg oral dose (2 x 20 mg tablets), with ~ 240 mL of water.

For dose administration in the fasted state, subjects were required to fast from 10 hours *prior* to dosing.

For dose administration in the fed state, subjects received a FDA-defined high fat, high calorie breakfast prior to dosing. The meal was ingested over a 30-minute period, such

that it was completed within 5 minutes prior to dosing, and no further food was permitted until at least 4 hours post-dose. The table below shows the details of the provided meal:

Two eggs (fried in blended oil)
Two rashers of bacon (grilled)
One slice of white toast with 10 g butter (2 pats)
Two hash brown potatoes
240 mL full-fat milk
Total calorific content: 927 kcal
Total fat content: 59 g (57% of total calorific content)
Total protein content: 45 g (19% of total calorific content)

Pharmacokinetic Blood Sampling

Venous blood samples were collected at pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 48, 72, and 96 hours post-dose.

Assay Method

An HPLC-MS/MS method with solid phase extraction was used to quantify tadalafil in plasma. During the analysis of the study unknown plasma samples, the precession of the quality control samples was < 7.6%, and the accuracy was between -4.3% and 2.9%.

Type	HPLC-MS/MS
Analyte	Tadalafil (IC351)
Calibration Range	0.5 – 500 ng/mL, weighted ($1/x^2$) quadratic fit
LLOQ	0.1 ng/mL
Specificity	Analysis of blank and spiked plasma confirms method selectivity for tadalafil. Chromatograms were provided
Precision (intra-day) %CV	≤ 7.1
Precision (inter-day) %CV	≤ 10.5
Accuracy (inter-day) %RE	-1.5 – 1.3
Accuracy (intra-day) %RE	-9.8 – 9.2
Recovery %	47.1 – 52.8
Dilution	10-fold dilution of plasma samples is acceptable
Stability	- Extracted plasma samples were stable for 41 hours - Plasma samples are stable up to 4 freeze/thaw cycles at ~ -70 °C - Plasma samples are stable for at least -425 days at -70 °C

Pharmacokinetics Data Analysis

Tadalafil PK parameters (C_{max}, T_{max}, AUC_{0-90}, $AUC_{0-\infty}$, $t_{1/2}$) were computed by standard non-compartmental methods of analysis

A mixed-effect analysis of variance (ANOVA) model was used to compare the pharmacokinetic parameters of tadalafil for each dietary condition (fed and fasted), with period and dietary condition as fixed effects and subject number as a random effect. Least squares geometric means for each dietary condition, the differences between the geometric means of the pharmacokinetic parameters in the fed and fasted conditions, and the corresponding 90% confidence intervals for the difference were estimated. Values of t_{max} were analyzed non-parametrically using the Wilcoxon signed-rank test. Median differences and 90% CI between the fed and fasted conditions were calculated.

Results

Fifteen subjects (9 males and 6 females) participated in the study with mean age of 38 ± 14.3 years. Twelve subjects completed the study.

Food has no effect on the pharmacokinetics of tadalafil. Mean AUC_{0-96} and $AUC_{0-\infty}$ were 11% and 14% higher, and mean C_{max} was 7% higher, following the administration of tadalafil in the fed states compared to the fasted conditions. These differences were not statistically significant as shown in the table below. There was no significant difference in t_{max} when tadalafil was administered with and without food.

	Geometric Mean (%CV)		Ratio	90% CI	
Parameter	Fed	Fast	Fed/Fast	Lower	Upper
AUC_{0-96}	16184(34.6)	14589(31.4)	1.14	1.05	1.22
$AUC_{0-\infty}$	17386(41.9)	15404(36.8)	1.11	1.04	1.19
C_{max}	586(18.0)	553(22.3)	1.07	0.99	1.15
T_{max}	3.0(1.0-6.0)*	2.0(1.0-6.0)*	0.5**	-0.008	2
$t_{1/2}$	20.7(38.6)	20.2(34.3)			

* Median and range, ** Median difference

Safety (Per Applicant)

No death or any other serious adverse events occurred during this study. Oral doses of tadalafil were reasonably well tolerated and adverse events were similar under fast and fed conditions.

All subjects reported adverse events during the study, all of which were either mild or moderate in severity. The most frequently reported adverse events following 40 mg tadalafil were headache, myalgia and back pain.

The occurrence (number of adverse events) of headache and back pain was approximately 2-fold higher in the fed condition compared to the fasted condition.

Conclusions

Based on AUC_{0-96}, $AUC_{0-\infty}$, and C_{max}, food does not alter the systemic exposure of tadalafil following the administration of a 40 mg single dose.

OFFICE OF CLINICAL PHARMACOLOGY: PHARMACOMETRIC REVIEW

1 SUMMARY OF FINDINGS

1.1 Key Review Questions

The purpose of this review is to address the following key questions.

1.1.1 What is the optimal dose of tadalafil for the treatment of pulmonary arterial hypertension (PAH) to improve exercise capacity?

The optimal dose of tadalafil for the treatment of PAH to improve exercise capacity is 20 mg. The median 6-minute walk distance (6-MWD) change from baseline appears to be similar for doses 10, 20 and 40 mg (Figure 1). The exposure-response relationship suggests the maximum effect on 6-MWD change from baseline is attained at tadalafil concentrations corresponding to the 20 and 40 mg doses (Figure 2). The effect of tadalafil on the secondary endpoint, pulmonary vascular resistance (PVR), also appears to be at its maximum at doses of 20 mg or greater (Figure 3). In vitro data suggest that the maximum free tadalafil concentration ranged from 52 to 92 times the PDE5 IC50 following the 20 and 40 mg doses. The most common dose-dependent adverse event was headache (placebo (14.6%), tadalafil 2.5 mg (18.3%), tadalafil 10 mg (37.5%), tadalafil 20 mg (31.7%) and tadalafil 40 mg (41.8%). The sponsor is proposing 40 mg of tadalafil for all patients. Long-term safety data are limited and incremental benefit in change from baseline 6-MWD at higher doses is not achieved. Therefore, 20 mg of tadalafil is recommended for majority of patients.

Figure 1. Change from Baseline in 6-Minute Walk Distance (meters): Median (25^{th} to 75th Percentiles)

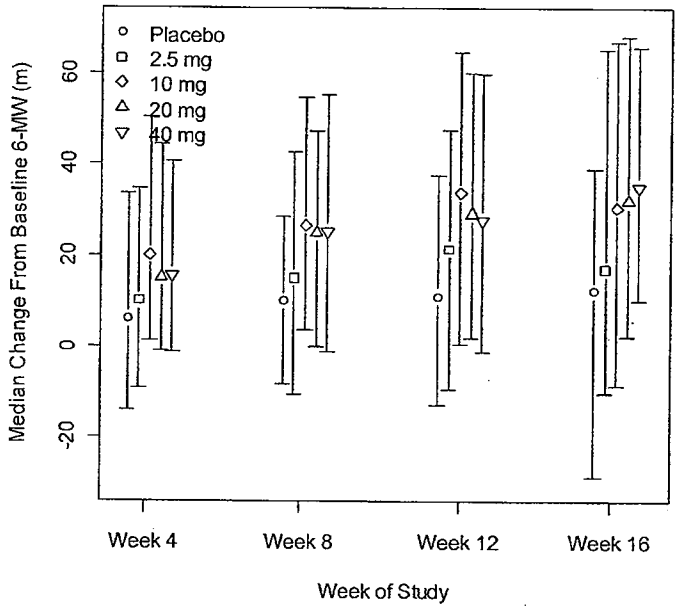


Figure 2: 6-MWD Mean (95% Confidence Interval) Change from Baseline (Week 16) by Binned Median C_{min}. Median (25^{th} – 75^{th} percentiles) C_{min} for the doses are noted.

