

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

202714Orig1s000

PHARMACOLOGY REVIEW(S)

**FOOD AND DRUG ADMINISTRATION
MEMORANDUM**

Date: June 28, 2012

From: Jeffrey Bray, PhD, Pharmacology/Toxicology Reviewer
Division of Reproductive and Urologic Products (DRUP)
For: Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Center for Drug Evaluation and Research

To: File for NDA 202714 Kyprolis (carfilzomib)

Re: Correction to Pharmacology/Toxicology NDA Review and Team Leader
Memorandum in DARRTS dated June 1, 2012 and June 6, 2012,
respectively

The purpose of this memo is to make corrections regarding the interpretation of data from embryo-fetal toxicity studies in rats and to provide a rationale for using doses in the package insert to compare animals to humans, rather than exposures (AUC). These corrections apply to both the Pharmacology/Toxicology NDA review by Drs. Bray and Hawes, as well as the Team Leader Memorandum by Dr. Palmby.

The first series of corrections are regarding the interpretation of the pivotal Embryofetal Toxicity study in Rats (#TR-0075-171). The original review and memorandum were based on the interpretation of the data submitted to NDA 202714 that was made during the review. However, upon reassessment of the data, this reviewer concludes that in the GLP Embryo-fetal Toxicity studies in rats (#TR-0075-171), carfilzomib did not cause significant embryo-fetal toxic effects. This reviewer simply misread across the columns in reaching the below conclusions.

Table 91: Selected Gestation Day 20 Laparohysterectomy Data Obtained from a Rat Embryo-Fetal Reproductive Toxicity Study of Daily IV PR-171 Administration (N=20-22/group)

Parameter	Dose (mg/kg)			
	0	0.5	1	2
No. Initial Females in Study	22	22	22	22
Pregnant Females	22	22	22	22
Pregnant Females at Termination	22	22	22	19
No. Females w/ Total Implantation Loss	0	0	0	1
Mean Gravid Uterine Weight (g)	77.05	77.92	80.07	82.82
Mean Early Resorptions/dam	0.8	0.7	1.0	1.4
Pre-implantation loss (%)	5.0	3.1	2.8	2.1
Post-implantation loss (%)	6.7	5.1	7.5	5.4
Mean Viable Fetuses/dam	12.5	12.7	12.6	13.1
Mean Fetal Weight (g)	4.0	4.0	4.1	4.1

*, P<0.05 v. control

In the Pharmacology/Toxicology NDA Review, the affected sentences are on pages 12, 163, 166, and 180 of the review submitted to DARRTS on June 1, 2012. These changes do not affect the overall conclusions reached by the nonclinical reviewers. The following statements can be modified as follows, since the interpretation is not entirely consistent with, nor supported by the data listed in **Table 91**, which are correctly extracted from Study #TR-0075-171:

- pg. 12 (last paragraph), **Executive Summary**: Delete ~~strikeout~~ text; add underlined text – “Carfilzomib caused no overt teratogenicity in pregnant rats at exposures (AUC) or in rabbits at doses that were lower than in patients receiving the recommended dose. Embryo-lethality occurred below human exposures or doses based on findings of increased pre-implantation loss and post-implantation loss from early resorptions in ~~rats and~~ rabbits. The negative effects on implantation ~~in both species~~, and fetal weight decreases in rabbits may be secondary due to maternal toxicities, such as body weight and feed consumption decreases.”
- pg. 163, **Key Study Findings**: delete sentence - “Increased pre-implantation loss and early resorptions at ≥ 1 mg/kg”.
- pg. 166, **Cesarean Data**: delete sentence - “There was an increase in early resorptions that resulted in a decreased % pre-implantation loss at ≥ 1 mg/kg”.
- pg. 180, **Reproductive Toxicity**: delete sentences - “Increased pre-implantation loss and early resorptions were observed at ≥ 1 mg/kg/day (approximately 10% the AUC in patients), although no findings were statistically significant compared to control.”
delete ~~strikeout~~ text; add underlined text – “Embryo-lethality occurred at doses equivalent to or below human exposures based on findings of increased pre-implantation loss and post-implantation loss from early resorptions in ~~rats and~~ rabbits.”

In the Team Leader Memorandum, the affected sentences are on page 2 submitted to DARRTS on June 6, 2012. Again, these changes did not affect the overall conclusions. The following statements can be modified as follows:

- pg. 2 (second to last paragraph): Delete ~~strikeout~~ text; add underlined text –
“Carfilzomib ~~caused embryo-fetal toxicity in rats and rabbits, but was not~~ teratogenic in rats or rabbits, but caused embryo-fetal toxicity in rabbits.”
“Therefore, this study was acceptable to ~~confirm similar~~ demonstrate toxicities in ~~a GLP embryo-fetal developmental toxicity study in rats, which included~~ toxicokinetics in rabbits.”

The second series of corrections are to justify the use of dose multiples between animals in toxicology studies and humans rather than exposures (AUC) and are directed at information provided on page 13 (section 1.3.3 Labeling). The Pharmacology/Toxicology NDA Review states that exposures (AUC) in animals were provided in the package insert for Kyprolis (carfilzomib) compared to exposures in patients who received the recommended dose of 27 mg/m² for two weeks in clinical trial PX-171-007 A1. However, during labeling negotiations, the Applicant provided additional information that sampling times in patients included an initial sampling immediately following completion of carfilzomib infusion, while in chronic repeat-dose toxicology studies in rats (study # TR-0072-171) and monkeys (study #TR-0073-171), the first sample for toxicokinetic analysis was taken 5 minutes following bolus intravenous (IV) carfilzomib administration.

Study TR-0383-171 in rats assessed the effects of different pharmacokinetic (PK) sampling times on plasma concentration and AUC. Following an 8 mg/kg IV dose, the plasma concentration of carfilzomib at 2 minutes post-dose was approximately 4% the concentration observed immediately after dosing. The observed C_{max} and AUC values were approximately 28- and 3-fold lower, respectively, if the first sampling time was 2 minutes post-dose compared to the first sample collection immediately following dosing. Therefore, the observed exposures (C_{max} and AUC) in the nonclinical toxicology studies represent an underestimate of the actual exposures as a result of the sampling times and short half-life of carfilzomib. Based on these findings, comparisons between animals in toxicology studies and humans in relevant sections of the package insert were made based on doses (body surface area) rather than based on exposure (AUC).

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JEFFREY D BRAY
06/29/2012

TODD R PALMBY
06/29/2012

I concur with Dr. Bray's conclusions and the amendments made to the Pharmacology/Toxicology NDA Review and the Team Leader Memorandum for NDA 202714 Kyprolis (carfilzomib) .

MEMORANDUM

Kyprolis (carfilzomib)

Date: June 7, 2012

To: File for NDA 202714

From: John K. Leighton, PhD, DABT

Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review of Drs. Bray and Hawes and labeling and secondary memorandum provided by Dr. Palmby. I concur with Dr. Palmby's conclusion that Kyprolis may be approved and that no additional nonclinical studies are needed for the proposed indication.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JOHN K LEIGHTON
06/07/2012

MEMORANDUM

Date: June 6, 2012
From: Todd R. Palmby, Ph.D.
Secondary Pharmacology/Toxicology Reviewer
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
To: File for NDA 202714 KYPROLISTM (carfilzomib)
Re: Approvability for Pharmacology and Toxicology
Indication: Treatment of patients with relapsed and refractory multiple myeloma who have received at least 2 prior lines of therapy that included a proteasome inhibitor and an immunomodulatory agent

Non-clinical pharmacology and toxicology studies to support KYPROLIS (carfilzomib) NDA 202714 for the treatment of relapsed and refractory multiple myeloma were reviewed by Jeffrey Bray, Ph.D. and Jessica J. Hawes, Ph.D. Studies conducted with intravenously administered carfilzomib included pharmacology, toxicokinetics and ADME, safety pharmacology, single- and repeat-dose toxicology (rat and monkey), and genetic toxicology (*in vivo* and *in vitro*). Reproductive and developmental toxicology studies were conducted in rats and rabbits to assess the effects of carfilzomib on embryo-fetal development. The studies cited in the review consist primarily of original research studies conducted by the Applicant.

Pharmacology studies submitted to the NDA support that carfilzomib is a proteasome inhibitor that irreversibly binds to the active sites of the 20S proteasome, the proteolytic core particle within the 26S proteasome. The mechanism of action of carfilzomib is the same as bortezomib (i.e., inhibition of the 20S proteasome), although differences exist in their abilities to inhibit the chymotrypsin-like, peptidyl-glutamyl peptide-hydrolyzing (PGPH)-like and trypsin-like activities of the 20S proteasome and in that bortezomib binding to the 20S proteasome is reversible. These differences may account for the distinct toxicity and activity profile of carfilzomib. As carfilzomib is an irreversible proteasome inhibitor, recovery of proteasome inhibition requires production of new proteasome protein, which occurred in ≥ 24 hours, depending on the tissue. The Established Pharmacologic Class (EPC) determined to be most appropriate for carfilzomib is "proteasome inhibitor" since data provided in the NDA demonstrate that it has a similar mechanism of action as previously approved products in this class.

The clinical route of administration for carfilzomib is an intravenous infusion over 2 to 10 minutes. The majority of studies in animals submitted to this NDA (e.g., safety pharmacology, ADME, repeat-dose toxicology, *in vivo* genetic toxicology, reproductive and developmental toxicology) were conducted with bolus intravenous injections of carfilzomib. A series of studies in rats were conducted comparing the pharmacodynamics, pharmacokinetics and toxicities of

intravenous carfilzomib administered as a bolus injection, a 10-minute infusion or a 30-minute infusion. The C_{max} and significant toxicities, including death and pre-renal azotemia (elevated blood urea nitrogen and creatinine), observed following a bolus injection were reduced when administered as a 10- or 30-minute infusion, while a similar level of proteasome inhibition was maintained. These results suggest that some toxicities observed following administration of carfilzomib may be related to the C_{max} . Adverse effects in rats and/or monkeys that were consistent with adverse events reported in clinical trials included cardiovascular (decreased blood pressure, increased heart rate, increased troponin-I, cardiac failure, cardiac fibrosis, pericardial fluid accumulation, cardiac hemorrhage/degeneration), renal (glomerulonephropathy, tubular necrosis, dysfunction), gastrointestinal (necrosis/hemorrhage), pulmonary (hemorrhage/inflammation), hepatic (changes in serum transaminases) and hematopoietic (decreased platelets) toxicities.

Carfilzomib was clastogenic in the *in vitro* chromosomal aberration test in peripheral blood lymphocytes, but was not mutagenic in the *in vitro* bacterial reverse mutation (Ames) assay or clastogenic in the *in vivo* mouse bone marrow micronucleus assay.

Carfilzomib caused embryo-fetal toxicity in rats and rabbits, but was not teratogenic. In rabbits, only a pilot, non-GLP embryo-fetal developmental toxicity study was conducted, which did not include a toxicokinetic analysis. In this study, carfilzomib caused embryo-fetal effects including increased pre- and post-implantation loss and early resorptions and a decrease in fetal weight as well as maternal toxicity. Therefore, this study was acceptable to confirm similar toxicities in a GLP embryo-fetal developmental toxicity study in rats, which included toxicokinetics. Section 8.1 Pregnancy in the package insert for KYPROLIS reflects the lack of toxicokinetic data in rabbits with the comparison to doses in patients based on body surface area rather than systemic exposure (AUC). The potential benefit of KYPROLIS in pregnant women in the indicated patient population may outweigh the potential risk to the developing fetus. Therefore, Pregnancy Category D is recommended.

Recommendation: I concur with Drs. Bray's and Hawes' conclusion that pharmacology and toxicology data support the approval of NDA 202714 for KYPROLIS. There are no outstanding nonclinical issues that would preclude the approval of KYPROLIS for the proposed indication.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

TODD R PALMBY
06/06/2012

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 202714
Supporting document/s: 1, 2
Applicant's letter date: 9/27/2011
CDER stamp date: 9/27/2011
Product: Kyprolis™ (carfilzomib)
Indication: Treatment of patients with relapsed and refractory multiple myeloma who have received at least 2 prior lines of therapy that included a proteasome inhibitor and an immunomodulatory agent
Applicant: Onyx Pharmaceuticals
Review Division: Division of Hematology Oncology Toxicology
(Division of Hematology Products)
Reviewer: Jeffrey Bray, PhD
Jessica J. Hawes, PhD
Secondary Reviewer: Todd R. Palmby, PhD
Division Director: John Leighton, PhD, DABT (Acting)
(Ann Farrell, MD (Acting))
Project Manager: Karen Bengtson

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202714 are owned by Onyx Pharmaceuticals or are data for which Onyx Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of NDA 202714 that Onyx Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 202714.

TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	10
1.1	INTRODUCTION	10
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	10
1.3	RECOMMENDATIONS	13
2	DRUG INFORMATION	14
2.1	DRUG	14
2.2	RELEVANT IND/s, NDA/s, AND DMF/s	14
2.3	DRUG FORMULATION	14
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	15
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN	16
2.7	REGULATORY BACKGROUND	17
3	STUDIES SUBMITTED.....	17
3.1	STUDIES REVIEWED.....	17
3.2	STUDIES NOT REVIEWED	20
3.3	PREVIOUS REVIEWS REFERENCED.....	20
4	PHARMACOLOGY.....	21
4.1	PRIMARY PHARMACOLOGY	21
4.2	SECONDARY PHARMACOLOGY	41
4.3	SAFETY PHARMACOLOGY	43
5	PHARMACOKINETICS/ADME/TOXICOKINETICS	44
5.1	PHARMACOKINETICS	44
5.2	ABSORPTION.....	49
5.3	DISTRIBUTION	51
5.4	METABOLISM.....	61
5.5	EXCRETION.....	75
5.6	PHARMACOKINETIC DRUG INTERACTIONS	78
5.7	TOXICOKINETICS	88
6	GENERAL TOXICOLOGY.....	97
6.1	SINGLE-DOSE TOXICITY	97
6.2	REPEAT-DOSE TOXICITY	99
7	GENETIC TOXICOLOGY	141
7.1	<i>IN VITRO</i> REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES).....	141
7.2	<i>IN VITRO</i> ASSAYS IN MAMMALIAN CELLS.....	149
7.3	<i>IN VIVO</i> CLASTOGENICITY ASSAY IN RODENT (MICRONUCLEUS ASSAY).....	154
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	161
9.2	EMBRYONIC FETAL DEVELOPMENT	161
10	SPECIAL TOXICOLOGY STUDIES.....	170

11	INTEGRATED SUMMARY AND SAFETY EVALUATION.....	171
12	ATTACHMENTS	181

Table of Tables

Table 1: IC ₅₀ fold differences between the proteasome chymotryptic activity assay and other proteasome activity assays	22
Table 2: $k_{\text{intact}}/K_i(\text{M}^{-1}\text{s}^{-1})$ values for inhibition of proteasome activities	22
Table 3: Calculated IC ₅₀ s for bortezomib and carfilzomib proteasome inhibition in bortezomib resistant cell lines	35
Table 4: <i>In vitro</i> activity of carfilzomib metabolites on the chymotrypsin-like activity of the constitutive proteasome and immunoproteasome	41
Table 5: Protease inhibition by PR-171 and bortezomib <i>in vitro</i>	42
Table 6: IC ₅₀ s for carfilzomib inhibition of neurokinin NK1 and NK2 receptors	43
Table 7: <i>In vitro</i> hERG inhibition by carfilzomib	44
Table 8: Pharmacokinetic parameters of carfilzomib following a single intravenous bolus and 30-minute infusion in rats	45
Table 9: Pharmacokinetic parameters of frozen and lyophilized carfilzomib drug product in rats following intravenous bolus administration	46
Table 10: Noncompartmental pharmacokinetic parameters for 1 mg/kg in Cynomolgus monkeys	47
Table 11: Noncompartmental pharmacokinetic parameters for 2 mg/kg in Cynomolgus monkeys	47
Table 12: Noncompartmental pharmacokinetic parameters for 4 mg/kg in Cynomolgus monkeys	48
Table 13: Pharmacokinetic parameters of carfilzomib in male mice following an intravenous bolus administration of 5 mg/kg	49
Table 14: Pharmacokinetic parameters of PR-58825 in female Cynomolgus monkeys after intravenous bolus administration at 2.5 mg/kg	50
Table 15: Pharmacokinetic parameters of PR-58825 in female Cynomolgus monkeys after nasogastric administration at 20 mg/kg	50
Table 16: Tissue:plasma concentration ratios after a single intravenous administration of ³ H-PR-171 to male rats	54
Table 17: Percentage of bound carfilzomib in rat, monkey and human plasma	60
Table 18: Percentage of remaining carfilzomib after 20 hour incubation in human, monkey and rat plasma	60
Table 19: Binding of carfilzomib to plasma proteins of rat, monkeys and human	61
Table 20: Stability of carfilzomib in rat, monkey and human plasma	61
Table 21: <i>In vitro</i> hepatic extraction ratio from liver microsomal incubation with NADPH	62
Table 22: <i>In vitro</i> intrinsic clearance (CL_{int} ($\mu\text{L}/\text{min}/\text{mg}$ proteins)) from liver cytosol incubation and liver microsome incubation with or without NADPH	62
Table 23: Phase I and II metabolic activities of primary hepatocytes	64
Table 24: Percentage of carfilzomib metabolites in rat hepatocytes	66
Table 25: Percentage of carfilzomib metabolites in monkey hepatocytes	66
Table 26: Percentage of carfilzomib metabolites in human hepatocytes	66
Table 27: Structures of carfilzomib and three metabolites	68
Table 28: Half-lives for <i>in vitro</i> metabolism of carfilzomib in rat blood and tissue homogenates	69