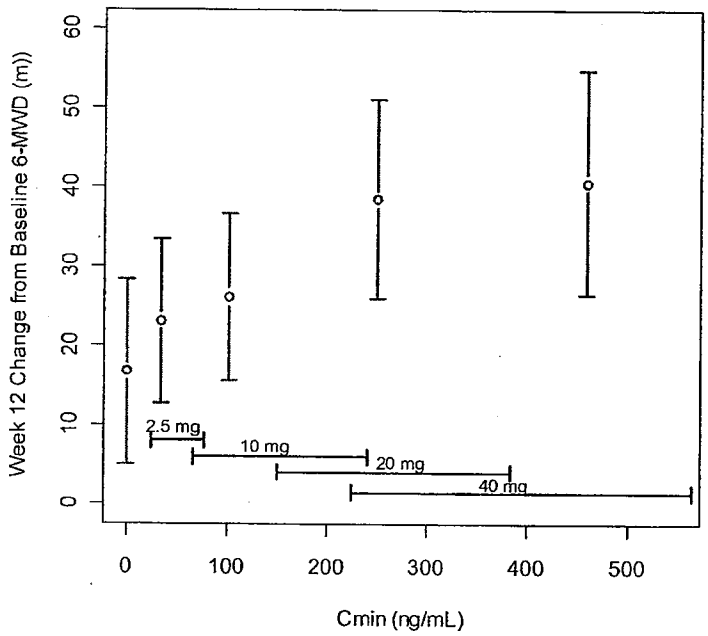
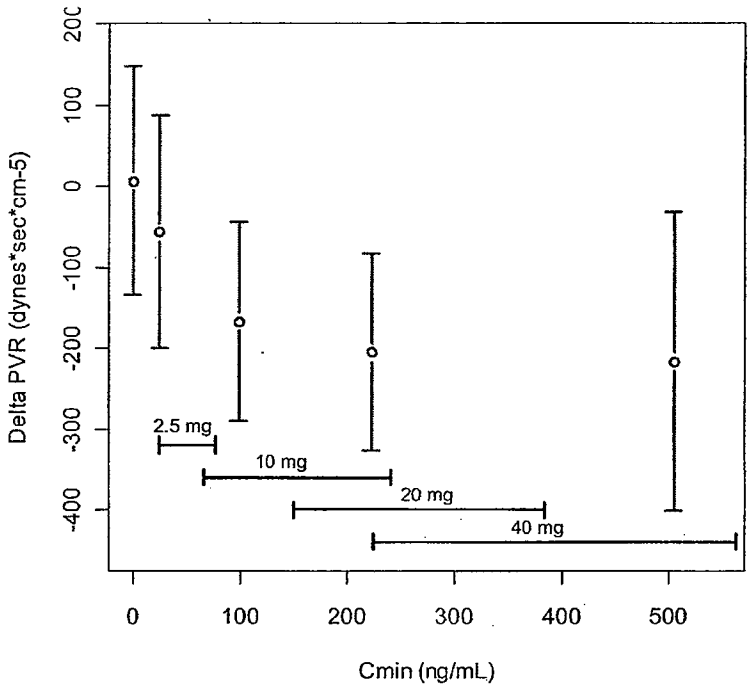


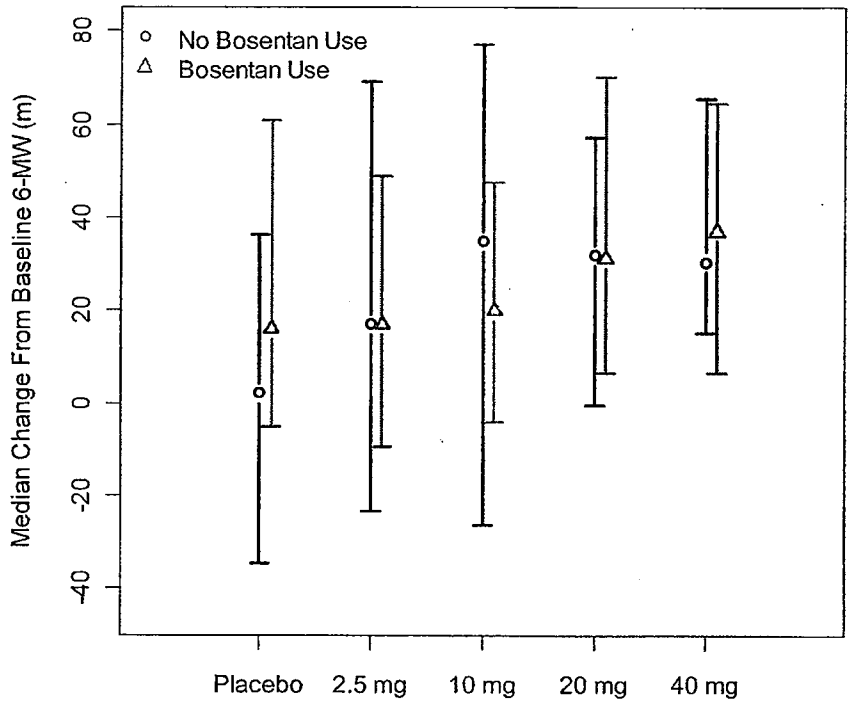
Figure 3: PVR Mean (95% Confidence Interval) Change from Baseline (Week 16) by Binned Median C_{min}. Median C_{min} for the doses are noted.



1.1.2 Is there need for dose adjustment of tadalafil with concomitant bosentan administration?

Dose adjustment of tadalafil is not required when administered with 20 mg or 40 mg bosentan. Figure 4 shows that median 6-MWD at Week 16 is not affected by concomitant bosentan administration, as long as the tadalafil dose is 20 mg or 40 mg. Median 6-MWD change from baseline for the 20 mg dose with and without concomitant bosentan administration was 31.25 and 32.0 meters, respectively. The median 6-MWD change from baseline for the 40 mg dose with and without concomitant bosentan administration was 37.05 and 30.5 meters, respectively. Concomitant bosentan administration only becomes relevant for the 10 mg dose where median 6-MWD change from baseline with concomitant bosentan is 20.0 meters compared to 35.0 meters without. These results suggest that the 20 mg and 40 mg doses are high enough on the dose-response relationship (Figure 2) that the decrease in tadalafil exposure with concomitant bosentan is not a significant factor.

Figure 4: Change from Baseline in 6-Minute Walk Distance (meters) at Week 16 by Bosentan Administration: Median (25th to 75th Percentiles)



1.2 Recommendations

- Patients should begin with a tadalafil dose of 20 mg. The dose can be increased to 40 mg if deemed necessary based on patient's and physician's assessment of effectiveness.
- No dose adjustment of tadalafil is needed when administered with bosentan.

1.3 Label Statements

Labeling statements to be removed are shown in ~~red strikethrough font~~ and suggested labeling to be included is shown in underline blue font.

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Population pharmacokinetics - In patients with pulmonary hypertension not receiving concomitant bosentan, the average tadalafil exposure at steady-state following 40 mg was 26% higher when compared to those of healthy volunteers. b(4)

The results suggest a lower clearance of tadalafil in patients with pulmonary hypertension compared to healthy volunteers.

2 PERTINENT REGULATORY BACKGROUND

Tadalafil was approved on November 21, 2003 for the treatment of erectile dysfunction (NDA 21-368) and is currently marketed in the United States as Cialis®. Cialis is available in 5 mg, 10 mg and 20 mg tablets for on-demand use and in 2.5 mg and 5 mg tablets for once daily use. The clinical development program for tadalafil for the treatment of PAH to improve exercise capacity began in 2005 under IND 71,871. A Pre-IND Meeting was held in April 2005 and an End of Phase 2a Meeting occurred in May, 2006.

3 RESULTS OF SPONSOR'S ANALYSIS

3.1 Study H6D-MC-LVGY

Study H6D-MC-LVGY was a randomized, double-blind, placebo-controlled, parallel-design study to evaluate the efficacy and safety of 2.5, 10, 20 and 40 mg tadalafil taken once daily for 16 weeks in 405 subjects with PAH. Primary efficacy was measured by the unencouraged 6-minute walk test conducted at baseline and Week 4, 8, 12 and 16. Secondary endpoints included World Health Organization functional class change from baseline, time to first occurrence of clinical worsening and Borg dyspnea score change from baseline. A subset of 93 subjects were included in a substudy in which cardiopulmonary hemodynamics were assessed at Week 16 by right heart catheterization.

3.2 Pharmacokinetic Model

The sponsor conducted a population pharmacokinetic analysis to characterize the pharmacokinetics of 2.5 mg, 10 mg, 20 mg, and 40 mg tadalafil once daily and identify covariates exerting significant influence on tadalafil exposure. The final dataset consisted of 1102 plasma concentration measurements from 305 patients administered tadalafil 2.5 mg (n = 77), 10 mg (n = 77), 20 mg (n = 77) or 40 mg (n = 74) once daily in study H6D-MC-LVGY. Sparse samples were collected at the Week 4, 8, 12 and 16 visits, where the Week 12 sample was a trough sample collected after withholding the morning dose.