Table 29: Metabolites of carfilzomib identified from incubation with cryopreserved human hepatocytes	71
Table 30: Effect of P450 inhibitors on the rate of carfilzomib metabolism	72
Table 31: Carfilzomib metabolites identified by LC/MS	73
Table 32: Pharmacokinetic parameters of carfilzomib following a single intravenous bolus administration of 2 mg/kg in male rats	77
Table 33: Renal excretion of carfilzomib and its metabolites in rats after an intravenous bolus administration of 2 mg/kg	77
Table 34: Biliary excretion of carfilzomib and its metabolites in rats after an intravenous bolus administration of 2 mg/kg	78
Table 35: <i>In vitro</i> CYP P450 inhibition by carfilzomib	79
Table 36: CYP2C activities in rats following intravenous bolus administration of carfilzomib	80
Table 37: CYP3A inhibition in rats following intravenous bolus administration of carfilzomib	80
Table 38: Microsomal protein yields and total cytochrome P450 content in Cynomolgus monkeys after intravenous bolus administration of carfilzomib	81
Table 39: Microsomal CYP1A activity in Cynomolgus monkeys after intravenous bolus administration of carfilzomib	82
Table 40: <i>In vitro</i> evaluation of carfilzomib as an inhibitor of human CYP enzymes	83
Table 41: <i>In vitro</i> CYP1A2 activity in human primary hepatocytes treated with carfilzomib	84
Table 42: <i>In vitro</i> CYP3A4 activity in human primary hepatocytes treated with carfilzomib	85
Table 43: Effect of carfilzomib on CYP3A4 mRNA expression in primary human hepatocytes	86
Table 44: Pharmacokinetic parameters of carfilzomib and its metabolites at 0.5 mg/kg in pregnant rats	89
Table 45: Pharmacokinetic parameters of carfilzomib and its metabolites at 1 mg/kg in pregnant rats	90
Table 46: Pharmacokinetic parameters of carfilzomib and its metabolites at 2 mg/kg in pregnant rats	90
Table 47: Pharmacokinetic parameters of carfilzomib in rats	91
Table 48: Pharmacokinetic parameters of the carfilzomib metabolite, PR-389, in rats	92
Table 49: Pharmacokinetic parameters of the carfilzomib metabolite, PR-413, in rats	92
Table 50: Pharmacokinetic parameters of the carfilzomib metabolite, PR-519, in rats	93
Table 51: Plasma concentration-time profile for carfilzomib in rats	93
Table 52: Pharmacokinetic parameters of carfilzomib in Cynomolgus monkeys	95
Table 53: Pharmacokinetic parameters of PR-389 in Cynomolgus monkeys	95
Table 54: Pharmacokinetic parameters of PR-413 in Cynomolgus monkeys	96
Table 55: Pharmacokinetic parameters of PR-519 in Cynomolgus monkeys	96
Table 56: Histopathological Findings of Note in Male Monkeys Given 2 mg/kg Intravenous PR-171 for Two Days	103
Table 57: Histopathological Findings of Note with Severity* in Rats Administered PR-171 by Intravenous Bolus (n=3 or 4/group)	108

Table 58: Notable Findings in FOB in Male Rats Administered Intravenous 2 mg/kg Carfilzomib or 0.2 mg/kg Bortezomib for 4 Weeks (n=20).....	112
Table 59: Probable Causes of Unscheduled Deaths in Main Study Based on Histopathology / Sacrifices Occurring during 6 Cycles of Dosing with PR-171	115
Table 60: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) in Rats Administered Intravenous PR-171 for 12 or 22 Weeks.....	117
Table 61: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) Following 10 Weeks of Recovery.....	117
Table 62: Percent or Fold (X) Change in Selected Mean Hematology Parameters at Termination Compared to Control in Rats Given 6 Months of Intravenous Doses of PR-171	119
Table 63: Percent or Fold (X) Change in Selected Clinical Chemistry Parameters at Termination Compared to Control in Rats Given 6 Months of Intravenous Doses of PR-171	120
Table 64: Percent Changes in Terminal Organ Weight Values Compared to Control Following 6 Months of Intravenous Administration of PR-171 to Rats.....	121
Table 65: Histopathological Findings of Note with Incidence and Severity in Rats Given 3 Months of Intravenous Doses of PR-171 (Interim)	124
Table 66: Histopathological Findings of Note with Incidence and Severity in Rats Given 6 Months of Intravenous Doses of PR-171 (Termination)	125
Table 67: Histopathological Findings of Note with Incidence and Severity in Rats Given 6 Months of Intravenous Doses of PR-171 (Recovery)	126
Table 68: Ex vivo Evaluation of CYP Activity in Rats Administered Intravenous PR-171	128
Table 69: Ex vivo Evaluation of CYP Expression in Rats Administered Intravenous PR-171	128
Table 70: TK Parameters of PR-171 in Rats	129
Table 71: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) in Monkeys Administered Intravenous PR-171 for 6 Months (n=4-6)	133
Table 72: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) Following 8 Weeks of Recovery (n=2)	133
Table 73: Change in Selected Mean Hematology Parameters Compared to Control in Monkeys Given 6 Months of Intravenous Doses of PR-171 on Day 241 (24 hours after last dose): % Difference or Fold Difference (n=4-6)	134
Table 74: Change in Selected Mean Clinical Chemistry Parameters Compared to Control in Monkeys Given 6 Months of Intravenous Doses of PR-171 on Day 241 (24 hours after last dose): % Difference or Fold Difference (n=4-6)	135
Table 75: Test Article-Related Absolute Organ Weight Changes on Day 241 (24 hours after last dose): % Difference from Concurrent Vehicle Controls	136
Table 76: Incidence of Macroscopic Findings in the Kidney 24 hours after the Last Dose of Carfilzomib (Day 241)	136
Table 77: Histopathological Findings of Note with Incidence and Severity at Termination in Monkeys Given 6 Months of Intravenous Doses of PR-171	138
Table 78: Histopathological Findings of Note with Incidence and Severity in Monkeys Given 6 Months of Intravenous Doses of PR-171 after 8 Weeks Recovery (n=2/sex/group)	139

Table 79: TK Parameters of PR-171 in Monkeys	140
Table 80: Bacterial Reverse Mutation Test Results for PR-171 in the Absence of Metabolic Activation (Test 2)	142
Table 81: Bacterial Reverse Mutation Test Results for PR-171 in the Presence of Metabolic Activation (Test 2)	144
Table 82: Bacterial Reverse Mutation Test Results for PR-171 in the Absence of Metabolic Activation (Test 2)	147
Table 83: Bacterial Reverse Mutation Test Results for PR-171 in the Presence of Metabolic Activation (Test 2)	148
Table 84: Summary of Metaphase Analyses ± S9 Metabolic Activation	151
Table 85: Metaphase Analysis Data - Number of Cell Examined	153
Table 86: Summary of Metaphase Analyses ± S9 Metabolic Activation	154
Table 87: Summary of Mouse Micronucleus Test Results for Intravenous PR-171 ...	157
Table 88: Summary of Mouse Micronucleus Test Results for Intravenous PR-171 Spiked with Three Process Impurities	160
Table 89: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) in Pregnant Rats Administered Intravenous PR-171 from Gestational Day 6-17 (n=20-22)	164
Table 90: TK Parameters of PR-171 and its Main Metabolites in a Rat Embryo-Fetal Toxicity Study	165
Table 91: Selected Gestation Day 20 Laparohysterectomy Data Obtained from a Rat Embryo-Fetal Reproductive Toxicity Study of Daily IV PR-171 Administration (N=20-22/group)	167
Table 92: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) in Pregnant Rabbits Administered Intravenous PR-171 from GD 6-19 (n=7-8)	169
Table 93: Selected Gestation Day 29 Laparohysterectomy Data Obtained from a Rabbit Embryo-Fetal Reproductive Toxicity Study of Daily IV PR-171 Administration (N=6-8/group)	170
Table 94: Exposure Multiples Based on AUC and Body Surface Area (BSA) for Carfilzomib Following Repeat Dose Intravenous Administration to Rats, Rabbits and Monkeys Compared to Humans at 27 mg/m ²	174

Table of Figures

Figure 1: Proteasome inhibition in a dose range-finding toxicology study in Cynomolgus monkeys.....	23
Figure 2: Proteasome inhibition in a 4-week toxicology study in rats	24
Figure 3: Sampling and dosing schedule for assessing proteasome inhibition in a 4-week toxicology study in Cynomolgus monkeys	26
Figure 4: Chymotrypsin-like Activity of the 20S Proteasome in Whole Blood in Cynomolgus monkeys	26
Figure 5: Chymotrypsin-like Activity of the 20S Proteasome in Heart in Cynomolgus monkeys.....	27
Figure 6: Chymotrypsin-like activity of the 20S Proteasome in Cynomolgus monkeys with unscheduled deaths.....	27
Figure 7: Pharmacodynamics of PR-171 in rat tissues.....	28
Figure 8: Recovery of proteasome activity in rats receiving QDx5 administration of PR-171	29
Figure 9: Body weights of rats following QDx5 dosing of PR-171	29
Figure 10: Chymotrypsin-like proteasome activity of whole blood in monkeys given PR-171	30
Figure 11: Pharmacodynamic of PR-171 in tissues following either a single or QDx2 dose administration	31
Figure 12: Pharmacodynamics of PR-171 in tissues following QDx2 dose administration.....	32
Figure 13: Carfilzomib treatment results in the activation of Caspase 3 in Molt4 and RPMI-8226 cells.....	33
Figure 14: Carfilzomib induces apoptotic cell death in Molt4 and HT-29 cells.....	33
Figure 15: Bortezomib and carfilzomib (PR-171) on bortezomib-resistant cell lines: HT-29 cultured in 300 nM bortezomib	34
Figure 16: Tumor volume in tumor bearing mice after carfilzomib administration twice weekly on non-consecutive days.....	36
Figure 17: Inhibition of tumor growth mediated by carfilzomib administration twice daily in tumor bearing mice.....	36
Figure 18: Chymotrypsin-like proteasome activity in blood samples from female Cynomolgus monkeys given PR-58825 or PR-171 IV.....	37
Figure 19: Chymotrypsin-like proteasome activity in brain from female Cynomolgus monkeys given PR-58825 or PR-171	38
Figure 20: Chymotrypsin-like proteasome activity in inguinal lymph node samples from female Cynomolgus monkeys given PR-58825 or PR-171	38
Figure 21: Inhibition of the chymotrypsin-like activity of the 20S proteasome by carfilzomib	39
Figure 22: Plasma concentration-time profiles of intravenous bolus and intravenous infusion of 8 mg/kg carfilzomib in rats	45
Figure 23: Average plasma concentration-time plot following intravenous bolus administration in male Cynomolgus monkeys	47
Figure 24: Plasma concentration-time profile following intravenous bolus of carfilzomib at 5 mg/kg in male mice	48

Figure 25: Mean plasma concentration-time profile following an intravenous bolus administration of carfilzomib at 2 mg/kg in female Cynomolgus monkeys.....	50
Figure 26: Mean plasma concentration-time profile following administration of PR-58825 via intravenous bolus at 2.5 mg/kg and nasogastric at 20 mg/kg in female Cynomolgus monkeys.....	51
Figure 27: Concentrations of radioactivity in plasma after a single intravenous dose of ³ H-PR171 to male rats	53
Figure 28: Amount of ³ H-PR-171 in urine and feces (%) after a single intravenous dose in male rats.....	53
Figure 29: Whole-body autoradiograph of male rat (C24122) administered a single intravenous dose of 3H-PR-171	56
Figure 30: Whole-body autoradiograph of male rat (C24123) administered a single intravenous dose of 3H-PR-171	58
Figure 31: Putative structures of metabolites found in hepatocytes	65
Figure 32: Formation of carfilzomib metabolites in rat blood and tissue homogenates	69
Figure 33: Disappearance of carfilzomib and formation of the diol metabolite M16 in cryopreserved human hepatocytes	72
Figure 34: Plasma concentration-time profiles of carfilzomib after a single intravenous bolus administration of 2 mg/kg in rats	76
Figure 35: Plasma concentration-time profile for carfilzomib in Cynomolgus monkeys	97
Figure 36: Body weights in male rats given multiple doses of PR-171	106
Figure 37: Group Mean Body Weights of Male Rats Treated with Carfilzomib or Bortezomib	110
Figure 38: Body Weights in Male Rats Given 6 Months of Intravenous Doses of PR-171	116
Figure 39: Body Weights in Female Rats Given 6 Months of Intravenous Doses of PR-171	117
Figure 40: Mean Feed Consumption in Males Given 6 Months of Intravenous Doses of PR-171	118
Figure 41: Mean Feed Consumption in Females Given 6 Months of Intravenous Doses of PR-171	118
Figure 42: Body Weights in Male Monkeys Given 6 Months of Intravenous Doses of PR-171	132
Figure 43: Body Weights in Female Monkeys Given 6 Months of Intravenous Doses of PR-171	132
Figure 44: Body Weight in Pregnant Rats Given Intravenous Administration of PR-171 from GD6-17	162
Figure 45: Gestational Body Weights in Pregnant Rats Treated with Intravenous PR-171 from Gestational Day 6 to 17 (n=20-22)	164
Figure 46: Feed Consumption in Pregnant Rats Treated with Intravenous PR-171 from Gestational Day 6 to 17 (n=20-22)	165
Figure 47: Protease Activity in Whole Blood Samples from Pregnant Rats.....	166
Figure 48: Body Weight in Pregnant Rabbits Given Intravenous Administration of PR-171 from GD6-19	169

1 Executive Summary

1.1 Introduction

NDA 202714 was submitted to the U.S. Food and Drug Administration for carfilzomib for the proposed indication of the treatment of patients with relapsed and refractory multiple myeloma who have received at least 2 prior lines of therapy that included a proteasome inhibitor and an immunomodulatory agent. Carfilzomib is a new molecular entity proteasome inhibitor, which irreversibly inhibits the chymotrypsin-like activity of the 20S proteasome. The proposed clinical dose of carfilzomib is 20 mg/m² in Cycle 1, escalating to 27 mg/m² in Cycle 2 administered intravenously over 2 to 10 minutes, twice weekly on consecutive days for 3 weeks (Days 1, 2, 8, 9, 15, and 16), followed by a 12-day rest period (Days 17 to 28). Nonclinical pharmacology, pharmacokinetic and toxicology studies were submitted to support the approval of carfilzomib for the proposed indication.

1.2 Brief Discussion of Nonclinical Findings

The ubiquitin-proteasome pathway is the major proteolytic mechanism in eukaryotic cells. It catalyzes the degradation of regulatory proteins and the rapid elimination of proteins with abnormal conformation.^{1,2} The major protease in this pathway is the 26S proteasome, an ATP-dependent proteolytic complex, which is formed by the barrel-shaped 20S catalytic core particle complexed with two 19S regulatory subunits.^{3,4} The 20S catalytic core particle within the 26S proteasome is also known as the 20S proteasome and is responsible for the degradation of key proteins involved in apoptosis, DNA repair, endocytosis and cell cycle control.^{4,5,6} Bortezomib was approved by the FDA in 2003 as a proteasome inhibitor for its activity against the 20S proteasome.

Nonclinical pharmacology studies were conducted to investigate the *in vitro* and *in vivo* ability of carfilzomib to inhibit proteasome activity. Like bortezomib, carfilzomib was shown to inhibit the 20S proteasome. Carfilzomib had the greatest inhibition of chymotrypsin-like activity, with less inhibition of peptidyl-glutamyl peptide-hydrolyzing (PGPH)-like activity, and the least inhibition of trypsin-like activity. Relative to bortezomib, carfilzomib had a lower inhibition of PGPH-like activity, suggesting it may be more specific for chymotrypsin-like inhibition. In addition, carfilzomib irreversibly binds to the N-terminal threonine-containing active sites of the 20S proteasome, which differs from bortezomib for which binding to the 20S proteasome is reversible. Multiple *in vitro* studies were conducted in which carfilzomib was demonstrated to have antiproliferative and proapoptotic activities in human tumor cell lines derived from solid

¹ Herschko, A and Ciechanover, A (1998) *Annu Rev Biochem*; **67**: 425-479.