The selection of a one compartment model as the structure of the basic pharmacokinetic model was informed by population pharmacokinetic analysis in male subjects with erectile dysfunction. The model parameterized in terms of absorption rate constant (k_a), apparent clearance (CL/F) and apparent volume of distribution (V/F). Inter-subject

variability was modeled as log-normal. A combined additive and proportional error model was used to describe residual variability. The first order conditional estimation (FOCE) method in NONMEM Version VI, Level 1.0 was used for parameter estimation. A summary of the parameter estimates of the basic model is provided in Table 1.

Table 1: Pharmacokinetic Parameter Estimates for Base Structural Model

Parameter Description	Population Estimate (%SEE)	Interindividual Variability (%SEE)
Rate of Absorption		
Parameter for k_a (h^{-1})	0.791 (14.2)	59.2% (69.7)
Clearance		
Parameter for CL/F (L/h)	2.36 (3.6)	59.7% (8.7)
Volume of Distribution		
Parameter for V/F (L)	85.5 (6.6)	53.7% (21.7)
Bioavailability		
Parameter for F	1 (fixed)	---
Intermdividual Variability		
Covariance Term (CL/F and V/F)	—	0.087 (38.8)
Residual Error (ratio of additive to proportional RV[a])	32.0 (33.1)	
Residual Error (proportional)	0.0646 (9.3)	

Abbreviations: CL/F = apparent clearance; F = bioavailability fraction; k_a = absorption rate constant; %SEE = percent standard error of the estimate; V/F = apparent volume of distribution.

[a] Residual variability was estimated to range from 35.21 %CV to 25.42 %CV at predicted concentrations ranging from 10 ng/mL to 2000 ng/mL.

Source: H6D-MC-LVGY Population Pharmacokinetics Report, Table LVGY.9.4, P49.

The covariates investigated for influence on inter-subject variability by forward selection and backward elimination are listed in Table 2.

Table 2: Potential Covariates Investigated in Pharmacokinetic Analysis

Categorical
Sex
Ethnicity
History of cardiovascular disease (CAD1, CAD2)
PAH history
Tadalafil dose, mg
Concomitant medications – bosentan, digoxin, warfarin[a]
Continuous
Age, years
Time on therapy, hours
Body weight, kg
PAH duration, years
Alanine aminotransferase (ALT), U/L
Aspartate aminotransferase (AST), U/L
Total bilirubin (TBIL), µmol/L
Creatinine clearance (CrCL), mL/min
Total serum protein, g/L

Source: H6D-MC-LVGY Population Pharmacokinetics Report, Table LVGY.8.1, P36.

The final model included the effect of concomitant bosentan use on CL/F and dose on F. A summary of the parameter estimates of the final model is provided in Table 3 and goodness of fit plots are provided in Figure 5.

Table 3: Pharmacokinetic and Covariate Parameter Estimates of the Final Model

Parameter Description	Population Estimate (%SEE)	Interindividual Variability (%SEE)
Rate of Absorption		
Parameter for k_a (h^{-1})	0.84 (12.6)	---
Clearance[a]		
Parameter for CL/F (L/h)	1.59 (5.0)	48.6% (12.1)
Effect of Concomitant Bosentan on CL/F (L/h)	1.20 (10.8)	
Volume of Distribution		
Parameter for V/F (L)	79.7 (6.7)	51.7% (21.9)
Bioavailability		
Parameter for F	1 (fixed)	---
Relative F for 40-mg Treatment	0.65 (5.3)	---
Residual Error (ratio of additive to proportional RV[b])	25.5 (41.6)	
Residual Error (proportional)	0.0685 (8.7)	

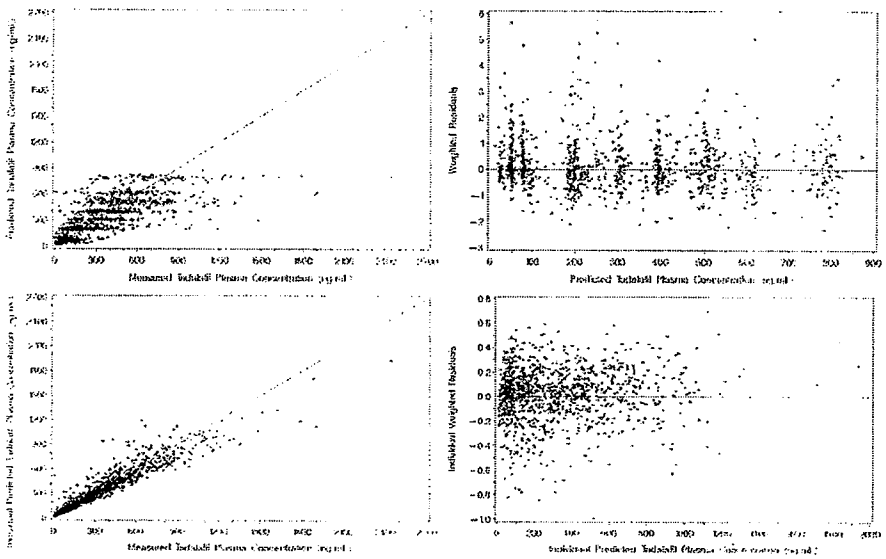
Abbreviations: CL/F = apparent clearance; F = bioavailability fraction; k_a = absorption rate constant; RV = residual variability; %SEE = percent standard error of the estimate; V/F = apparent volume of distribution.

[a] Individual CL/F = 1.59 +1.2 (BOS) where BOS = 0 when no concomitant bosentan use is reported and BOS = 1 for those receiving concomitant bosentan.

[b] Residual variability was estimated to range from 71.7 %CV to 26.2 %CV at predicted concentrations ranging from 10 ng/mL to 2000 ng/mL.

Source: H6D-MC-LVGY Population Pharmacokinetics Report, Table LVGY.9.7, P53.

Figure 5: Goodness of Fit Plots of the Final Pharmacokinetic Model

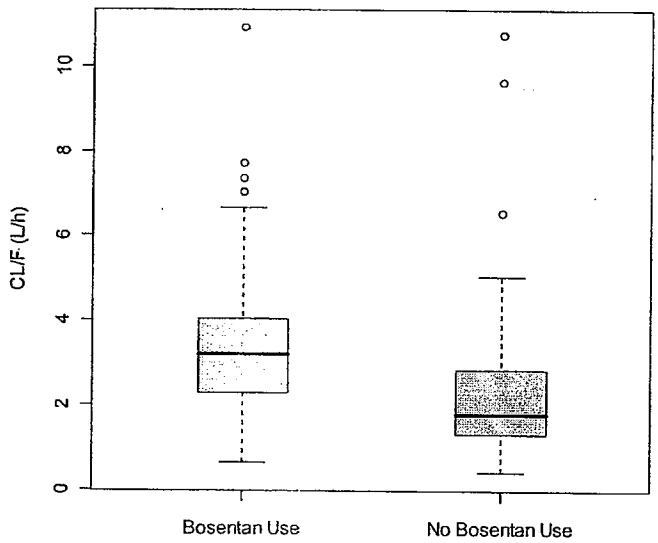


Source: H6D-MC-LVGY Population Pharmacokinetics Report, Figure LVGY.9.5, P53.

Reviewer's Comment: The parameter estimates and goodness of fit plots indicate an adequate performance of the final model to describe tadalafil pharmacokinetics. The sponsor also performed parameter sensitivity analysis and leverage analysis to further support the robustness of the final model.

The parameter estimate for the relative bioavailability of the 40 mg dose was 0.65, indicating a 35% reduction in bioavailability of this dose compared to lower doses. Therefore, a 2-fold change in dose from 20 mg to 40 mg results in a 1.48-fold increase in exposure. Furthermore, the sponsor calculated that approximately 81% of the observed tadalafil AUC_{ss} values for the 40 mg dose were within the 5th to 95th percentiles of those estimated for the 20 mg dose. Apparent tadalafil clearance was estimated to increase from 1.59 L/h to 2.79 L/h in subjects receiving concomitant bosentan. This corresponds to a 35% decrease in systemic exposure of tadalafil in this population (Figure 6).