² Hochstrasser, M (1996) *Annu Rev Genet*; **30**: 405-439.

³ Baumeister, W, et al. (1998) *Cell*; **92**: 367-380.

⁴ Coux, O, Tanaka, K, Goldberg, AL (1996) *Annu Rev Biochem*; **65**: 801-847.

⁵ Hoffman, L and Rechsteiner, M (1996) *Curr Top Cell Regul*; **34**: 1-32.

⁶ Hochstrasser, M (1995) *Curr Opin Cell Biol*; **7**(2): 215-223.

and hematologic malignancies. *In vivo* studies showed that carfilzomib resulted in proteasome inhibition shortly after administration, which was sustained in blood and some tissues, likely due to irreversible binding to the 20S proteasome requiring new protein production to restore activity. Mouse xenograft studies resulted in delayed tumor growth of multiple myeloma, hematologic and solid tumor cells following carfilzomib administration.

The potential for cardiac effects resulting from carfilzomib administration was identified in safety pharmacology studies. Carfilzomib inhibited the hERG channel potassium current at doses $\geq 1.5 \mu\text{M}$. A single bolus intravenous injection of 3 mg/kg carfilzomib in monkeys resulted in increased ventricular premature complex, ST segments and T wave amplitudes in one male, and increased heart rate and troponin-T levels and decreased blood pressure, PR interval, QRS interval and QT interval in the other male tested. Both males administered 3 mg/kg died within 73 hours after dosing.

The half-life of carfilzomib in animals is very short (≤ 20 min). Therefore, increases in exposure that were observed with repeat dosing were likely due to decreased elimination rather than drug accumulation. Carfilzomib was rapidly and widely distributed to most tissues in rats, but did not cross the blood-brain barrier. Carfilzomib was highly protein bound in the plasma. Carfilzomib was highly metabolized by two main mechanisms: peptide cleavage and epoxide hydrolysis. No unique human metabolites were identified *in vitro*, and none of the major metabolites inhibited *in vitro* proteasome activity significantly. Although elimination mechanisms are similar between species, there are also some species-specific clearance and/or metabolism mechanisms that may ultimately lead to differences in exposures after repeat dosing between species. The majority of carfilzomib elimination occurred through metabolism and incorporation into cellular proteins. Renal and biliary clearance accounted for about 30% of carfilzomib elimination via each route following a single bolus intravenous administration in rats, which occurred within 4 hours. It is unclear if renal impairment (pre-existing or carfilzomib-related) would increase the risk of drug toxicity with repeat dosing.

Significant dose-dependent mortality and toxicities were observed in all studies conducted with carfilzomib. Overall, at the significantly toxic doses there were signs of cardiac failure and related renal failure (prerenal azotemia), with acute phase response, gastrointestinal toxicity and hematological effects (e.g., thrombocytopenia, erythrocytosis, and leukocytosis) observed in shorter studies.

In repeat-dose toxicology studies, rats were administered bolus intravenous carfilzomib at 1, 2, or 4 mg/kg for 3 or 6 months, and monkeys were administered bolus intravenous carfilzomib at 0.5, 1, or 2 mg/kg for 9 months. Carfilzomib-related mortality was observed in rats and monkeys following the proposed clinical administration schedule at doses of ≥ 2 mg/kg/dose and 2 mg/kg/dose, respectively. Deaths were preceded by body weight gain decrease or weight loss and decreased feed consumption with common clinical signs that included piloerection, lethargy, paleness, and hunched posture (emesis in monkeys). Identified causes of death included multiple organ failure, cardiac failure, cardiac fibrosis, pericardial fluid accumulation, cardiac hemorrhage/degeneration, gastrointestinal necrosis/hemorrhage, and renal failure (see below). Additionally, necropsies showed signs of edema in many organs and tissues. The systemic exposures (AUCs) following ≥ 2 mg/kg/dose in rats were approximately

40% of those observed in patients who received 27 mg/m² of carfilzomib twice weekly for two weeks. The dose of 2 mg/kg/dose in monkeys resulted in systemic exposures approximately 10% of those observed in patients.

Rats and monkeys receiving repeated administration of carfilzomib often had reduced evidence of significant toxicity at the end of dosing compared to animals that died during the studies. However, significant toxicities were observed in the cardiovascular, gastrointestinal and renal systems. Cardiac toxicity in rats and monkeys was observed at 2 mg/kg/dose and 1 mg/kg/dose (approximately 40% and 10% the systemic exposures observed in patients), respectively, including enlarged hearts and increased heart weights (both species), elevated troponin I (monkeys) and CRP (both), and a number of histopathological correlates of cardiomyopathy with only partial recovery. Renal toxicity consistent with prerenal azotemia in both species was noted at all doses, indicated by increased BUN, creatinine, and phosphorus with enlarged kidneys, and microscopic findings of chronic progressive nephropathy in rats and glomerulonephropathy in both species. In rats, there were three deaths attributed to hemorrhage/necrosis of the intestine. No animals in the chronic repeat-dose toxicology studies that reached scheduled necropsy had any findings related to gastrointestinal hemorrhage/necrosis, but in a 3-week study in rats, one animal at 6 mg/kg had a number of findings in the large intestine including hyperplasia, fibrosis, and dilatation.

Many findings in nonclinical repeat-dose toxicity studies were related to the proteasome inhibition activities of carfilzomib, consisting of effects on hematological parameters (decreased platelets, increased red cell and white cell types) and hematopoietic organs (such as bone marrow, lymph nodes and spleen) in rats and monkeys. Both species had increased liver weights, serum CRP, neutrophils, monocytes, and fibrinogen and decreased ALT and albumin in the chronic studies. Many of these findings are consistent with Acute Phase Response. These occurred at all doses in rats while the monkeys tended only to have these findings at the high dose of 2 mg/kg. Additionally, rats had some histopathological correlates noted, whereas monkeys had none. Based on the studies of shorter duration, it appears that the effects on the liver may be adaptive or exposure-dependent. Findings of note with no dose-dependency were observed in the lung, pancreas, and intestinal fat, but only in rats. No signs of neurotoxicity (functional or histopathological) were observed.

Carfilzomib was not genotoxic in the reverse mutation bacterial test (Ames) and the mouse micronucleus test. Carfilzomib causes an increase in chromosome structural aberrations in human peripheral blood cells at ≥ 0.04 $\mu\text{g/mL}$ and at ≥ 2.4 $\mu\text{g/mL}$ in vitro in the absence and presence of metabolic activation, respectively. The difference noted with metabolic activation suggests that some inactivation of carfilzomib has occurred. Findings of clastogenicity for PR-171 are consistent with the pharmacology.

Carfilzomib caused no overt teratogenicity in pregnant rats at exposures (AUC) or in rabbits at doses that were lower than in patients receiving the recommended dose. Embryo-lethality occurred below human exposures or doses based on findings of increased post-implantation loss from early resorptions in rats and rabbits. The negative effects on implantation in both species, and fetal weight decreases in rabbits may be secondary due to maternal toxicities, such as body weight and feed consumption decreases.

Overall, the findings are consistent among species and expected based on the mechanism of action. The relevant and significant nonclinical toxicities, such as cardiac and renal toxicities, have been observed in humans at comparable AUC exposure. The majority of deaths in rats occurred early and during the first cycle, so appropriate clinical monitoring should be in place. Body weight decreases and a number of clinical signs were also observed almost immediately after dosing was initiated. The expected pharmacodynamic effects on hematological parameters are monitorable, occurred rapidly after dosing initiated, and appear to be maximal at doses lower than those that resulted in significant toxicities. In the 9-month repeat dose study in monkeys, an ECG result correlated with other pathological findings in one animal at 2 mg/kg, which suggests that developing cardiotoxicity may be monitored by this manner along with CRP and troponin I levels. For monitoring of renal toxicity, urinalysis and CBC are useful, especially since the BUN and creatinine increases suggest prerenal azotemia. The Applicant suggests that the transiently observed acute phase response and thrombocytopenia may be ameliorated by decreasing the infusion rate. In both rats and monkeys, reversibility at the high dose was partial in organ systems with the most severe toxicities, i.e., heart and kidney, suggesting the potential for permanent impairment.

1.3 Recommendations

1.3.1 Approvability

Approvable

There are no nonclinical findings that would preclude the approval of carfilzomib for the proposed indication.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Exposures in animals were provided in the package insert as an AUC at a given dose compared to AUC in patients who received the recommended dose of 27 mg/m² for two weeks. In clinical study PX-171-007 A1, carfilzomib was administered as an intravenous bolus injection (over 2-10 minutes) to patients with solid tumors, multiple myeloma or lymphoma. Five patients received carfilzomib at 20 mg/m² on Day 1 and Day 2, then at 27 mg/m² on Days 8, 9, 15, and 16. Pharmacokinetic parameters were determined following blood collection on Days 1 and 16. The AUC_{inf} in these patients was 349 ng*hr/mL, or 20,940 ng*min/mL, which was used to determine exposure multiples compared to animals in relevant sections of the package insert (see Section 11 Integrated Summary and Safety Evaluation). All nonclinical data referred to in the relevant nonclinical sections of the package insert for Kyprolis (carfilzomib) are contained within this review. At the time this review was completed, labeling negotiations with the Applicant were ongoing.

2 Drug Information

2.1 Drug

CAS Registry Number: 868540-17-4

Generic Name: carfilzomib

Code Name: PR-171

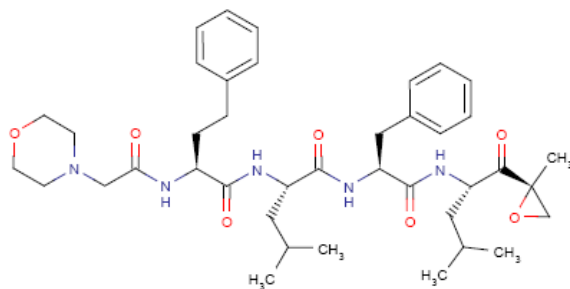
Chemical Name:

(2S)-N-((S)-1-((S)-4-methyl-10((R)-2-methyloxiran-2-yl)-1-oxopentan-2-ylcarbamoyl)-2-phenylethyl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-4-methylpentanamide

synonym: Morpholinoacetyl-hPhe-Leu-Phe-Leuketoepoxide

Molecular Formula/Molecular Weight: C₄₀H₅₇N₅O₇/ 719.9 g/mol

Structure or Biochemical Description:



Pharmacologic Class: Proteasome inhibitor

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 71057; DMF (b) (4); DMF (b) (4)

2.3 Drug Formulation

Lyophilized carfilzomib in (b) (4) 60 mg single-use vials, to be reconstituted with water for injection, USP; reconstituted product consists of 2 mg/mL solution of carfilzomib free base in (b) (4) sulfobutylether-®-cyclodextrin (Captisol).

Component	Nominal amount per milliliter of PR-171 for Injection
PR-171 Free Base	2.0 mg
SBE- β -CD	100 mg
Citric acid	1.9 mg
NaOH	QS to pH 3.5
WFI (USP)	QS to 1.0 mL

(Table excerpted from Applicant's package)

2.5 Comments on Impurities/Degradants of Concern

There were a number of related substances listed in the drug product specification. The following includes the rationale for the acceptability of the drug product specification for each of these impurities:

(b) (4)

The above specifications are acceptable from a Pharmacology/Toxicology perspective to provide for adequate safety given the indicated patient population of relapsed/refractory multiple myeloma based on the justifications provided by the Applicant.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication for carfilzomib is for the treatment of patients with relapsed and refractory multiple myeloma who have received at least 2 prior lines of therapy that included a proteasome inhibitor and an immunomodulatory agent.

Carfilzomib is administered intravenously at 20/27 mg/m² (20 mg/ m² in Cycle 1, escalating to 27 mg/ m² in Cycle 2) over 2 to 10 minutes, twice weekly on consecutive days for 3 weeks (Days 1, 2, 8, 9, 15, and 16), followed by a 12-day rest period (Days 17 to 28).

2.7 Regulatory Background

The original IND 71057 for carfilzomib was submitted in 2005 for a spectrum of hematological malignancies including multiple myeloma, (b) (4)

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

Primary Pharmacology

Study Title	Study #
Selectivity of PR-171: Testing Against Proteasome Active Sites	TR-0004-171
Dose Range Finding Toxicology Study of PR-171 in Male Cynomolgus Monkeys: Pharmacodynamics of Proteasome Activity	TR-0011-171 / TXC-003
Pharmacodynamics of IV bolus of PR-171 in Male Sprague Dawley Rats: Comparison of Single doses to QDx5 Administration	TR-0021-171 / PDR-019 AND pdr-020
A Single Dose Cardiovascular Pharmacology and Toxicity Study of PR-171 in Cynomolgus Monkeys: PD Appendix	TR-0024-171 / TXC-005
Pharmacodynamics of IV Bolus Administration of PR-171 in Rats: Comparison of Single Doses to QDx2 Administration	TR-0027-171 / PDR-016
Biochemical Evaluation of Carfilzomib (PR-171): PR-171 Effects on Mammalian Cells in Vitro	TR-0031-171
Generation of Cells Resistant to Bortezomib: Effects on PR-171 Sensitivity	TR-0032-171
In vivo Efficacy Study of PR-171 in HT-29 Human Colorectal Adenocarcinoma Xenografts	TR-0037-171 / PDM-013
A Single Dose Pharmacodynamic Study Comparing PR-171 Administered Intravenously to PR-58825 Administered Both Intravenously and Nasogastrically to Female Cynomolgus Monkeys	TR-0046-171-PD
Pharmacodynamic Evaluation of Carfilzomib in Rats: Comparison of Bolus Injection vs. IV Infusion Delivery	TR-0089-171 / R1005-SN252
The activity carfilzomib metabolites on the chymotrypsin-like activity of the constitutive proteasome and immunoproteasome	TR-0206-171

Secondary Pharmacology

Study Title	Study #
Selectivity of PR-171: Testing Against a Panel of Proteases	TR-0002-171
Selectivity of PR-171: Testing Against a Panel of Receptors and Enzymes	TR-0003-171

Safety Pharmacology

Study Title	Study #
Effects of Carfilzomib (PR-171) on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	TR-0088-171/ 070914.QTQ

Pharmacokinetics/ADME/Toxicokinetics**Pharmacokinetics**

Study Title	Study #
Pharmacokinetics of carfilzomib in rats: comparison of frozen and lyophilized drug products	TR-0079-171
Pharmacokinetics of Carfilzomib in Sprague Dawley Rats Following a Single Intravenous Bolus or Infusion Administration	TR-0383-171
Dose Range Finding Toxicology Study of PR-171 in Male Cynomolgus Monkeys: PR-171 Quantitation in Plasma Samples and Estimation of Non-Compartmental Pharmacokinetic Parameters	TR-0012-171
Pharmacokinetics of PR-171 in Mouse Following 5 mg/kg Intravenous Bolus Administration	TR-0053-171

Absorption

Study Title	Study #
A Single Dose Pharmacodynamic Study Comparing PR-171 Administered Intravenously to PR-58825 Administered Both Intravenously and Nasogastrically to Female Cynomolgus Monkeys: Pharmacokinetics of PR-171 and PR-58825	TR-0046-171-PK

Distribution

Study Title	Study #
Absorption, Metabolism, and Excretion of ³ H-PR-171 Following Intravenous Administration to Rats	TR-0041-171
<i>In vitro</i> protein binding of PR-171 in human, rat and monkey plasma using equilibrium dialysis	TR-0049-171
<i>In vitro</i> Protein Binding of Carfilzomib in Rat, Monkey and Human Plasma Using Rapid Equilibrium Dialysis (RED) Device	TR-0457-171

Metabolism

Study Title	Study #
<i>In vitro</i> metabolic stability of PR-171 in human, monkey, rat and mouse liver microsomes and cytosols	TR-0033-171
<i>In Vitro</i> Metabolism of [³ H]-PR-171 by Rat, Monkey, and Human Hepatocytes	TR-0040-171
<i>In Vitro</i> Metabolism of Carfilzomib in Rat Blood and Tissue Homogenates	TR-0184-171
<i>In Vitro</i> Metabolism of Carfilzomib in Human Hepatocytes	TR-0212-171
Searching and Identification of Carfilzomib (PR-171) Metabolites in Rat Plasma, Bile, and Urine and in Monkey Plasma	TR-0271-171

Excretion

Study Title	Study #
Pharmacokinetics and Renal and Biliary Excretion of Carfilzomib (PR-171) and Its Metabolites in Sprague Dawley Rats Following a Single Intravenous Bolus Administration	TR-0294-171