Figure 6: Influence of Bosentan Use on Tadalafil Apparent Clearance



3.3 Pharmacokinetic/Pharmacodynamic Model

The sponsor used the output from the pharmacokinetic model to perform a pharmacodynamic analysis in order to describe the relationship between tadalafil exposure and change in 6-minute walk distance (6-MWD). The final dataset consisted of 1827 6-minute walk records from 389 subjects administered placebo (n = 82), tadalafil 2.5 mg (n = 77), 10 mg (n = 77), 20 mg (n = 78) or 40 mg (n = 75) once daily in study H6D-MC-LVGY. 6-minute walk tests were performed at baseline and the Week 4, 8, 12 and 16 visits.

The time course of the 6-MWD was described by the following equation in the basic model:

$$Walk = BaselineWalk + \left(\frac{E\max \times WSFD}{T_{50} + WSFD} \right) \text{ where,}$$

Walk = the observed 6-MWD

Baseline Walk = the observed 6-MWD at baseline

E_{max} = the maximum 6-MWD response to treatment

WSFD = week since first dose of tadalafil

T_{50} = the time for half-maximal response due to treatment

A linear function of weeks since first dose of tadalafil was used to describe the time course of 6-MWD in placebo subjects. Tadalafil AUC_{ss} was included as a power function on the E_{max} parameter to incorporate the effect of tadalafil exposure on 6-MWD. Inter-subject variability was modeled as log-normal. An additive error model was used to describe residual variability. The FOCE method in NONMEM Version VI, Level 1.0 was used for parameter estimation. A summary of the parameter estimates of the basic model is provided in Table 4.

Table 4: Parameter Estimates for Base Pharmacokinetic/Pharmacodynamic Model

Parameter Description	Population Estimate (%SEE)	Interindividual Variability (%SEE)
Baseline 6-Minute Walk (meters)	339 (1.2)	0.0453 (9.8)[a]
Slope for Placebo Time-Course (meters/week)	0.863 (45.1)	3820 (25.9)[b]
Active Treatment E_{max} (meters)	51.4 (15.6)	3190 (32.3)[c]
Power for AUC_{ss} on E_{max}	0.199 (51.3)	
T_{50} for Active (weeks)	8.12 (38.4)	NE
Residual Error (SD in meters)	29.88 (7.9)	

Abbreviations: AUC_{ss} = steady-state area under the concentration-time curve; E_{max} = maximum response to treatment; NE = not estimated; SD = standard deviation; %SEE = percent standard error of the estimate; T_{50} = time at which 50% maximal response is achieved.

[a] The estimate provided in the table (0.0453) is a variance term. The corresponding %CV = 21.28%.

[b] The estimate provided in the table (3820) is a variance term. The corresponding SD = 61.81 meters.

[c] The estimate provided in the table (3190) is a variance term. The corresponding SD = 56.48 meters.

Placebo 6-Minute Walk = 339 + 0.863 × WSFD

$$\text{Active Tadalafil 6-Minute Walk} = 339 + \frac{\left[51.4 \times \left(\frac{AUC_{ss}}{5955.3} \right)^{0.199} \times WSFD \right]}{\left[8.12 + AUC_{ss} \right]}$$

Source: H6D-MC-LVGY Population Pharmacokinetics Report, Table LVGY.9.15, P72.

The covariates investigated for influence on inter-subject variability by forward selection and backward elimination are listed in Table 5.

Table 5: Potential Covariates Investigated in Pharmacodynamic Analysis

Categorical
Sex
Ethnicity
PAH history
Tadalafil dose, mg
WHO Class
Concomitant medications: calcium channel blockers, bosentan, and digoxin[a]
Continuous
Age, years
PAH duration, years
Body weight, kg
Baseline 6-minute walk, meters

Source: H6D-MC-LVGY Population Pharmacokinetics Report, Table LVGY.8.2, P40.

The final model no longer included a time-dependent component for the placebo response. Instead, 6-MWD was described as a linear function of age and baseline 6-MWD in this group. For subjects receiving tadalafil treatment, age and AUC_{ss} were found to significantly influence E_{max}. Baseline 6-MWD, PAH etiology and concomitant calcium channel blockers were found to significantly affect T_{50}. For all subjects, WHO Class was a significant predictor of baseline 6-MWD, where clinical worsening, defined as WHO Class III and IV, predicted a decrease of 50 meters.

Table 6: Pharmacodynamic and Covariate Parameter Estimates of the Final Model

Parameter Description	Population Estimate (%SEE)	Interindividual Variability (%SEE)
Baseline 6-Minute Walk (meters)	321 (1.6)	0.0372 (9.6)[a]
Additive Shift for WHO Class I and II on Baseline 6-Minute Walk (meters)	50.4 (13.8)	
Slope for Baseline 6-Minute Walk on Placebo Response (meters)	186 (6.4)	NE
Slope for Age on Placebo Response (meters)	-150 (8.7)	
Active Treatment E_{max} (meters)	60.9 (17.2)	3870 (34.6)[b]
Power for AUC_{ss} on E_{max}	0.225 (45.3)	
Slope for Age on E_{max} (meters/year)	-1.96 (25.1)	
T_{50} for Active (weeks)	14.2 (33.5)	NE
Additive Shift for PAH Related to Collagen Disorders on T_{50} (weeks)	14.0 (42.4)	
Additive Shift for Other PAH on T_{50} (weeks)	-3.11 (22.5)	
Exponent for Baseline Walk on T_{50}	1.51 (21.5)	
Additive Shift for Concomitant Calcium Channel Blockers on T_{50} (weeks)	-5.45 (34.1)	
Residual Error (SD in weeks)	29.51 (7.8)	

Abbreviations: AUC_{ss} = steady-state area under the concentration time curve; E_{max} = maximum response to treatment; NE = not estimated; PAH = pulmonary arterial hypertension; SD = standard deviation; %SEE = percent standard error of the estimate; T_{50} = time at which 50% maximal response is achieved; WHO = World Health Organization.

[a] The estimate provided in the table (0.0372) is a variance term. The corresponding %CV = 19.29%.

[b] The estimate provided in the table (3870) is a variance term. The corresponding SD = 62.21 meters.

Placebo 6-Minute Walk $= 321 + 50.4 \times WHO2 + 186 \times \left(\frac{BaselineWalk}{363}\right) - 150 \times \left(\frac{Age}{54.4}\right)$ where WHO2 = 1 for WHO Class I or II and WHO2 = 0 for WHO Class III or IV.

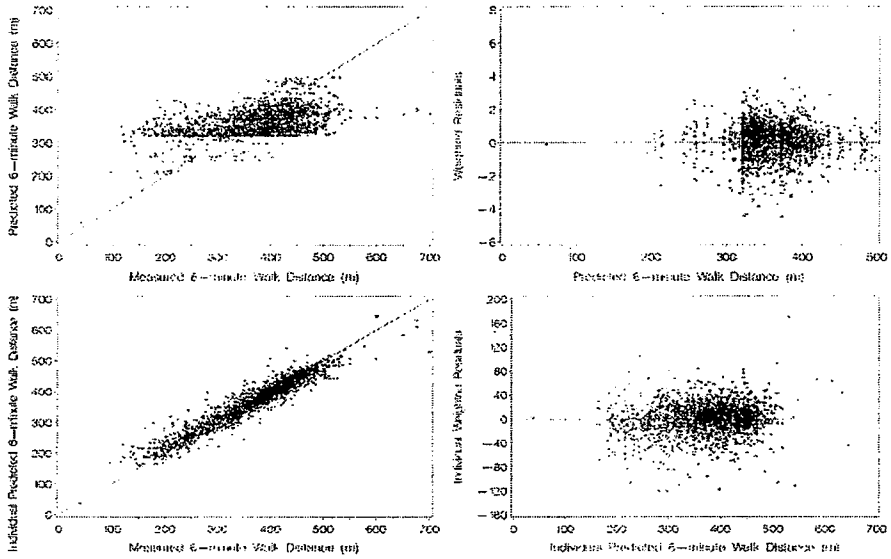
Active Tadalafil 6-Minute Walk =

$$(321 + 50.4 \times WHO2) + \frac{\left[\left(60.9 \times \left(\frac{AUC_{ss}}{5955.3}\right)^{0.225} - 1.96 \times (Age - 53.9)\right) \times WSFD\right]}{\left(14.2 \times \left(\frac{BaselineWalk}{359}\right)^{1.51} + 14 \times PAHC - 3.11 \times PAHO - 5.45 \times CCB\right) + WSFD}$$

where WHO2 = 1 for WHO Class I or II and WHO2 = 0 for WHO Class III or IV; PAHC = 1 for PAH related to collagen disorders and PAHC = 0 otherwise; PAHO = 1 for other PAH and PAHO = 0 for idiopathic and related to collagen disorders; CCB = 1 for those receiving concomitant calcium channel blockers and CCB = 0 when no concomitant calcium channel blockers were reported.