Pharmacokinetic Drug Interactions

Study Title	Study #
Inhibition of cytochrome P4501A2, cytochrome P4502C19, cytochrome P4502D6 and cytochrome P4503A4 catalytic activities by PRX-002	TR-0034-171
The Effect of PR-171 on Hepatic Microsomal Levels of Total Cytochrome P450 and Selected Enzyme Activities in Male and Female Sprague Dawley Rats Following Repeat IV Bolus Administration at Doses of 0, 2, 4, and 6 mg/kg/day for 18 and 19 Days	TR-0042-171
The Effect of PR-171 on Hepatic Microsomal Levels of Total Cytochrome P450 and Selected Enzyme Activities in Male and Female Sprague Dawley Rats Following Repeat IV Bolus Administration at Doses of 0, 0.5, 1, and 2 mg/kg/day for 19 and 28 Days	TR-0043-171
<i>In Vitro</i> Evaluation of Carfilzomib as an Inhibitor of Human Cytochrome P450 Enzymes	TR-0081-171
Evaluation of Induction Potential of Cytochrome P450 Isoforms by Carfilzomib in Cultured Human Hepatocytes	TR-0086-171
Assessment of Carfilzomib as Substrate and Inhibitor of P-glycoproteins in a Caco-2 Monolayer System	TR-0087-171
<i>Ex-Vivo</i> Evaluation of Cytochrome P450 Expression in Sprague-Dawley Rats Treated with Carfilzomib	TR-0196-171
<i>Ex-Vivo</i> Evaluation of Cytochrome P450 Expression in Cynomolgus Monkeys Treated with Carfilzomib	TR-0197-171

Toxicokinetics

Study Title	Study #
Toxicokinetics of Carfilzomib in a 3/6-Month Intravenous Toxicity Study in Rats (GLP)	TR-0251-171
Toxicokinetics of Carfilzomib in a 9-Month Intravenous Toxicity Study in Cynomolgus Monkeys	TR-0252-171
Toxicokinetics of Carfilzomib in an Intravenous Embryo-Fetal Toxicity Study in Rats (GLP)*	TR-0250-171

* Reviewed as part of the Toxicity study# TR-0075-171

General Toxicology

Single-Dose Toxicity

Study Title	Study #
Single Dose Toxicity and Pharmacodynamic Studies of PR-171 in Rats: Determination of Maximum Tolerated Dose	TR-0006-171
Single Dose PK/PD and Toxicology Studies of PR-171 in Male Sprague Dawley Rats: Determination of Maximum Tolerated Dose	TR-0010-171
Pharmacodynamics and Toxicity of Carfilzomib in Rats: Effect of Infusion Delivery	TR-0356-171
Dose Range Finding Toxicity Study of PR-171 in Male Cynomolgus Monkeys	TR-0008-171

Repeat-Dose Toxicity

Study Title	Study #
A 2-Week Intravenous Dose Range-Finding Study of PR-171 in Rats	TR-0009-171
Intravenous Multi-Dose Range-Finding Study of PR-171 in Rats	TR-0020-171
A 4 Week Intravenous Injection Neurotoxicity Study of Bortezomib and Carfilzomib in Male Sprague Dawley Rats with a 4 Week Recovery Period	TR-0297-171
A 3/6 Month Intravenous Toxicity Study of Carfilzomib in Rats with an 8-Week Recovery Period	TR-0072-171
A Two Dose, 7-Day Intravenous Toxicity Study of Carfilzomib in Cynomolgus Monkeys	TR-0157-171
A 9-Month Intravenous Toxicity Study of Carfilzomib in Cynomolgus Monkeys with an 8-week Recovery Period	TR-0073-171

Genetic Toxicology

Study Title	Study #
Carfilzomib: Bacterial Reverse Mutation Test	TR-0069-171
Carfilzomib: Bacterial Reverse Mutation Test	TR-0425-171
Carfilzomib: In Vitro Mammalian Chromosome Aberration Test In Human Lymphocytes	TR-0070-171
Carfilzomib: In Vitro Mammalian Chromosome Aberration Test In Human Lymphocytes	TR-0426-171
Carfilzomib Mouse Micronucleus Test	TR-0071-171
Carfilzomib: Mouse In Vivo Micronucleus Test	TR-0422-171

Reproductive and Developmental Toxicology

Study Title	Study #
Preliminary Intravenous Embryo-Fetal Toxicity Study in Rats	TR-0074-171
Carfilzomib: An Intravenous Embryo-Fetal Toxicity Study in Rats	TR-0075-171
Preliminary Intravenous Embryo-Fetal Toxicity Study in Rabbits	TR-0123-171

Other Toxicity Studies

Study Title	Study #
Effect of Lipopolysaccharide on the Tolerability of PR-171 in BALB/c Mice	TR-0424-171

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

IND 71057, Review#1 dated 7/13/2005 by Dr. M. Anwar Goheer (See Section 12 Attachments).

Pharmacology**Safety Pharmacology**

Study Title	Study #
Cardiovascular, Respiratory, and Central Nervous System Assessments of PR-171 When Administered Intravenously to Conscious Monkeys	TR-0023-171 / 04-6576

Pharmacokinetics/ADME/Toxicokinetics**Absorption**

Study Title	Study #
A 4-Week Repeat Dose Intravenous Toxicity Study of PR-171 in Sprague Dawley Rats: Toxicokinetics	Study TR-0016-171 / TXR-003
A 4-Week Repeat Dose Intravenous Toxicity Study of PR-171 in Cynomolgus Monkeys: Toxicokinetics	Study TR-0019-171

General Toxicology

Repeat-Dose Toxicity

Study Title	Study #
A 4-week Repeat Dose Intravenous Toxicity Study of PR-171 in Rats	TR-0014-171 / TXR-003
A 4-week Repeat Dose Intravenous Toxicity Study of PR-171 in Cynomolgus Monkeys	TR-0017-171

4 Pharmacology

4.1 Primary Pharmacology

Selectivity of PR-171: Testing Against Proteasome Active Sites (Study TR-0004-171)**Key Study Findings**

- PR-171 is an inhibitor of chymotryptic active sites of the 20S proteasome, similar to Bortezomib.
- PR-171 IC_{50} for PGPH-like site inhibitory activity is 22-fold lower than Bortezomib,
- PR-171 inhibits the chymotrypsin- and trypsin-like activities of the 20S proteasome with similar IC_{50} 's as bortezomib.
- PR-171 potency: chymotrypsin-like > PGPH-like > trypsin-like

METHODS

In vitro proteasome activity/inhibition assays were used to assess the chymotryptic, PGPH-like, and tryptic activities of the 20S proteasome. Positive control = bortezomib.

RESULTS

PR-171 inhibits the chymotrypsin-like (IC_{50} = 4 nM) and trypsin-like (IC_{50} = 2400 nM) activities of the 20S proteasome with similar potency to bortezomib. However, PR-171 (IC_{50} = 1650 nM) inhibitory activity at the PGPH-like site of the 20S proteasome is 22-fold lower than bortezomib (IC_{50} = 75 nM). The IC_{50} values for PR-171 inhibition of chymotrypsin-like sites are 545-fold and 375-fold lower than that of trypsin-like and PGPH-like sites. These findings suggest that PR-171 is an inhibitor of the 20S proteasome with the greatest activity for chymotryptic enzymatic sites.

Table 1: IC₅₀ fold differences between the proteasome chymotryptic activity assay and other proteasome activity assays

	Chymotrypsin-like	PGPH-like		Trypsin-like	
Inhibitor	IC ₅₀ (nM)	IC ₅₀ (nM)	Fold Diff. wrt Chymo	IC ₅₀ (nM)	Fold Diff. wrt Chymo
PR-171	4.4 ± 2.7	1650 ± 480	375	2400 ± 60	545
Bortezomib	3.9 ± 1.7	75 ± 28	19	2840 ± 1740	728
PR-514	22 ± 14	2500 ± 1450	114	4930 ± 5090	224

For the bortezomib determinations N=24 (chymotrypsin-like), N=7 (PGPH-like) and N=4 (trypsin-like). For PR-171 determinations N=43 (chymotrypsin-like), N=6 (PGPH-like) and N=2 (trypsin-like). For PR-514 determinations N=2 (chymotrypsin-like), N=2 (PGPH-like) and N=2 (trypsin-like).

Table 2: k_{intact}/K_i (M⁻¹s⁻¹) values for inhibition of proteasome activities

Inhibitor	Inhibition of Proteasome Activities k_{intact}/K_i (M ⁻¹ s ⁻¹)		
	Chymotrypsin-like	Trypsin-like	PGPH
PR-171	33,800	<100	<100
bortezomib	38,000	<100	5,700

(Tables excerpted from Applicant's package)

Dose Range Finding Toxicology Study of PR-171 in Male Cynomolgus Monkeys: Pharmacodynamics of Proteasome Activity (Study TR-0011-171 / TXC-003)

Key Study Findings

- No dose-dependency in PR-171 inhibition of chymotrypsin-like activity of the 20S proteasome *in vivo*.
- Binding of PR-171 is irreversible, consistent with recovery of proteasome activity dependent on new protein synthesis.
- Proteasome inhibition does not increase following repeated dosing as compared to single doses.

METHODS

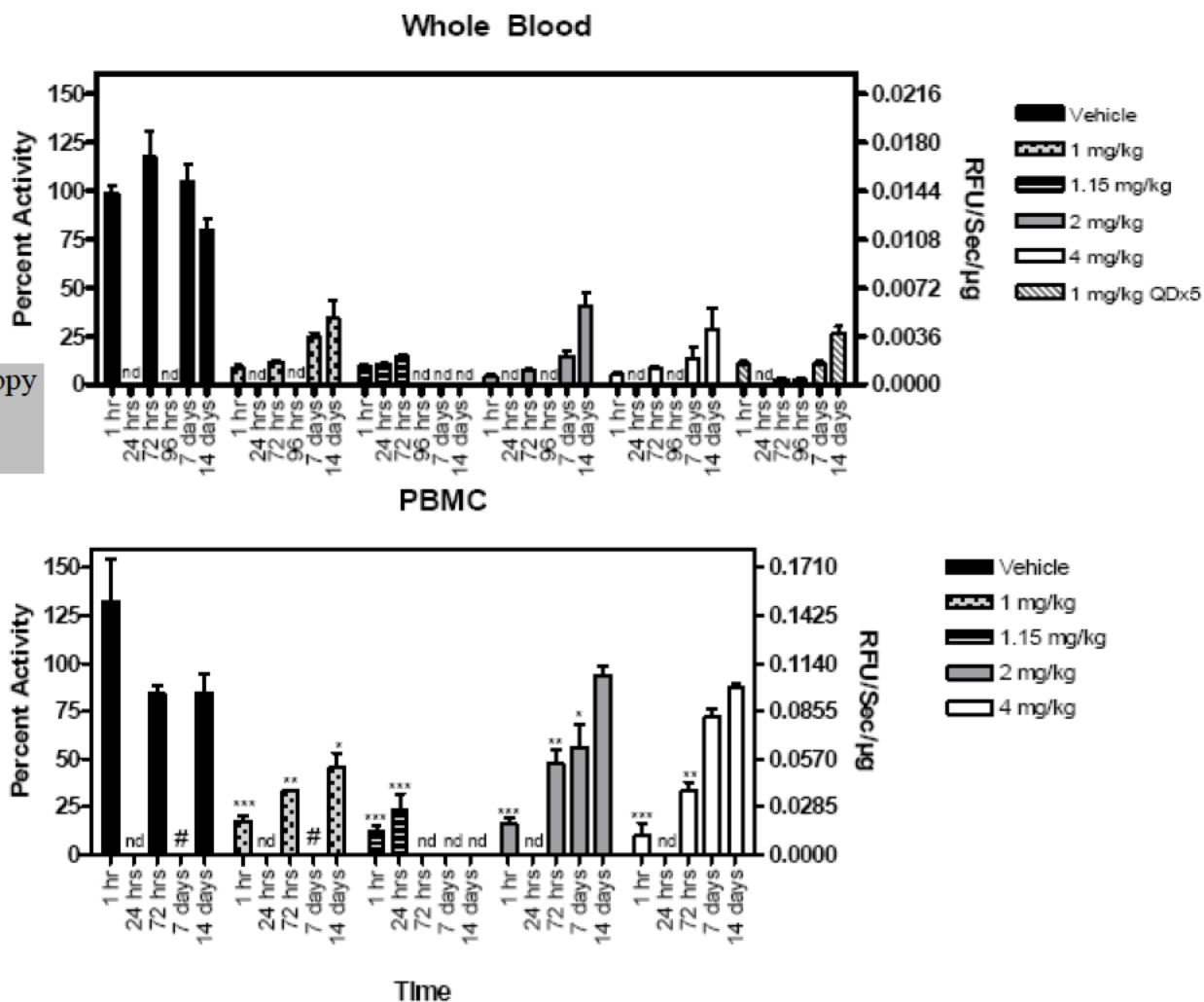
Male Cynomolgus monkeys received a single IV bolus of 0, 1.0, 1.15, 2.0, or 4.0 mg/kg PR-171, followed by a repeat dose of 1.0 mg/kg for 5 days. Chymotrypsin-like proteasome activity was determined in whole blood (primarily erythrocytes) and peripheral blood mononuclear cells (PBMCs) at 1 hr, 24 hr, 72 hr, 96 hr, 7 days, and/or 14 days post-dose of single administrations. Activity in the bone marrow was determined 1 hr and 7 days after 0 and 1.0 mg/kg administration only.

RESULTS

PR-171 inhibited chymotryptic proteasome activity 80-90% in PBMCs and whole blood by 1 hr at all doses. By Day 14, proteasome activity returned to normal levels in PBMCs, but only recovered by 60% in whole blood. Slower recovery time in whole blood is consistent with the time required for replacement with new red blood cells due to the lack of new protein synthesis in erythrocytes. These findings are also consistent

irreversible inhibition. No changes in proteasome activity were apparent in bone marrow at 1 hr and 7 days post-dose. Daily administration for 5 days resulted in >90% inhibition with recovery in whole blood similar to 4 mg/kg single administration.

Figure 1: Proteasome inhibition in a dose range-finding toxicology study in Cynomolgus monkeys



(Figure excerpted from Applicant's package)

A 4-Week Repeat Dose Intravenous Toxicity Study of PR-171 in Sprague Dawley Rats: PD Appendix (Study TR-0015-171 / TR-0015 / TXR-003)

Key Study Findings

- PR-171 inhibited proteasome activity in all tissues examined (whole blood, adrenal glands, bone marrow, liver and PBMCs) within 1 hr of dosing.
- Recovery within 24 hours in adrenal gland, liver and PBMC
- No recovery at 24 hours in whole blood (erythrocytes) or bone marrow
- No proteasome inhibition in brain

METHODS

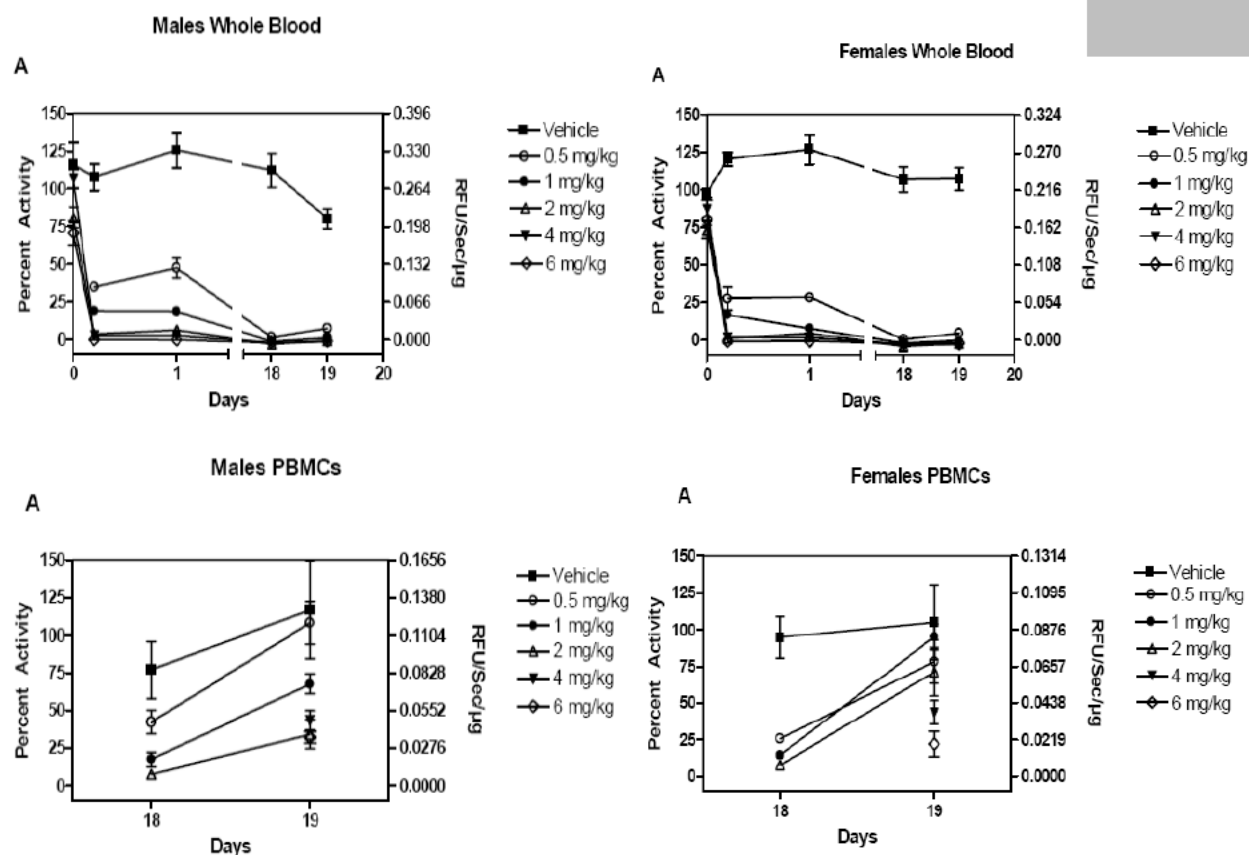
Proteasome activity was determined in whole blood, PBMCs, bone marrow, adrenal glands, brain, and liver at 0, 1, and 24 hr post-dose (Days 1 and 18) in male and female Sprague Dawley Rats administered 0.5, 1, 2, 4, and 6 mg/kg PR-171 (Days 0-4 and 14-18).