Source: H6D-MC-LVGY Population Pharmacokinetics Report, Table LVGY.9.16, P76.

Figure 7: Goodness of Fit Plots of the Final Pharmacodynamic Model



Source: H6D-MC-LVGY Population Pharmacokinetics Report, Figure LVGY.9.21, P77.

Reviewer's Comment: The goodness of fit plots suggest an adequate fit of the pharmacodynamic model to the 6-MWD data. There are, however, some weaknesses in the model parameterization. First, the final estimate of T_{50} for tadalafil treatment is 14.0 weeks, which suggests that many of the subjects would not have reached their maximum effect at the end of the 16 week study. Therefore, the interpretation of the E_{max} parameter is unclear. Given this model parameterization, it is unlikely one would be able to reliably estimate both T_{50} and E_{max}. Second, the inclusion of covariates on T_{50} is dubious, considering inter-subject variability of this parameter can not be estimated. Third, if the value of AUC_{ss} is set to zero in the final model, 6-MWD is still dependent on weeks from first dose of tadalafil. This is at odds with the placebo subjects, who will also have $AUC_{ss}=0$, yet a constant 6-MWD throughout the study. Predictions from this model should therefore be interpreted with caution. Lastly, the choice of AUC_{ss} as the measure of exposure is open to debate, even if there only a 40% peak to trough fluctuation. The concentration at the time of the 6-minute walk test may be a more appropriate predictor of response.

The Week 16 model-predicted 6-MWD at each dose is presented in Table 7.

Table 7: Model Predicted 6-MWD at Week 16

Dose	No Bosentan		Concomitant Bosentan	
	AUC_{ss} (ng × h/mL)[a]	Increase in 6-minute walk (m)	AUC_{ss} (ng × h/mL)[a]	Increase in 6-minute walk (m)
2.5 mg	1950.4 (913.4 – 3737.6)	25.10 (21.16 – 29.05)	1092.1 (680.46 – 2128.0)	22.03 (19.80 – 25.60)
10 mg	6936.9 (2870.4 – 10898.0)	33.39 (27.38 – 36.96)	2902.8 (2193.5 – 4279.0)	27.45 (25.77 – 29.95)
20 mg	11524.5 (6179.6 – 15449.0)	37.43 (32.53 – 39.98)	6874.60 (4390.0 – 10595.0)	33.32 (30.13 – 36.73)
40 mg	14825.5 (10017.0 – 26792.0)	39.61 (36.27 – 45.26)	9600.0 (5906.3 – 17306.0)	35.92 (32.21 – 41.02)

Abbreviations: AUC_{ss} = steady-state area under the concentration time curve.
[a] Median (10^{th} – 90^{th} percentile)

Source: H6D-MC-LVGY Population Pharmacokinetics Report, Table LVGY.9.20, P94.

Reviewer's Comment: Notwithstanding the limitations of the model parameterization mentioned previously, the model appears to provide a reasonable fit to the data. The predicted increase in 6-minute walk in Table 7 *are fairly consistent with observed median change from baseline 6-MWD.*

4 REVIEWER'S ANALYSIS

4.1 Introduction

The pre-specified statistical criterion for improvement in 6-minute walk distance (6-MWD) in Study H6D-MCLVGY was set to $p < 0.01$. The tadalafil 40 mg group met this criterion with $p = 0.0004$ and a placebo-adjusted treatment difference of 32.8 meters (95% confidence interval: 15.2 to 50.3 meters). The 20 mg treatment groups, however, failed the pre-specified criteria, providing a 27.5 meter (95% confidence interval: 10.6 to 44.3 meters) at $p = 0.0278$. An initial review of the data suggested a similar improvement in 6-MWD is the 10 mg, 20 mg and 40 mg tadalafil treatment groups. In addition, the results of the sponsor's population pharmacokinetic report indicated, "subjects who benefited most from tadalafil treatment received doses of at least 20 mg tadalafil once daily." The purpose of the review was therefore to explore whether 40 mg tadalafil was the optimal dose, or if lower doses could provide the same benefit.

4.2 Objectives

Analysis objectives are:

1. Determine the optimal dose of tadalafil for treatment of PAH to improve exercise capacity.
2. Asses the need for a dose adjustment of tadalafil in patients receiving concomitant bosentan.

4.3 Methods

4.3.1 Data Sets

Data sets used are summarized in Table 8.

Table 8. Analysis Data Sets

Study Number	Name	Link to EDR
H6D-MC-LVGY	wakbrg.xpt	\\Cdsesub1\evsprod\NDA022332\0000\m5\datasets\h6d-mc-lvgy\analysis
H6D-MC-LVGY	rhc.xpt	\\Cdsesub1\evsprod\NDA022332\0000\m5\datasets\h6d-mc-lvgy\analysis
H6D-MC-LVGY	disposit.xpt	\\Cdsesub1\evsprod\NDA022332\0000\m5\datasets\h6d-mc-lvgy\analysis
H6D-MC-LVGY	dataset-pkcovf.xpt	\\Cdsesub1\evsprod\NDA022332\0000\m5\datasets\h6d-mc-lvgypk\analysis
H6D-MC-LVGY	dataset-pkpdf.xpt	\\Cdsesub1\evsprod\NDA022332\0000\m5\datasets\h6d-mc-lvgypk\analysis

4.3.2 Software

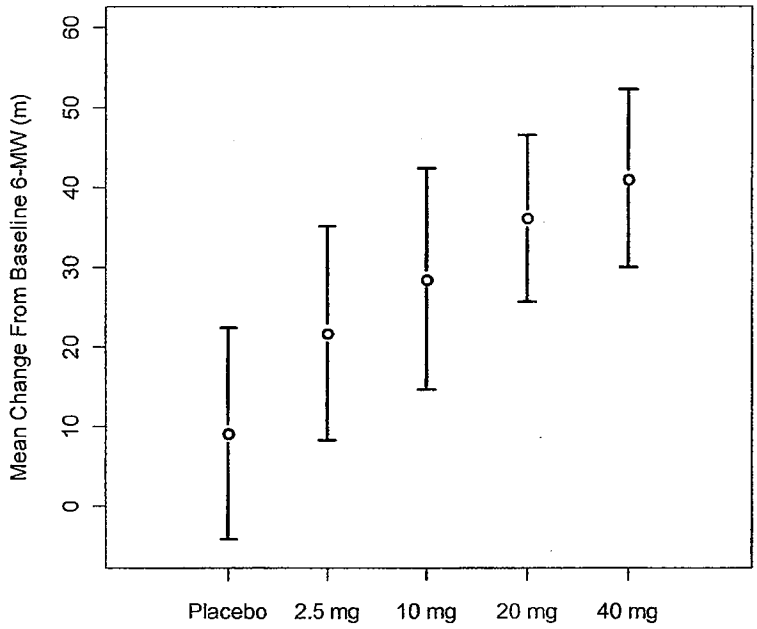
NONMEM Version VI was used to evaluate sponsor's pharmacokinetic/pharmacodynamic modeling. R was used for data manipulation and creating graphs. SAS Version 9.1 was used for the mixed effects models with repeated measures.

4.4 Results

4.4.1 What is the optimal dose of tadalafil for the treatment of pulmonary arterial hypertension (PAH) to improve exercise capacity?