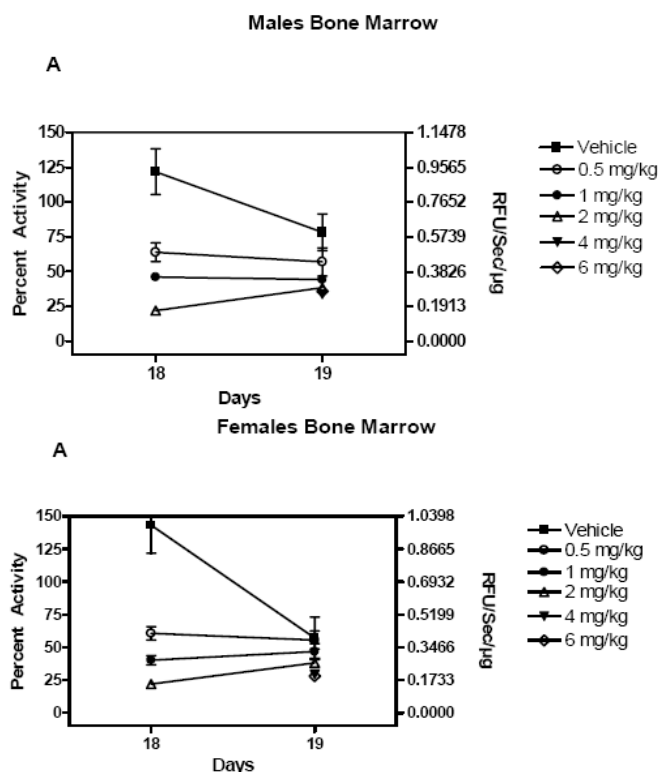
RESULTS

Initial dose-dependent proteasome inhibition in whole blood was evident between 0.5 and 2 mg/kg only at 1 hr post-dose on Day 1, but reached near complete inhibition at doses > 2 mg/kg at all other time points. Dose-dependent proteasome inhibition was seen at 1 hr post-dose (all doses) on Day 18 in PBMC and bone marrow. Non-dose-dependent inhibition occurred in the adrenal gland and liver, whereas no inhibition was evident in the brain. Recovery was seen after 24 hr in PBMC, adrenal gland, and liver tissues, but not in whole blood and possibly bone marrow. There were no clear gender-related differences.

Figure 2: Proteasome inhibition in a 4-week toxicology study in rats

Best Available Copy





Best Available Copy

(Figures excerpted from Applicant's package)

A 4-Week Repeat Dose Intravenous Toxicity Study of PR-171 in Cynomolgus Monkeys: Pharmacodynamics of PR-171 (Study TR-0018-171 / TXC-004)

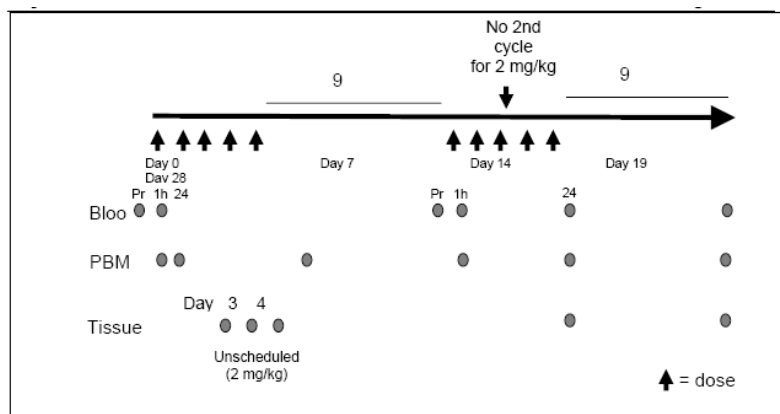
Key Study Findings

- >80% inhibition of chymotrypsin activity in whole tissues with a single dose and complete inhibition with repeated dosing (≥ 0.5 mg/kg, ≥ 6 mg/m²).
- Minimal recovery in whole blood after 9 days due to irreversibility of PR-171 binding.
- Significant inhibition (~50%) in heart tissue at 24 hr post-dose, but with complete recovery after 9 days.

METHODS

Cynomolgus monkeys (♂, ♀) received 0.5, 1.0, and 2.0 mg/kg IV bolus infusions on Days 0-4 (all groups) and Days 14-18 (≤ 1.0 mg/kg) over a 4 week period. Whole blood was collected 1 hr post-dose on Days 0 and 14, 24 hr after last dose (Day 19), and 9 days (Day 28) after the last dose. Adrenal, brain, heart, and liver samples were also collected on Day 19 and 28. 20S proteasome inhibition was determined using a fluorescent tetrapeptide.

Figure 3: Sampling and dosing schedule for assessing proteasome inhibition in a 4-week toxicology study in Cynomolgus monkeys



Best Available Copy

(Figure excerpted from Applicant's package)

RESULTS

Significant inhibition (>80%) of 20S proteasome chymotryptic activity in whole blood occurred at all doses and increased near 100% with dosing, suggesting accumulation of inhibition. Only slight recovery (65-80% inhibition) was evident at all doses 9 days post-dose (Day 28). Dose-dependent decreases (↓28-56%) in chymotrypsin-like activity were observed in the heart 24 hrs after the last dose which recovered to control levels by Day 28. Protease activity was highly variable in samples from the 6 fatalities at 2 mg/kg. However, it appears that significant proteasome inhibition was not present in all tissues of all fatalities and that significant inhibition was evident in the brain of at least one animal. There were no significant changes in proteasome activity in the brains, liver, or adrenal glands of surviving animals, which could be due to recovery or incomplete exposure. Variability in PBMCs was too high to reach significance, which the sponsor suspects is due to RBC and platelet contamination.

Figure 4: Chymotrypsin-like Activity of the 20S Proteasome in Whole Blood in Cynomolgus monkeys

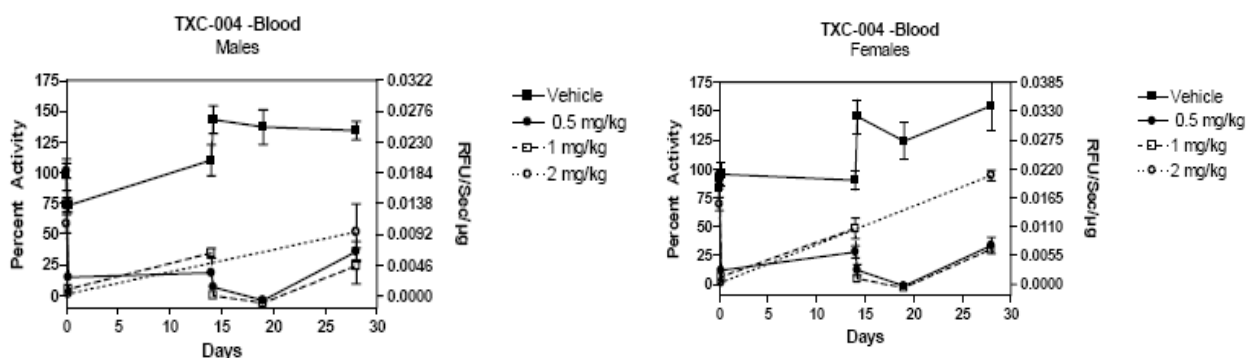
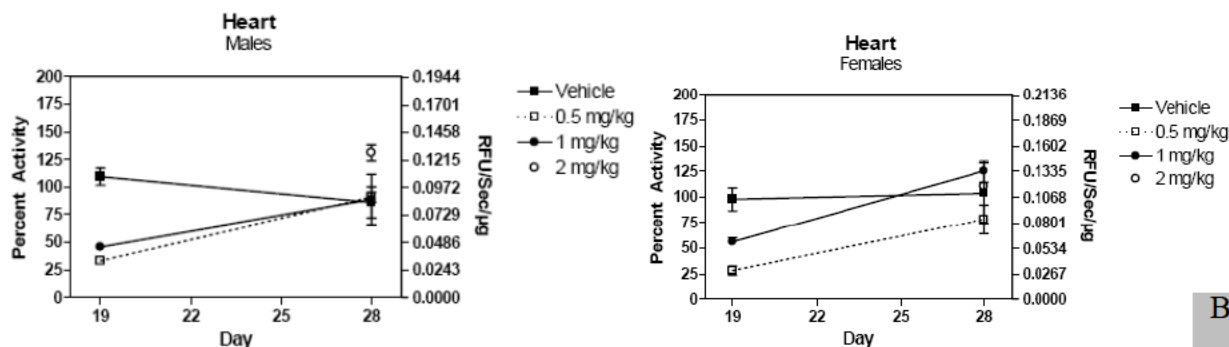


Figure 5: Chymotrypsin-like Activity of the 20S Proteasome in Heart in Cynomolgus monkeysBest Available
Copy**Figure 6: Chymotrypsin-like activity of the 20S Proteasome in Cynomolgus monkeys with unscheduled deaths**

Dose Group	MALES				FEMALES					
	Day 3 4 hours post-dose		Day 4 4 hour post-dose		Day 3 4 hours post-dose		Day 4 4 hours post-dose		Day 5 20 hours post-dose	
	Animal No.	% Act.	Animal No.	% Act.	Animal No.	% Act.	Animal No.	% Act.	Animal No.	% Act.
PBMC	4491	54.8	4493	< LLOQ ²	4990	#	4993	229		
Adrenal	4491	108	4493	128	4990	145	4993	216	4992	91
Brain	4491	84.6	4493	88.7	4990	131	4993	100	4992	55
Heart	4491	80.8	4493	112	4990	59.4	4993	49.3	4992	27.6
Liver	4491	140	4493	155	4990	140	4993	115	4992	81.1

¹ Control activity is the average of all vehicle post-dose values from scheduled harvest times.

² The Lower Limit of Quantitation (LLOQ) was defined as five times the standard deviation of negative controls (containing no lysate). For the PBMC monkey samples, this corresponds to 0.0026 RFU/sec/µg total protein (9.7 µg was the average total protein used in these assays) or to 2.1 % of the mean in Vehicle samples. No values reported for Adrenal, Brain, Heart or Liver were below their defined LLOQ.

This PBMC sample was excluded due to low protein concentration (< 0.2 mg/ml).

(Figures excerpted from Applicant's package)

Pharmacodynamics of IV bolus of PR-171 in Male Sprague Dawley Rats: Comparison of Single doses to QDx5 Administration (Study TR-0021-171 / PDR-019 AND PDR-020)

Key Study Findings

- Weight gain inhibition with weight loss at 2 mg/kg.
- Dose-dependent proteasome inhibition in all tissues, except brain.
- Cumulative effects of multiple doses evident in whole blood and the heavily perfused tissues heart and lung.
- Partial to complete recovery evident in all tissues except whole blood 4 days post-dose, due to irreversible inhibition of PR-171 and the lack of protein synthesis in erythrocytes.

METHODS

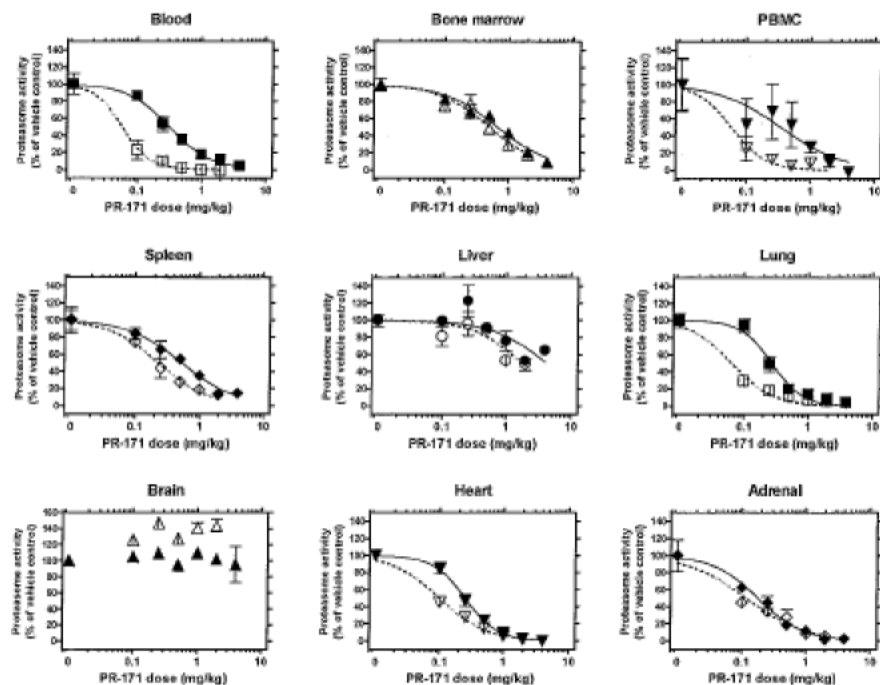
Chymotrypsin-like proteasome inhibition was measured in male Sprague Dawley rats 1 hour after administration of 0.1, 0.25, 0.5, 1, 2 and 4 mg/kg in whole blood, PBMCs, brain, spleen, erythrocyte-depleted bone marrow, adrenal, heart, liver and lung. Proteasome inhibition was also measured 1 hour after the 5th dose of 0.1, 0.25, 0.5, 1, and 2 mg/kg daily administrations, as well as at 24, 72, and 216 (9 days) after the 5th dose of 2 mg/kg.

RESULTS

No inhibition was evident in the brain. Dose-dependent inhibition (>80% at ≥ 1 mg/kg) was evident in all other tissues. Inhibition was significantly increased after the 5th dose in blood, PBMC, lung, and heart, but not in bone marrow, spleen, liver or adrenal tissues. It is possible that increased inhibition of the heart and lung could be due to the presence of blood in the samples. The recovery after repeated dosing was slow, but similar to that of single doses with a $t_{1/2}$ of 24 hours for most tissues. Although the recovery plateaus between 60 and 80% inhibition, possibly due to the presence of blood which undergoes only minimal recovery due to the lack of protein synthesis and irreversible binding of PR-171.

Weight gain was also inhibited with daily administration of 2 mg/kg PR-171, but recovered with cessation of dosing.