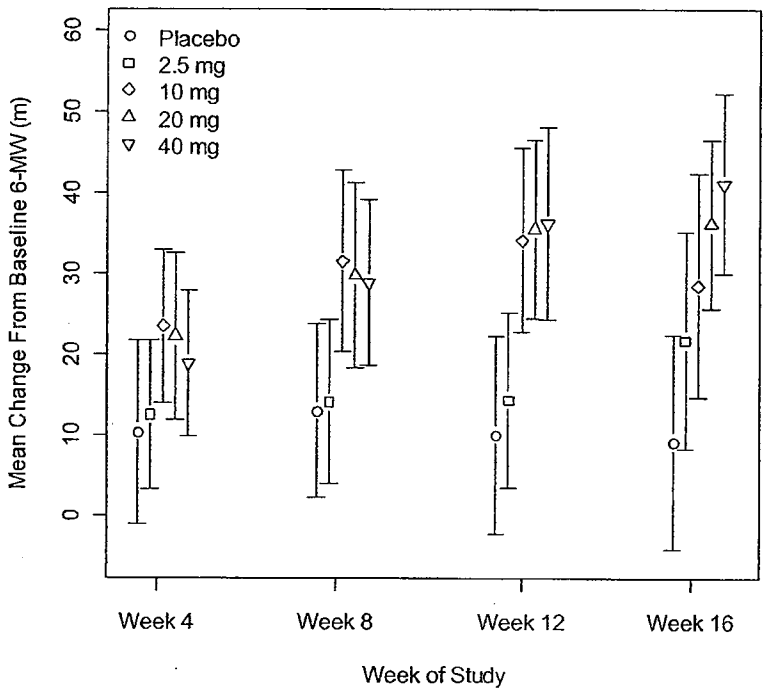
A plot of the mean change from baseline in 6-MWD at Week 16 suggests a clear dose response relationship, with the 40 mg tadalafil treatment group showing the largest response (Figure 8).

Figure 8: Week 16 Change from Baseline in 6-MWD (meters): Mean (95% Confidence Interval)



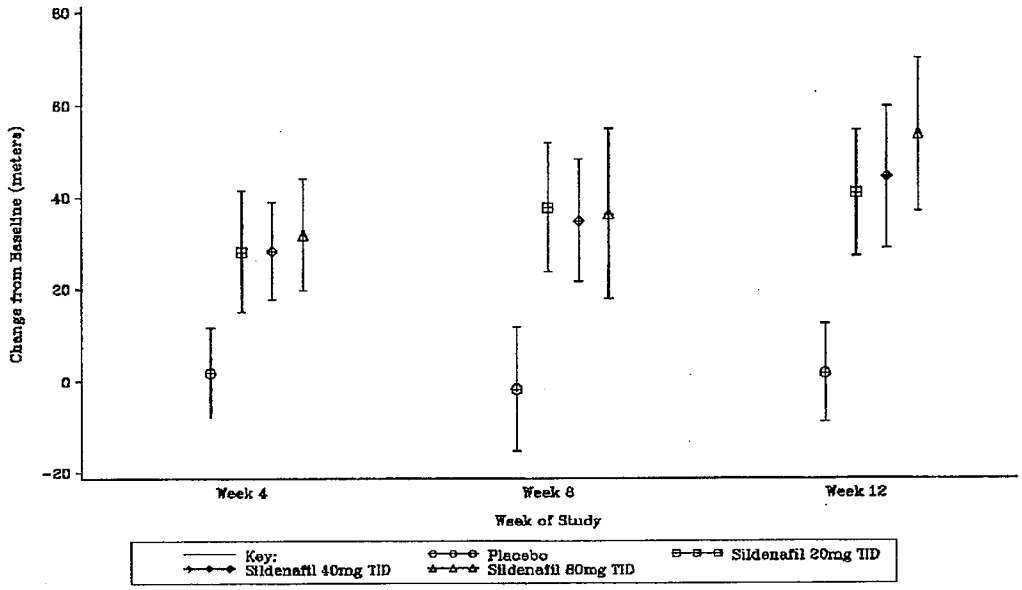
When change from baseline in 6-MWD is plotted for Weeks 4, 8, 12 and 16, a different picture emerges (Figure 9). The apparent dose-response relationship observed in Week 16 is not present in any of the previous weeks of the study. At Weeks 4, 8 and 12, the 2.5 mg tadalafil treatment group has a similar change from baseline in 6-MWD as the placebo group. The 10 mg, 20 mg and 40 mg treatment groups all exhibit a similar improvement in 6-MWD throughout the first twelve weeks. The main differences between Weeks 4-12 and Week 16 appear to be: (1) an enhanced response of the 2.5 mg tadalafil treatment group at Week 16 and (2) a diminished response in the 10 mg tadalafil treatment group in Week 16 compared to previous weeks.

Figure 9: Change from Baseline in 6-Minute Walk Distance (meters): Mean (95% Confidence Interval)



To put these results in context, an analogous plot from another phosphodiesterase type 5 (PDE5) inhibitor, sildenafil (Revatio®) is reproduced in Figure 10. The recommended dose of Revatio® is 20 mg TID. Applying the same standards used for Revatio® to tadalafil would result in the recommendation of the 10 mg dose.

Figure 10: Change from Baseline in 6-Minute Walk Distance (meters): Mean (95% Confidence Interval) - Revatio®



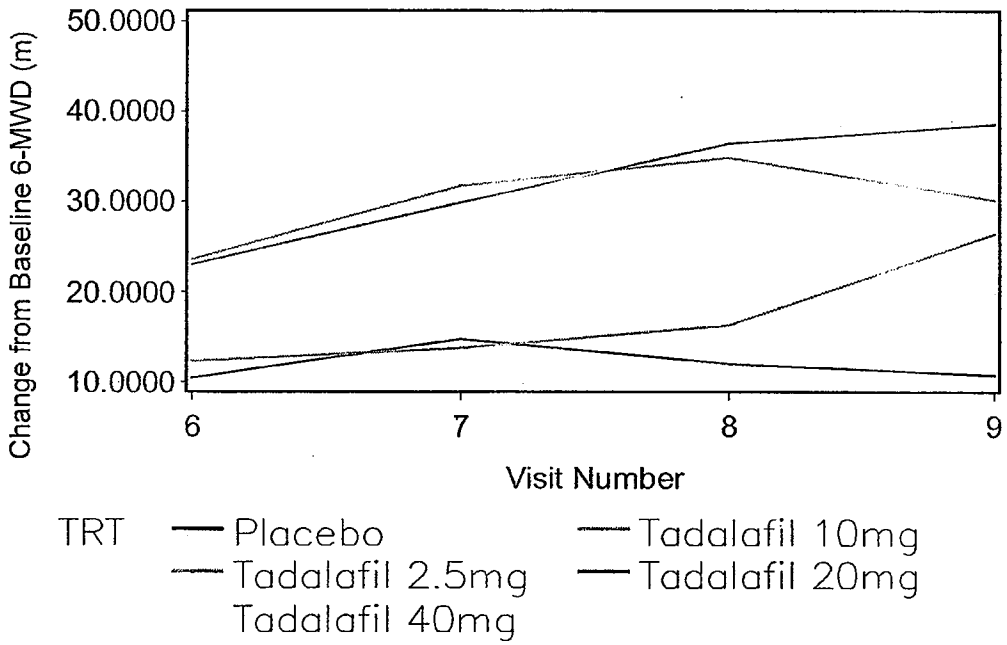
Source: Revatio® label

To attempt to resolve the discrepancy observed at Week 16 in study H6D-MC-LVGY, the impact of the imputation method, last observation carried forward (LOCF), was investigated. Mixed-effects model repeated-measures (MMRM) analyses were carried out on the observed data, without imputation. MMRM use likelihood-based estimation where subject-specific effects and correlations between the repeated measurements are modeled via the within-subject error correlation structure. A comparison between LOCF and MMRM is provided in Table 9 and Figure 11. Mean changes from baseline in 6-MWD at Week 16 were larger using the MMRM method. A similar dose-response trend was observed using LOCF and MMRM. Of note, the 10 mg tadalafil treatment group showed a diminished response at Week 16 and the 2.5 mg tadalafil treatment group showed a sudden increase in 6-MWD at Week 16. Mean change in 6-MWD were similar for the 20 mg and 40 mg tadalafil treatment groups throughout the course of the study,

Table 9: Comparison of Mean Change from Baseline 6-MWD at Week 16 Calculated with LOCF and MMRM

	LOCF	No Imputation (repeated measures model)
Dose	**Mean**	**Mean**
2.5 mg	**21.8**	**26.5**
10 mg	**28.6**	**30.1**
20 mg	**36.2**	**38.6**
40 mg	**41.1**	**42.4**

Figure 11: Mean Change from Baseline 6-MWD (meters) using MMRM



The impact of LOCF for the 20 mg and 40 mg tadalafil treatment groups was further explored by examining the reasons for withdrawal from the study (Figure 12 and Figure 13). It was thought that the greater number of subjects with LOCF values at Week 16 in the 20 mg group (11/80) compared to the 40 mg group (4/76) might be due to worsening of disease in these subjects. From Figure 12 it is clear that only two of the 11 subjects in the 20 mg group dropped out to due to worsening of WHO class. Of the other 9 subjects, there does not appear to be any trend which would significantly affect the calculation of the mean effect. Two of the four subjects in the 40 mg group who had LOCF values at

Week 16 dropped out of the study due to worsening of WHO class. One of these subjects had a change from baseline 6-MWD value of 23.8 meters imputed at Week 16, which is likely to overestimate the true benefit for this individual.

Figure 12: Patients with LOCF at Week 16 and Reason for Withdrawal: 20 mg Tadalafil

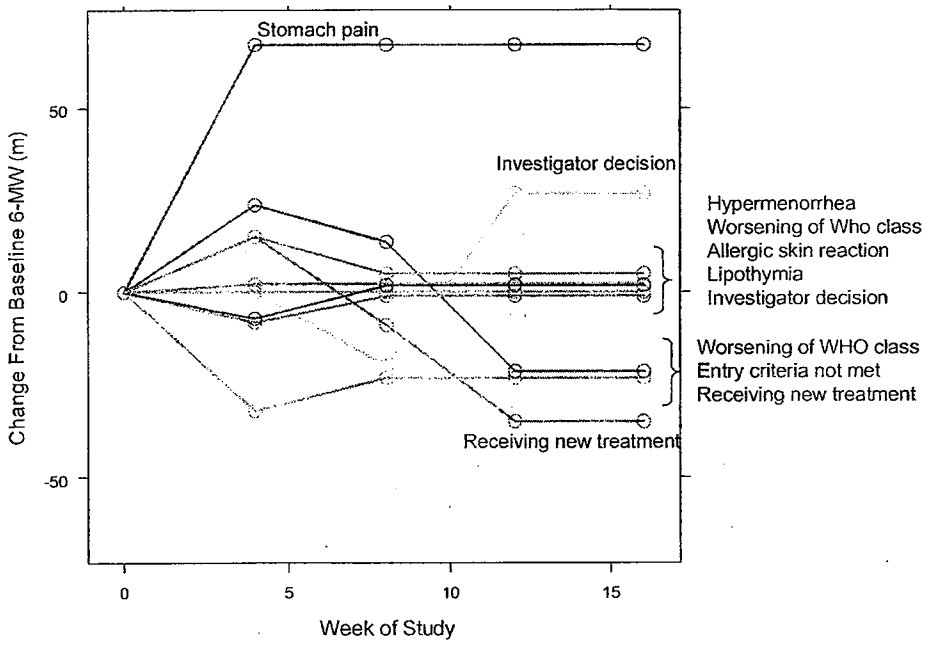
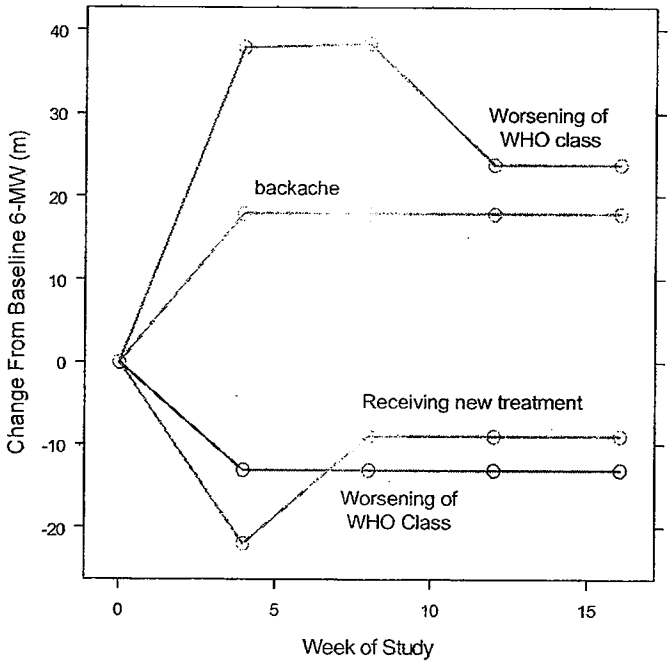


Figure 13: Patients with LOCF at Week 16 and Reason for Withdrawal: 40 mg Tadalafil



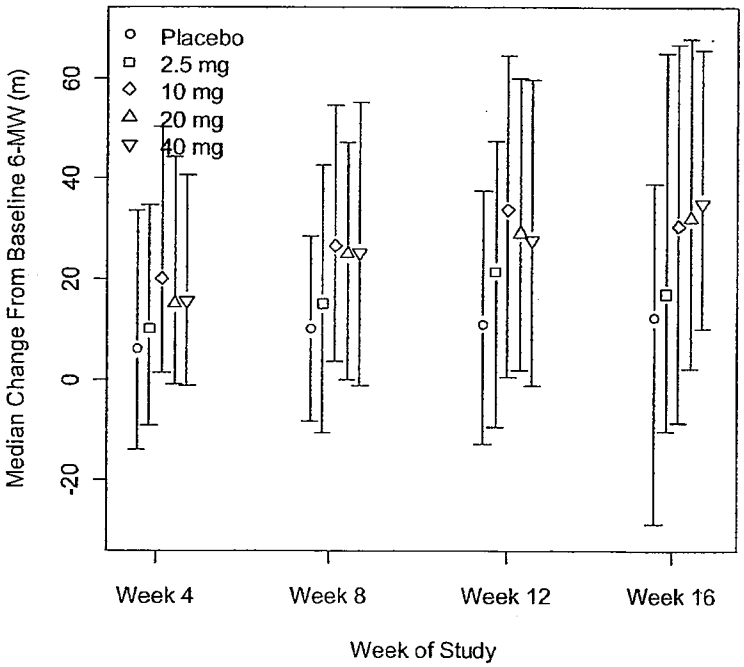
Lastly, the impact of the imputation method was investigated by using the worst observation carried forward (WOCF). Change from baseline in 6-MWD using WOCF at Week 16, however, was similar to that from that produced using LOCF (Table 10), providing more evidence that the choice of imputation method did not cause the unexpected results at Week 16.

Table 10: Comparison of Mean Change from Baseline 6-MWD at Week 16 Calculated with LOCF and WOCF

	LOCF	WOCF
Dose	Mean	Mean
2.5 mg	21.8	20.2
10 mg	28.6	26.9
20 mg	36.2	34.9
40 mg	41.1	41.0

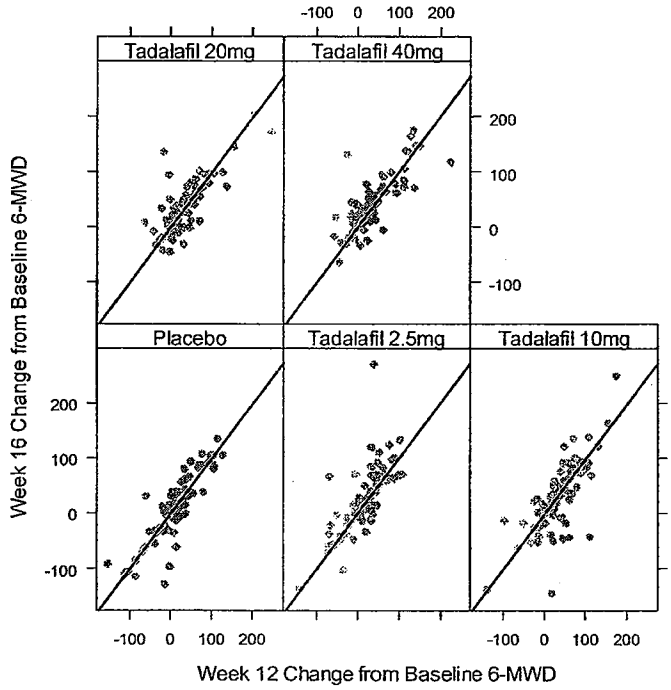
A second possible explanation for the inconsistency of the Week 16 results is the presence of outliers, or an asymmetrical distribution of 6-MWD observations. This possibility was first explored by examining the median value of change from baseline in 6-MWD. The resulting plot (Figure 14) no longer exhibits the inconsistent behavior at Week 16. The 10 mg, 20 mg and 40 mg tadalafil treatment groups show similar change from baseline in 6-MWD throughout the 16 weeks of the study.