Figure 7: Pharmacodynamics of PR-171 in rat tissues



Best Available Copy

Figure 8: Recovery of proteasome activity in rats receiving QDx5 administration of PR-171

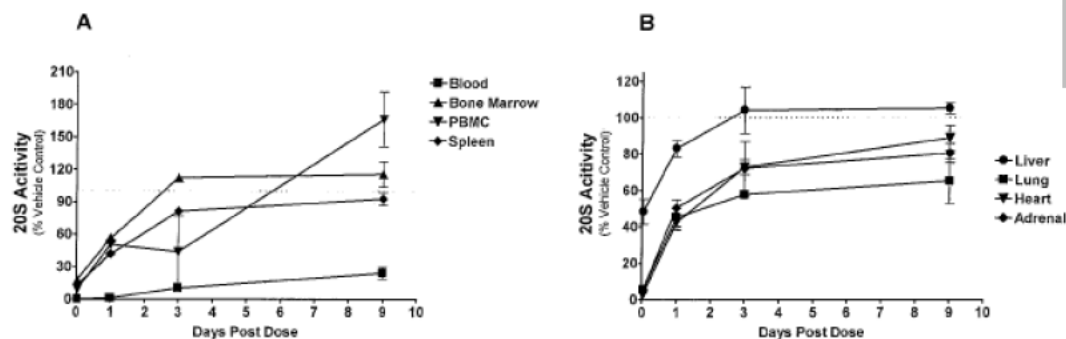
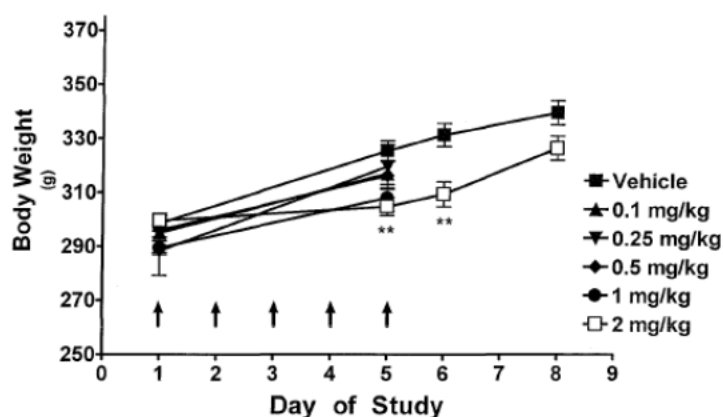


Figure 9: Body weights of rats following QDx5 dosing of PR-171



(Figures excerpted from Applicant's package)

A Single Dose Cardiovascular Pharmacology and Toxicity Study of PR-171 in Cynomolgus Monkeys: PD Appendix (Study TR-0024-171 / TXC-005)

Key Study Findings

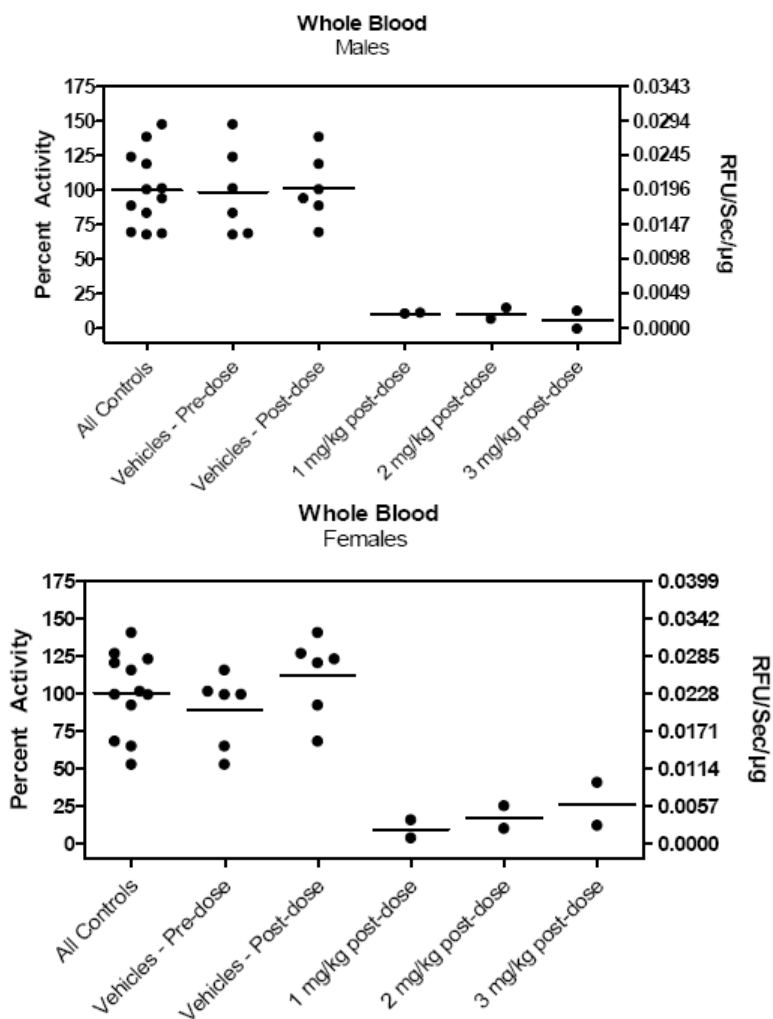
- PR-171 significantly inhibits ($\geq 75\%$) chymotrypsin-like protease activity in Cynomolgus monkey blood 24 hr after a single dose.

METHODS

Telemetered, conscious Cynomolgus monkeys (σ , ϕ) were administered 1.0, 2.0, and 3.0 mg/kg PR-171 48 to 72 hours after vehicle administration. Chymotryptic protease activity was measured in whole blood 24-25 hr post-dose. Neurological tests were also conducted after dosing for a different study.

RESULTS

Chymotrypsin-like activity was inhibited ($\geq 75\%$) in whole blood at all doses, but without a dose-response. There were no significant gender effects.

Figure 10: Chymotrypsin-like proteasome activity of whole blood in monkeys given PR-171

Best Available Copy

(Figures excerpted from Applicant's package)

Pharmacodynamics of IV Bolus Administration of PR-171 in Rats: Comparison of Single Doses to QDx2 Administration (Study TR-0027-171 / PDR-016)

Key Study Findings

- PR-171 significantly inhibits chymotrypsin-like activity of 20S proteasome in all the tissues examined after both single and two daily administrations.
- A cumulative effect was evident in liver
- Only partial recovery occurs at 24 hr post-dose
- No recovery after 3 days in whole blood

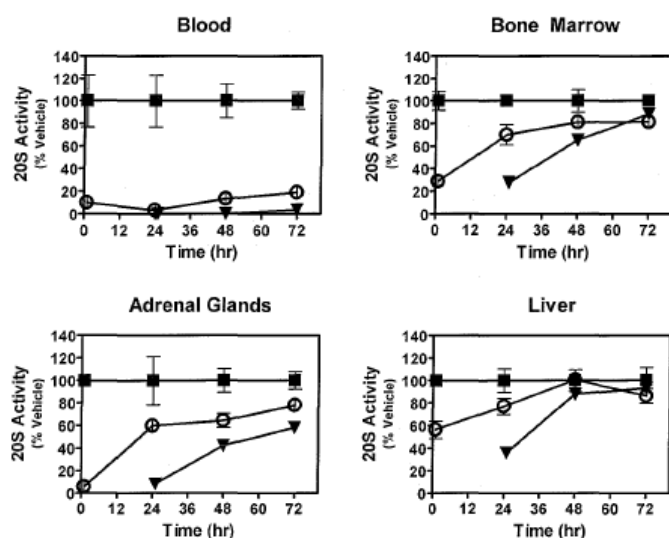
METHODS

Male Sprague Dawley rats received either a single or 2 daily doses of 2 mg/kg PR-171 (or vehicle) followed by measurement of chymotrypsin-like protease activity at 1, 24, 48, and 72 (single dose only) hours post-dose in whole blood, adrenal gland, erythrocyte depleted bone marrow, and liver.

RESULTS

Chymotryptic protease activity was inhibited >80% in whole blood and adrenal gland, 75% in bone marrow, and 45% in liver. A cumulative response was evident in the liver after a second dose, but not in the other tissues. Similar responses were evident after the second administration, except in the liver. Only partial recovery is evident at 24 hr post-dose prior to administration of the second dose. Recovery in blood was not observed throughout the study up to 3 days post-dose.

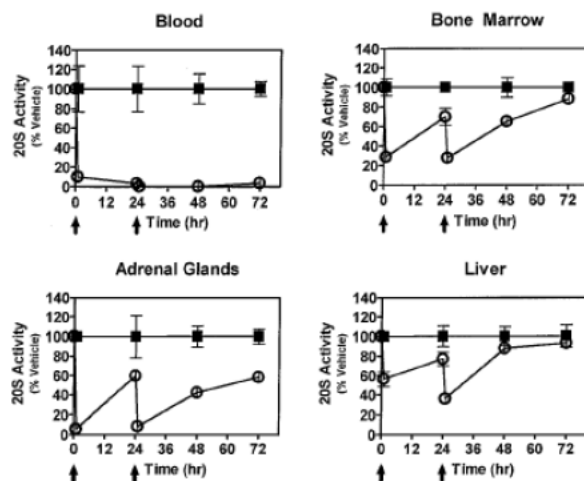
Figure 11: Pharmacodynamic of PR-171 in tissues following either a single or QDx2 dose administration



Best Available Copy

A single dose of vehicle (filled squares) or 2 mg/kg PR-171 (open circles) or two daily doses of 2 mg/kg PR-171 (filled triangles) were administered to male rats (n=3). Proteasome chymotrypsin-like activity was determined in tissues harvested at 1, 24, 25, 48 and 72 hours after the first dose.

Figure 12: Pharmacodynamics of PR-171 in tissues following QDx2 dose administration



Best Available Copy

Data from Figure 11 was combined to show the inhibition and recovery of proteasome activity following two daily administrations of 2 mg/kg PR-171 (indicated by arrows).

(Figures excerpted from Applicant's package)

Biochemical Evaluation of Carfilzomib (PR-171): PR-171 Effects on Mammalian Cells in Vitro (Study TR-0031-171)

Key Study Findings

- PR-171 leads to activation of the apoptotic pathway and cell death in human tumor cell lines *in vitro*.

METHODS

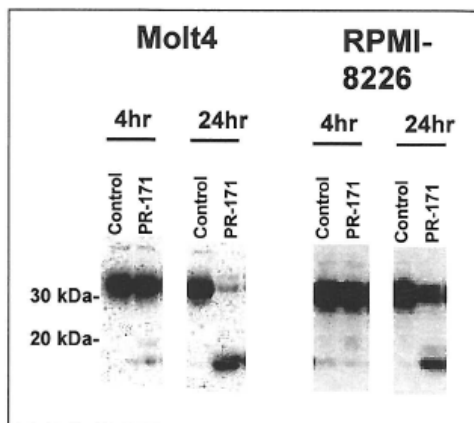
RPMI-1640, HT29, Molt4, and RPMI-8226 cells were treated with 500 nM Carfilzomib for 1 hr and harvested at 4, 24, and 72 hours post-treatment initiation. Western analysis was used to determine levels of caspase-3 and protein ubiquitination. Phosphatidyl exposure on the cell surface, a marker of apoptosis, was determined using staining for annexin and propidium iodide (PI) followed by FACS analysis.

RESULTS

Carfilzomib treatment led to accumulation of polyubiquitin chains within 4 hr post-treatment, indicative of proteasome inhibition. Significantly higher levels of cleaved caspase-3 (active form) were seen 24 hours after induction of carfilzomib treatment, but not after 4 hours, indicating activation of pro-apoptotic pathways. Carfilzomib treatment in Molt4 and HT29 cells resulted in increased annexin/PI double staining after 24 hr and 72 hr exposures, respectively, indicating apoptotic cell death. The Applicant suggests that high background staining in RPMI-8226 cells obstructed interpretation, but it appears that carfilzomib did not increase apoptosis in these cells after 24 hrs treatment. It is unclear why increased apoptosis would not be seen in RPMI-8226 cells even

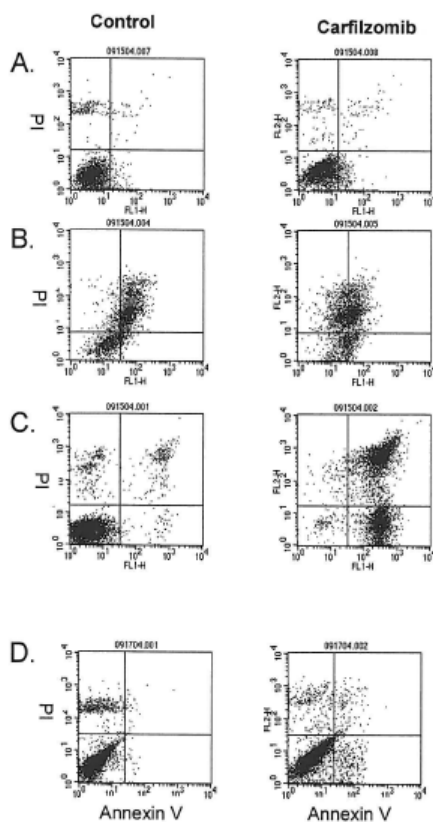
though increased caspase-3 levels are present, unless there is a delay in the induction of apoptosis which may be visible at later time points, similarly to HT29 cells.

Figure 13: Carfilzomib treatment results in the activation of Caspase 3 in Molt4 and RPMI-8226 cells



Best Available Copy

Figure 14: Carfilzomib induces apoptotic cell death in Molt4 and HT-29 cells



HT-29 (A and D), RPMI-8226 (B) and Molt4 (C) cells were exposed to 500 nM carfilzomib for 1 hr; carfilzomib was then washed out and cells returned to 37 °C.

(Figures excerpted from Applicant's package)

Generation of Cells Resistant to Bortezomib: Effects on PR-171 Sensitivity (Study TR-0032-171)

Key Study Findings

- Although bortezomib is also an inhibitor of the 20S proteasome, resistance to bortezomib does not necessarily confer cross-resistance to PR-171.

METHODS

A bortezomib-resistant cell line was generated *in vitro* and tested for sensitivity to carfilzomib (PR-171).

RESULTS

Three independent cell lines were generated with IC_{50} values 2- to 115-fold higher than the parental line, suggesting resistance to bortezomib. Although the bortezomib-resistant cell lines were less sensitive to PR-171 as well, they remained significantly more sensitive to PR-171 than bortezomib. Thus, cells resistant to bortezomib are not similarly resistant to PR-171 to the same degree even though both compounds target the 20S proteasome. These findings suggest that PR-171 differs functionally enough to circumvent resistance to bortezomib. It is unclear if these differences are purely due to increased specificity of PR-171 to the chymotryptic active sites, off-target binding, or compound-specific resistance mechanisms.

Figure 15: Bortezomib and carfilzomib (PR-171) on bortezomib-resistant cell lines: HT-29 cultured in 300 nM bortezomib

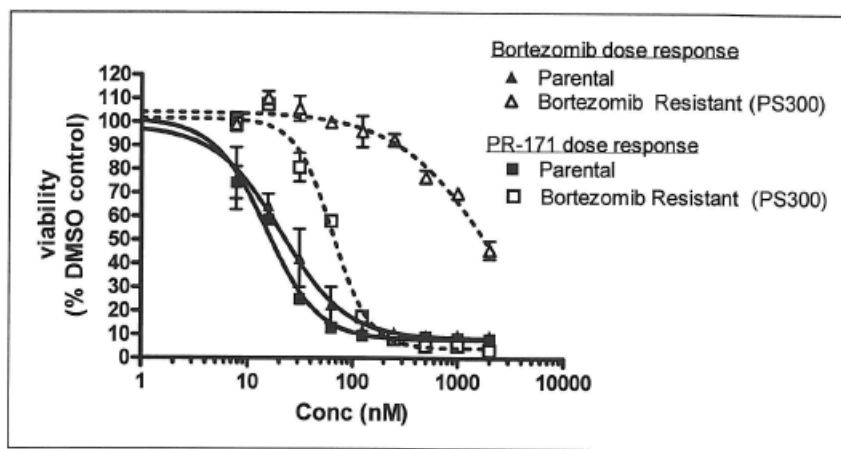


Table 3: Calculated IC₅₀s for bortezomib and carfilzomib proteasome inhibition in bortezomib resistant cell lines

Cell Line (Bortezomib Concentration)	Bortezomib		Carfilzomib	
	IC ₅₀	Resistance Factor	IC ₅₀	Resistance Factor
HT-29 parental (0 nM)	17.3 nM	n/a	32.9 nM	n/a
HT-29 PS 100 (100 nM)	313.4 nM	18.1	104.2 nM	3.2
HT-29 PS 200 (200 nM)	298.6 nM	17.2	41.7 nM	1.3

Cell Line (Bortezomib Concentration)	Bortezomib		Carfilzomib	
	IC ₅₀	Resistance Factor	IC ₅₀	Resistance Factor
HT-29 parental (0 nM)	20.7 nM	n/a	14.9 nM	n/a
HT-29 PS 300 (300 nM)	2,373 nM	114	64.4 nM	4.3

(Figure and table excerpted from Applicant's package)

In vivo Efficacy Study of PR-171 in HT-29 Human Colorectal Adenocarcinoma Xenografts (Study TR-0037-171 / PDM-013)

Key Study Findings

- Only mice on the twice a day schedule (15 mg/m² X 2 per day) exhibited a significant anti-tumor response, whereas up to twice a week (30 mg/m² X 2 per week) did not.
- PR-171 administered twice a week did not inhibit tumor growth.

METHODS

Female BNX mice with subcutaneous H-29 (human colon cancer) xenografts (Day 7 post-implantation) received PR-171 IV bolus for 4 weeks; 10 mg/kg once weekly (QW), 5 mg/kg twice daily (QDx2), or 1, 5, and 10 mg/kg twice a week (BIW; Day1/Day4). The inhibitor of the 20S proteasome, bortezomib (PS-341), was also administered BIW at 1 mg/kg. Tumor growth, body weights, and clinical signs were assessed 2 to 3 times a week.

RESULTS

Tumors in mice receiving 5 mg/kg twice a day were 40% smaller than vehicle controls and correlate with significant tumor growth inhibition. Animals given PS-341 biweekly or PR-171 weekly and biweekly did not inhibit tumor growth. It is unknown if PS-341 administered twice daily, similarly to the QDx2 PR-171 group, would exhibit anti-tumor activity. Note that the drop in tumor volume in 10 mg/kg group (Figure 16) is due to early euthanasia of 1 animal with a tumor above allowable limits and is not due to anti-tumor activity of PR-171. There were no significant changes in body weight or clinical observations.