Figure 14: Change from Baseline in 6-Minute Walk Distance (meters): Median (25th to 75th Percentiles)



The cause of the unexpected behavior of the Week 16 mean change from baseline in 6-MWD seen in Figure 9 and in the MMRM analysis can be partly attributed to the presence of a few outliers in the Week 16 data. These outliers are visualized in Figure 15 where points lying along the line of unity indicate no change in 6-MWD from the Week 12 to Week 16 visits. In the 2.5 mg group, there is one point lying far above the line of unity, indicating a vastly improved 6-MWD at Week 16 in one patient. This one point is a cause of the enhanced response of the 2.5 mg tadalafil treatment group at Week 16. Conversely, there are a few points in the 10 mg group that fall far below the line of unity. These few patients are a cause of the diminished response observed in this group. Week 16 6-MWD observations which differed by more than 100 meters from the corresponding Week 12 6-MWD observations were subsequently removed from the database to investigate their influence on mean calculations. Mean change from baseline 6-MWD distance for the placebo and 2.5 mg groups were 11 meters and 18 meters, respectively. The 10, 20 and 40 mg treatments groups now had more similar mean change from baseline 6-MWD values of 33, 35 and 39 meters, respectively.

Figure 15: Week 16 vs. Week 12 Change from Baseline 6-MWD (meters)



Concentration 6-MWD Relationship

The concentration 6-MWD relationship was constructed using C_{min} values and Week 12 change from baseline 6-MWD. Week 12 was chosen because the 6-minute walk test at this visit was performed at trough tadalafil concentrations. Figure 16 shows an evident relationship between increasing tadalafil C_{min} values and increasing change from baseline 6-MWD. Since concentrations varied widely, patients were binned by concentrations percentiles of 0, <0-25th, >25th – 50th, >50th – 75th, and >75th – 100 so that an approximate equal number of patients composed each percentile. The largest 6-MWD change from baseline occurs in the bins corresponding to the predicted mean C_{min} for the 20 and 40 mg doses, 284 and 432 ng/mL, respectively (Figure 2 and Table 11).

Figure 16: 6-MWD Change from Baseline (Week 12) vs. Tadalafil C_{min}

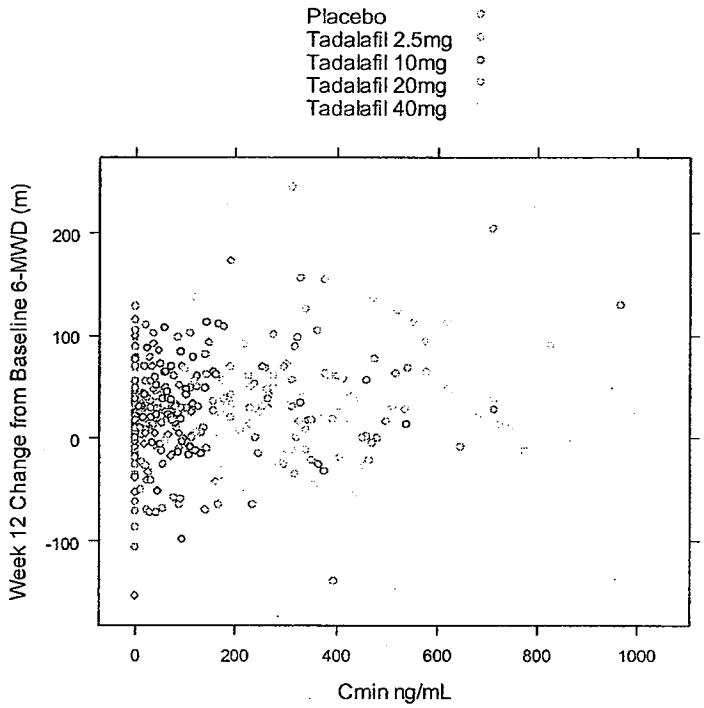


Table 11: 6-MWD Change from Baseline by C_{min} (binned)

Bin (# patients)	Median C_{min} (ng/mL)	Mean Change in 6-MWD (m)
1 (74)	0	16.7
2 (70)	34.6	23.1
3 (70)	102.3	26.1
4 (70)	250.6	38.5
5 (71)	459.2	40.6

Concentration Pulmonary Vascular Resistance (PVR) Relationship

The concentration PVR relationship was investigated using C_{min} values and PVR calculated at the Week 16 visit from the subset of subjects (n = 93) who took part in the cardiopulmonary hemodynamic substudy. Again, patients were binned by concentrations percentiles of 0, <0-25th, >25th – 50th, >50th – 75th, and >75th – 100 so that an approximate equal number of patients composed each percentile. The results in Table 12 and illustrated in Figure 3 suggest a maximum PVR change from baseline is achieved at C_{min}

greater than 200 ng/mL, which corresponds with the predicted C_{min} for the 20 mg dose (276.4 ng/mL).

Table 12: PVR Change from Baseline by Median C_{min} (binned)

Bin (# patients)	Median C_{min} (ng/mL)	Median Change in PVR (dynes*sec*cm^{-5})
1 (13)	0	-16.0
2 (14)	25.2	-47.0
3 (14)	99.0	-137.78
4 (14)	222.9	-203.4
5 (14)	505.0	-165.9

Relationship of PDE5 Inhibition to In Vivo Plasma Tadalafil Concentrations

Tadalafil inhibits PDE5 with an IC50 of 0.94 nM. Maximum free tadalafil concentration ranged from approximately 52 to 92 times the PDE5 IC50 following the 20 and 40 mg doses. For further information, please see the PharmTox Review by John Koerner.

4.4.2 Should there be a dose adjustment of tadalafil with concomitant bosentan administration?

The population pharmacokinetic analysis found that concomitant bosentan increases apparent oral clearance of tadalafil by 75%. Given the decrease in tadalafil exposure with bosentan administration, the reviewer explored the need for dose adjustment of tadalafil. Figure 4 shows that no dose adjustment is necessary for the 20 mg and 40 mg doses. Median 6-MWD change from baseline for the 20 mg dose with and without concomitant bosentan administration was 31.25 and 32.0 meters, respectively. The median 6-MWD change from baseline for the 40 mg dose with and without concomitant bosentan administration was 37.05 and 30.5 meters, respectively. Concomitant bosentan administration only becomes relevant for the 10 mg dose where median 6-MWD change from baseline with concomitant bosentan is 20.0 meters compared to 35.0 meters without. These results suggest that the 20 mg and 40 mg doses are high enough on the dose-response relationship that the decrease in tadalafil exposure with concomitant bosentan is not a significant factor.

5 LISTING OF ANALYSES CODES AND OUTPUT FILES

File Name	Description	Location in \\cdsnas\pharmacometrics\
analysis.sas	Mixed effects model with repeated measures	Tadalafil\Reviewer\SAS\code
wocf.R	Worst observation carried forward analysis	Tadalafil\Reviewer\PKPDdatacheck
locf.R	Last observation carried forward analysis	Tadalafil\Reviewer\PKPDdatacheck

make.locfplots.R	Plots investigating influence of LOCF	Tadalafil\Reviewer\PKPDdatacheck
medianlocf.R	Calculation and plotting of median 6-MWD	Tadalafil\Reviewer\PKPDdatacheck
hemo.R	PVP exposure-response analysis and plots	Tadalafil\Reviewer\Hemo
er.R	6-MWD exposure-response analysis and plots	Tadalafil\Reviewer\PKPDdatacheck
run1.mod	Sponsor's base pharmacokinetic model (control file)	Tadalafil\Reviewer\NM
run1.lst	Sponsor's base pharmacokinetic model (output file)	Tadalafil\Reviewer\NM
run2.mod	Sponsor's final pharmacokinetic model (control file)	Tadalafil\Reviewer\NM
run2.lst	Sponsor's final pharmacokinetic model (output file)	Tadalafil\Reviewer\NM
run3.mod	Sponsor's base pharmacokinetic-pharmacodynamic model (control file)	Tadalafil\Reviewer\NM
run3.lst	Sponsor's base pharmacokinetic-pharmacodynamic model (output file)	Tadalafil\Reviewer\NM
run4.mod	Sponsor's final pharmacokinetic-pharmacodynamic model (control file)	Tadalafil\Reviewer\NM
run4.lst	Sponsor's final pharmacokinetic-pharmacodynamic model (output file)	Tadalafil\Reviewer\NM

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