Figure 16: Tumor volume in tumor bearing mice after carfilzomib administration twice weekly on non-consecutive days

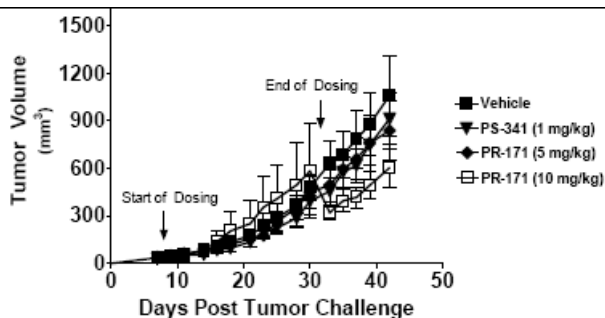
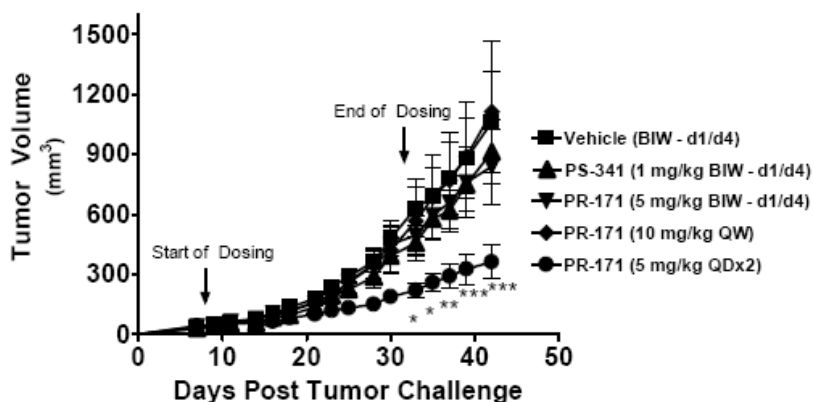


Figure 17: Inhibition of tumor growth mediated by carfilzomib administration twice daily in tumor bearing mice



(Figures excerpted from Applicant's package)

A Single Dose Pharmacodynamic Study Comparing PR-171 Administered Intravenously to PR-58825 Administered Both Intravenously and Nasogastrically to Female Cynomolgus Monkeys (Study TR-0046-171-PD)

Key Study Findings

- PR-171 does not inhibit brain proteasome activity; whereas PR-58825 crosses the blood-brain barrier after IV administration.
- PR-171 has a faster rate (within 15 min) of proteasome inhibition in whole blood than PR-58825.
- PR-171 and PR-58825 have similar levels of proteasome inhibition when administered via IV and nasogastrically.

METHODS

Non-naïve female Cynomolgus monkeys that received prior doses of PR-171 or PR-58825, were administered a single dose of 2.0 mg/kg PR-171 (IV), 2.5 mg/kg PR-58825 (IV), or nasogastric (NG) 10 or 20 mg/kg PR-58825. Chymotrypsin-like proteasome activity via proteolysis of a fluorescent tetrapeptide was determined in whole blood,

erythrocyte-depleted bone marrow, adrenal, brain, liver, heart, lung, and inguinal lymph nodes at 1 hr post-dose.

RESULTS

PR-171 showed a shorter (15 min) T_{max} for inhibition in whole blood than PR-58825 (1 hr). PR-58825 inhibited proteasome function in all tissues examined; whereas, PR-171 did not inhibit proteasome activity in the brain or inguinal lymph nodes. Proteasome inhibition was similar between PR-171 and PR-58825 in the whole blood and bone marrow.

Figure 18: Chymotrypsin-like proteasome activity in blood samples from female Cynomolgus monkeys given PR-58825 or PR-171 IV

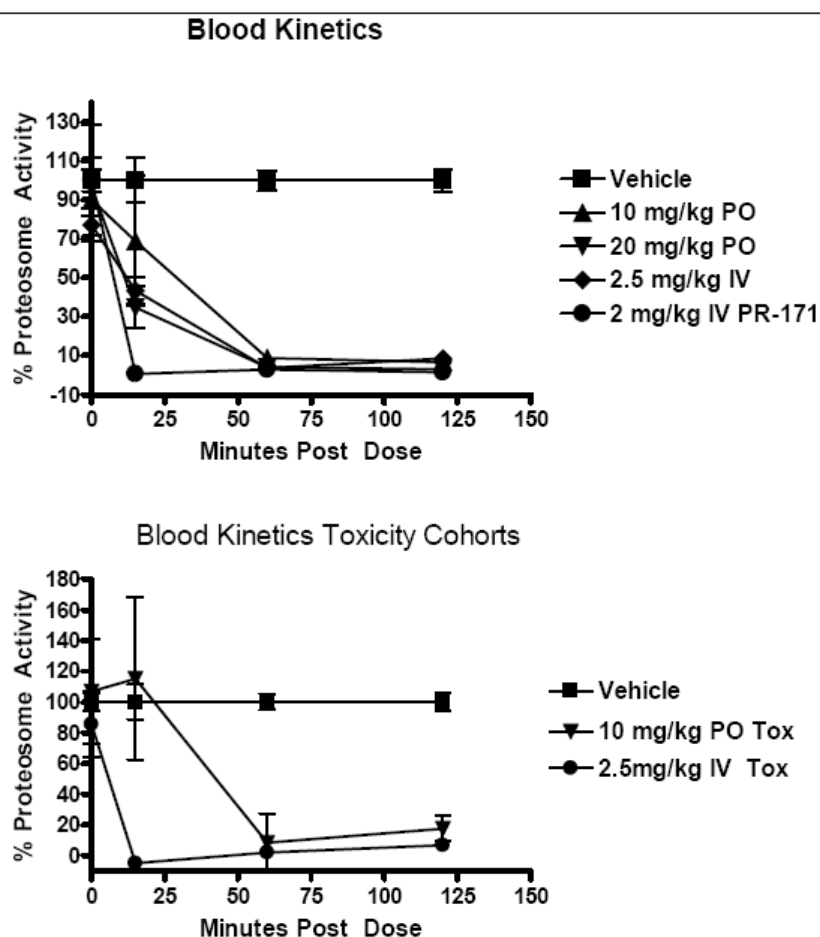


Figure 19: Chymotrypsin-like proteasome activity in brain from female Cynomolgus monkeys given PR-58825 or PR-171

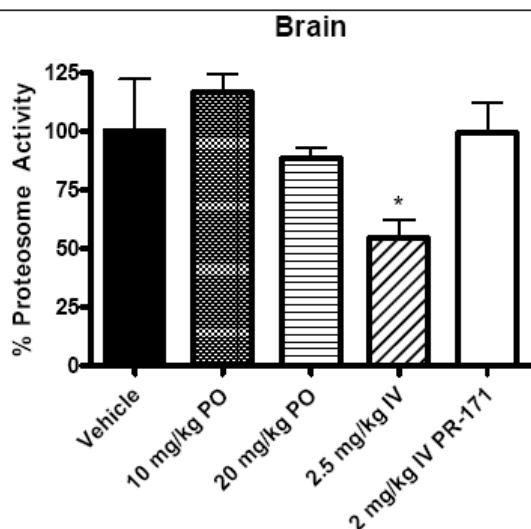
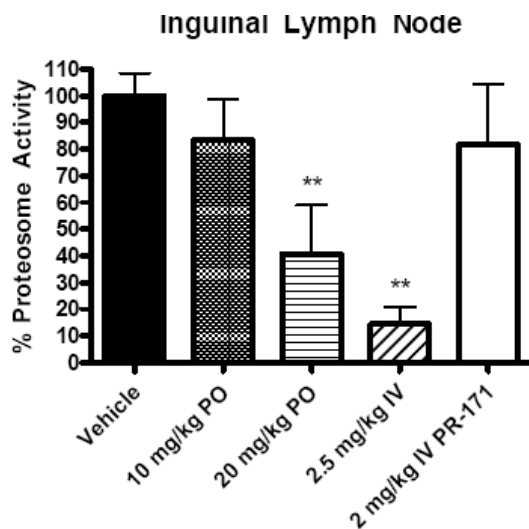


Figure 20: Chymotrypsin-like proteasome activity in inguinal lymph node samples from female Cynomolgus monkeys given PR-58825 or PR-171



(Figures excerpted from Applicant's package)

Pharmacodynamic Evaluation of Carfilzomib in Rats: Comparison of Bolus Injection vs. IV Infusion Delivery (Study TR-0089-171 / R1005-SN252)

Key Study Findings

- The pharmacodynamics of carfilzomib-mediated proteasome inhibition is equivalent after IV bolus and IV infusions.
- Although carfilzomib inhibits primarily the chymotrypsin-like activity in most tissues, it also inhibits caspase-like and trypsin-like activity in the heart.

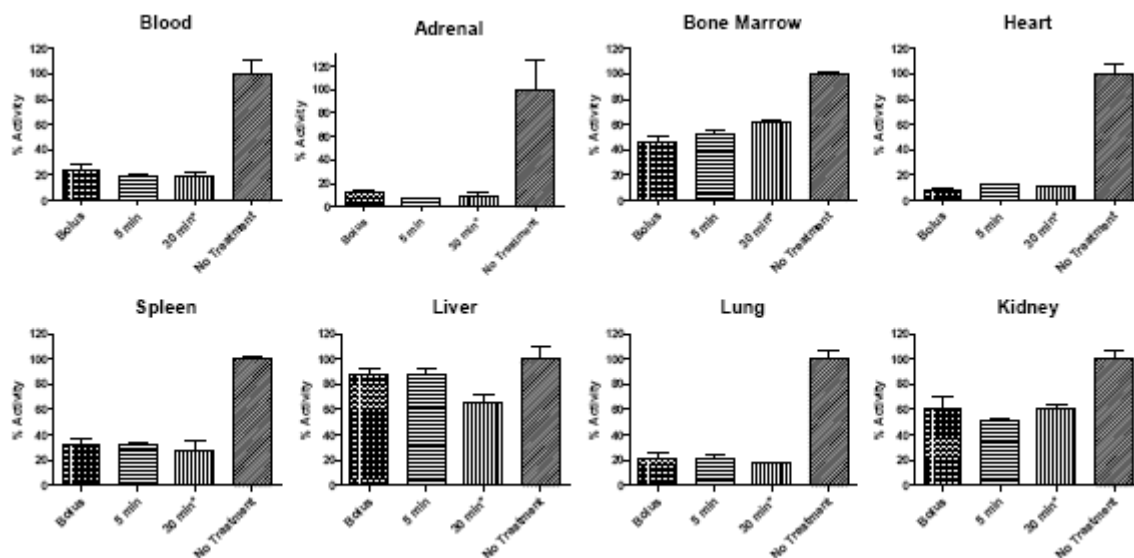
METHODS

To examine the pharmacodynamic properties of carfilzomib after various modes of administration, male Sprague Dawley rats were administered 1 mg/kg carfilzomib by either a bolus IV injection or 1, 5, or 30 minute IV infusions, followed by evaluation of chymotrypsin-like, caspase, and trypsin-like activities in blood, adrenal, heart, lung, spleen, kidney, bone marrow, and liver tissues at 1 hr post-dose.

RESULTS

There were no significant differences in chymotrypsin-like inhibition between bolus injection and infusions of carfilzomib. Carfilzomib administration via all modes resulted in significant inhibition of chymotrypsin-like activity by $\geq 80\%$ in the blood, adrenal, heart, and lung, 70% in the spleen, and 50-60% in the kidney and bone marrow, but not in the liver. Carfilzomib administration also inhibited ($\sim 40\%$) caspase-like and trypsin-like activities in the heart, but not in other tissues.

Figure 21: Inhibition of the chymotrypsin-like activity of the 20S proteasome by carfilzomib



(Figure excerpted from Applicant's package)

The activity carfilzomib metabolites on the chymotrypsin-like activity of the constitutive proteasome and immunoproteasome (Study TR-0206-171)

-study reviewed by Todd R. Palmby, Ph.D.

Key Study Findings:

- Metabolites of carfilzomib did not significantly inhibit the chymotrypsin-like activity of the 20S proteasome under the conditions tested

METHODS

Five human metabolites of carfilzomib were tested for inhibitory activity against chymotrypsin-like activity in an assay measuring activity of human purified 20S constitutive proteasome and immunoproteasome: abundant metabolites found in human plasma PR-059389 (M14) and PR-000519 (M16), and abundant metabolites found in human urine PR-059411 (M3), PR-059409 (M4), PR-059389 (M14) and PR-059413 (M15). *In vitro* inhibition of the 20S constitutive proteasome and immunoproteasome by serial dilutions of carfilzomib and each metabolite was measured by changes in fluorescence resulting from proteasome-mediated cleavage of a fluorogenic substrate, Suc-LLVY-AMC, over 2 minutes intervals for approximately 1 hour. Background values from no enzyme control wells were subtracted from the sample values and then normalized to DMSO only controls to determine percent activity. Percent activity was plotted against concentrations for each metabolite. To determine the IC₅₀'s, the percent proteasome activity values versus concentration were plotted and fit with a sigmoidal dose-response. The complete assay was repeated on a second day to provide a total number of data points for each metabolite at a given concentration of n=4.

RESULTS

Four of the metabolites [PR-059389 (M14), PR-059411 (M3), PR-059409 (M4), PR-059413 (M15)] showed no inhibition of the chymotrypsin-like activity of the constitutive proteasome or immunoproteasome up to the highest concentration tested of 15 μ M. One, metabolite [PR-000519 (M16)] showed weak inhibition of the chymotrypsin-like activity of the constitutive and immunoproteasome. The IC₅₀ of PR-000519(M16) for the chymotrypsin-like activity of the constitutive proteasome was approximately 2500-fold higher than carfilzomib. The IC₅₀ of PR-000519(M16) for the chymotrypsin-like activity of the immunoproteasome was approximately 300-fold higher than carfilzomib (see Table 4).

Reviewer's note: M3 and M4 were removed from the report for clinical trial TR-0077-171 in the discussion of carfilzomib metabolites in human plasma and urine through an amendment because they were detected in pre-dose human plasma samples at levels similar to carfilzomib-treated human plasma samples in clinical trial TR-0201-171 and in the nonclinical study 09609, and because their relative abundances reported originally in TR-0077-171 were found to be over-represented. In addition, M2 was not investigated in the current study for activity on the chymotrypsin-like activity of the proteasome. The relative abundance of M2 in human plasma from subjects receiving 27 mg/m² was slightly lower than that of M15 (clinical trial TR-0077-171), but was the only other detectable metabolite present in plasma aside from M14, M15 and M16.

Table 4: *In vitro* activity of carfilzomib metabolites on the chymotrypsin-like activity of the constitutive proteasome and immunoproteasome

Proteolix ID	IC50 (μM) human constitutive proteasome	IC50 (μM) human immuno- proteasome
carfilzomib	0.003	0.027
PR-000519 (M16)	7.4	8.2
PR-059389 (M14)	>15	>15
PR-059409 (M4)	>15	>15
PR-059411 (M3)	>15	>15
PR-059413 (M15)	>15	>15

(Table excerpted from Applicant's package)

4.2 Secondary Pharmacology

Selectivity of PR-171: Testing Against a Panel of Proteases (Study TR-0002-171)

Key Study Findings

- PR-171 did not significantly inhibit (>50%) any of the enzymes tested at 10 μM. Nevertheless, there was a trend for a 3-fold higher activity against human pancreatic chymotrypsin compared to all other enzymes tested.

METHODS

The inhibitory activities of 10 μM PR-171 and bortezomib were tested against a panel of 21 proteases, including serine proteases, metalloproteases, cysteine proteases and aspartyl proteases.

RESULTS

PR-171 only inhibited human pancreatic chymotrypsin by 38% and did not meet the criteria for significant inhibition (≥50% inhibition) compared to control. Nevertheless, PR-171 only inhibited all other enzymes by ≤11% and had 3-fold higher activity for chymotrypsin over the other enzymes. Conversely, bortezomib inhibited human pancreatic chymotrypsin as well as cathepsin G (neutrophils) and chymase (mast cells and skeletal muscle) by ≥90%. Bortezomib also inhibited rennin, angiotensin converting enzyme (ACE), and human leukocyte elastase by >25%.

Table 5: Protease inhibition by PR-171 and bortezomib *in vitro*

Protease Class	Protease	Source	% Inhibition	
			PR-171	Bortezomib
Aspartyl	Cathepsin D	Human	2	-1
Aspartyl	Renin	Human	-8	34
Cysteine	Calpain-1	Human	-1	-1
Cysteine	Caspase 3	Human	0	-8
Cysteine	Cathepsin B	Human	1	11
Metalloprotease	ACE	Rabbit	-6	31
Metalloprotease	MMP-1	Human	2	-9
Serine	Cathepsin G	Human	-2	93
Serine	Chymase	Human	11	94
Serine	Chymotrypsin	Human	38	90
Serine	DPP IV	Pig	7	-14
Serine	Elastase	Human	6	40
Serine	Factor VIIa	Human	7	17
Serine	Factor Xa	Human	-2	5
Serine	Kallikrein	Human	-9	2
Serine	Plasmin	Human	-5	16
Serine	Prolyl Oligopeptidase	Bacteria	8	-20
Serine	Thrombin	Human	-1	-22
Serine	tPA	Human	0	-14
Serine	Trypsin	Human	-1	0
Serine	Tryptase	Human	-9	-20

(Table excerpted from Applicant's package)

Best Available Copy

Selectivity of PR-171: Testing Against a Panel of Receptors and Enzymes (Study TR-0003-171)

Key Study Findings

- PR-171 inhibited the neurokinin NK₂ receptor, but with 100-fold less potency than for the 20S proteasome.

METHODS

Binding and inhibitory activity of 10 μ M PR-171 was examined against a broad specificity (b) (4) Diversity Panel, which includes 67 receptors and 16 enzymes.

RESULTS

PR-171 binds to neurokinin NK₁ (61%, IC₅₀ = 22 μ M) and NK₂ (120%, IC₅₀ = 0.7 μ M) receptors and the sodium channel (51%), but with 100-fold lower potency than towards the proteasome. No significant activities (>50%) were seen with other receptors or enzymes.

Table 6: IC₅₀s for carfilzomib inhibition of neurokinin NK1 and NK2 receptors

Assay (b)(4)	Compound I.D.	Client Compound I.D.	IC ₅₀ (M)	K _i (M)	n _H
NK ₁ (h)					
9013-1		PRX-003	2.2E-05	9.9E-06	1.0
NK ₂ (h)					
9013-1		PRX-003	6.6E-07	3.6E-07	1.2

(Table excerpted from applicant's package)

4.3 Safety Pharmacology

Brief Summary of the findings in Study 04-6576: Cardiovascular, respiratory, and central nervous system assessments of PR-171 when administered intravenously to conscious monkeys (see Section 12 Appendix/Attachments in this review)

Cardiovascular

↑ST segments, ↑T wave amplitude, ↓blood pressure, ↑heart rate, ↓PR interval, ↓QRS interval, ↓QT interval, ↑Troponin-T myocardial biomarker, hydropericardium, discoloration of the left ventricle and interventricular septum, soft cardiac ventricles at 3 mg/kg.

Pulmonary

Peribronchial edema in the soft tissue of the hilus in two animals at 3 mg/kg.

Effects of Carfilzomib (PR-171) on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study TR-0088-171 / 070914.QTQ)

Key Study Findings

- PR-171 significantly inhibited the hERG channel potassium current at $\geq 1.5 \mu\text{M}$
- IC₅₀ = 2.1 μM

METHODS

Single HEK293 cells stably transfected with hERG cDNA were sequentially exposed to increasing doses of PR-171 (0, 0.7, 1.5 and 2.2 μM) while patch clamp was used to measure the peak potassium current until steady state was achieved or 12 minutes of exposure time had elapsed.

RESULTS

PR-171 dose-dependently inhibited potassium currents at all doses, reaching statistical significance at 1.5 μM with 12.7% inhibition. At 2.2 μM , 54.2% of the potassium current was inhibited with PR-171 treatment, which is comparable to the positive control (60 nM terfenadine) at 85.4% inhibition. IC₅₀ = 2.1 μM .

Table 7: *In vitro* hERG inhibition by carfilzomib

Concentration (μM)	Mean	SD	SEM	N
0	0.1%	0.1%	0.0%	3
0.7	6.9%	0.7%	0.4%	3
1.5	12.7%*	2.3%	1.2%	4
2.2	54.2%*	5.5%	3.2%	3

* Value is statistically different than vehicle alone.

(Table excerpted from Applicant's package)

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 Pharmacokinetics

Pharmacokinetics of Carfilzomib in Sprague Dawley Rats Following a Single Intravenous Bolus or Infusion Administration (Study TR-0383-171)

Key Study Findings

- Rapid elimination of PR-171 ($t_{1/2} < 20$ min) thru non-hepatic mechanisms.
 - Therefore, immediate sampling after bolus injection is necessary to avoid underestimating C_{max} and overestimating clearance.
- AUC exposure, clearance, and $t_{1/2}$ are similar after intravenous bolus and infusion administration.

METHODS

The pharmacokinetics of PR-171 was evaluated in Sprague Dawley rats receiving IV bolus injection or IV infusion of PR-171 (2, 4, or 8 mg/kg). For bolus injections, plasma samples were collected Predose, immediately after dosing (0.1 min), and at 1, 2, 5, 15, 30, and 60 min post-dose. Samples at 120 min post bolus dose were also evaluated in an additionally cohort. For infusions, animals received 8 mg/kg PR-171 infused over 30 minutes, and samples were collected pre-dose and 15, 30, 32, 35, 45, 60, 90, and 150 post start of infusion.

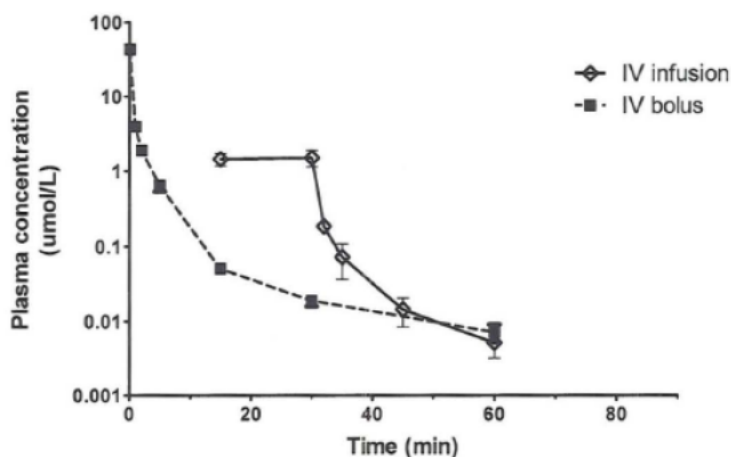
RESULTS

C_{max} for bolus injection was achieved immediately after administration and was rapidly cleared, wherein plasma clearance was much higher than reported rat hepatic blood flow, suggesting that PR-171 is eliminated primarily through non-hepatic mechanisms. C_{max} after infusion (1.55 μM) was achieved within 15 min post the start of infusion, but was 28-fold lower than for bolus injection. Conversely, AUC_{inf} exposure, clearance, and half life (<20 min) were similar for both infusion and bolus administration.

Table 8: Pharmacokinetic parameters of carfilzomib following a single intravenous bolus and 30-minute infusion in rats

Dose Administration	Infusion	Bolus
Dose	8 mg/kg	8 mg/kg
$t_{1/2}$ (min)	10 ± 6	17 ± 3
t_{max} (min)	25 ± 9	0.1
C_{max} (μ M)	1.55 ± 0.37	42.94 ± 4.45
C_0 (μ M)	NA	49.45 ± 4.66
AUC_{last} (min* μ mol/L)	35.8 ± 7.3	37.6 ± 2.8
AUC_{inf} (min* μ mol/L)	35.9 ± 7.2	37.8 ± 2.8
AUC_{inf}/D (min*kg* μ mol/L/ μ mol)	3.22 ± 0.66	3.39 ± 0.25
CL (ml/min/kg)	319 ± 66	296 ± 22
V_{ss} (L/kg)	2.0 ± 0.5	0.62 ± 0.12

Best Available Copy

Figure 22: Plasma concentration-time profiles of intravenous bolus and intravenous infusion of 8 mg/kg carfilzomib in rats*(Table and figure excerpted from Applicant's package)***Pharmacokinetics of carfilzomib in rats: comparison of frozen and lyophilized drug products (Study TR-0079-171)**

The pharmacokinetic profiles of frozen and lyophilized PR-171 are similar after IV administration in Sprague Dawley rats. Average plasma concentration at all time points, AUCs, plasma clearance, volume of distribution at steady state, and half-lives were not statistically different.

Table 9: Pharmacokinetic parameters of frozen and lyophilized carfilzomib drug product in rats following intravenous bolus administration

PK parameter	Units	Frozen product		Lyophilized product	
		Mean	S.D.	Mean	S.D.
$T_{1/2}$	min	6.9	2.7	7.8	1.4
MRT_{INF}	min	3.5	1.0	3.4	0.8
C_{max}	$\mu\text{mol/L}$	0.19	0.05	0.19	0.02
AUC_{last}	$\text{min} \cdot \mu\text{mol/L}$	3.36	0.97	3.48	0.57
AUC_{INF}	$\text{min} \cdot \mu\text{mol/L}$	3.43	0.99	3.54	0.58
CL	mL/min/kg	855	196	804	136
V_{ss}	L/kg	3.1	1.2	2.8	1.1

(Table excerpted from Applicant's package)

Dose Range Finding Toxicology Study of PR-171 in Male Cynomolgus Monkeys: PR-171 Quantitation in Plasma Samples and Estimation of Non-Compartmental Pharmacokinetic Parameters (Study TR-0012-171)

Key Study Findings

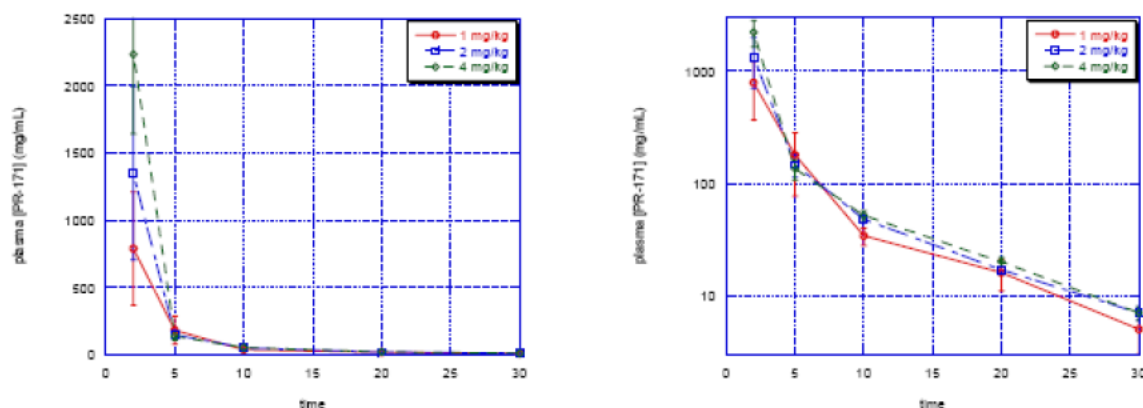
- Biphasic half life: $t_{1/2\alpha}$ =0.6 to 1.3 min and $t_{1/2\beta}$ =7.5 to 8.5 min
- Large distribution into peripheral tissues.
- Majority of drug clearance occurs in the first minute.

METHODS

Non-compartmental pharmacokinetic parameters were estimated from a range-finding IV bolus toxicity study in Cynomolgus monkeys receiving 0, 1, 2, or 4 mg/kg PR-171 (n=3). Plasma samples were collected at intervals up to 1 hr post dose. Data was also examined by a two compartment analysis ($t_{1/2\alpha}$ and $t_{1/2\beta}$).

RESULTS

Plasma concentration vs. time profiles appear biphasic, fitting a two compartment model (2 comp) with $t_{1/2\alpha}$ =0.6 to 1.3 min and $t_{1/2\beta}$ =7.5 to 8.5 min. AUC exposures increased dose-dependently. PR-171 cleared rapidly with the majority clearing during the short alpha phase. Steady state volumes of distribution (V_{ss}) were 3- to 16-fold higher than blood volume, suggesting distribution of PR-171 into peripheral tissues. This is also consistent with widespread proteasome inhibition previously reported in peripheral monkey tissues. The mechanism of clearance of PR-171 was unknown during the conduct of this study.

Figure 23: Average plasma concentration-time plot following intravenous bolus administration in male Cynomolgus monkeysBest Available
Copy

Left panel: linear plot; right panel: semi-logarithmic plot

Table 10: Noncompartmental pharmacokinetic parameters for 1 mg/kg in Cynomolgus monkeys

Parameter	Units	Animal #			Mean	Stdev
		40131	41002	40101		
Terminal $t_{1/2}$	min	7.1	7.7	6.0	6.9	0.7
C_{max}	ng/mL	593	1373	400	789	421
AUC_{INF_obs}	min*ng/mL	4137	8993	2876	5335	2637
AUC_{INF}/D_{obs}	min*kg*ng/mL/mg	4137	8993	2876	5335	2637
Cl_{obs}	mL/min/kg	242	111	348	234	97
V_{ss_obs}	mL/kg	691	298	1754	914	615
$t_{1/2}(\alpha)$ (2 comp)	min	1.0	1.2	1.6	1.3	0.2
$t_{1/2}(\beta)$ (2 comp)	min	7	10	7	8	1

Table 11: Noncompartmental pharmacokinetic parameters for 2 mg/kg in Cynomolgus monkeys

Parameter	Units	Animal #			Mean	Stdev
		030925	041011	040977		
Terminal $t_{1/2}$	min	7.46	7.45	7.37	7.43	0.04
C_{max}	ng/mL	436	1831	2467	1578	848
AUC_{INF_obs}	min*ng/mL	3183	17797	17296	12759	6774
AUC_{INF}/D_{obs}	min*kg*ng/mL/mg	1592	8898	8648	6379	3387
Cl_{obs}	mL/min/kg	628	112	116	285	242
V_{ss_obs}	mL/kg	2858	163	210	1077	1260
$t_{1/2}(\alpha)$ (2 comp)	min	1.1	0.7	0.8	0.9	0.2
$t_{1/2}(\beta)$ (2 comp)	min	7.6	7.6	7.3	7.5	0.1

Table 12: Noncompartmental pharmacokinetic parameters for 4 mg/kg in Cynomolgus monkeys

Parameter	Units	Animal #			Mean	Stdev
		030387	040950	040997		
Terminal $t_{1/2}$	min	6.9	7.3	7.5	7.2	0.3
C_{max}	ng/mL	1422	2815	1782	2006	590
AUC_{INF_obs}	min*ng/mL	23274	25546	16990	21937	3619
AUC_{INF/D_obs}	min*kg*ng/mL/mg	5818	6387	4247	5484	905
Cl_{obs}	mL/min/kg	172	157	235	188	34
V_{ss_obs}	mL/kg	162	188	308	219	64
$t_{1/2}(\alpha)$ (2 comp)	min	0.65	0.56	0.58	0.60	0.04
$t_{1/2}(\beta)$ (2 comp)	min	10	7	8	8	1

(Tables and figure excerpted from Applicant's package)

Pharmacokinetics of PR-171 in Mouse Following 5 mg/kg Intravenous Bolus Administration (Study TR-0053-171)

Key Study Findings

- $T_{1/2} = 19$ min
- High plasma clearance comparable to liver blood flow.

METHODS

Pharmacokinetics of PR-171 as determined in balb/c mice after 5 mg/kg IV bolus administration at pre-dose, 2, 5, 10, 30, 30, and 60 min post-dose.

RESULTS

PR-171 exposures rapidly decreased in a multi-exponential manner with a half-life of 19 min. In contrast to other species (rat, monkey), the high plasma clearance was comparable with liver blood flow in the mouse (90 mL/min/kg), which does not necessarily implicate non-hepatic clearance of PR-171 in mice.

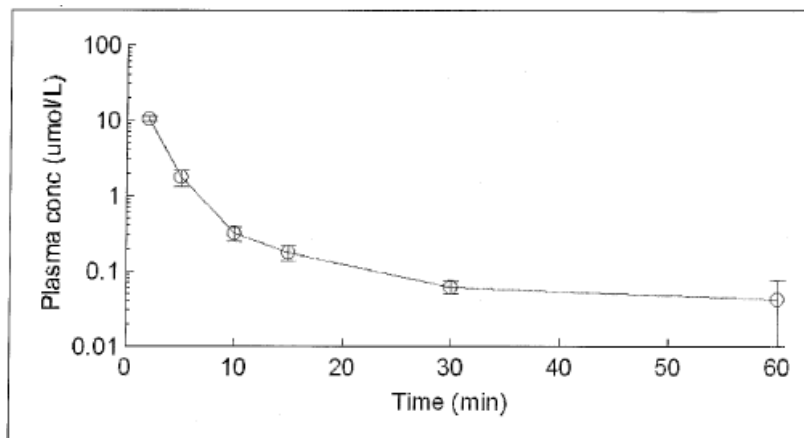
Figure 24: Plasma concentration-time profile following intravenous bolus of carfilzomib at 5 mg/kg in male mice

Table 13: Pharmacokinetic parameters of carfilzomib in male mice following an intravenous bolus administration of 5 mg/kg

PK parameter	Units	Value
T _{1/2}	min	19
MRT _{INF}	min	4
C _{max}	μmol/L	10.4
AUC _{last}	min*μmol/L	72.4
AUC _{INF}	min*μmol/L	73.6
CL	mL/min/kg	94
V _{ss}	L/kg	0.39

(Table and figure excerpted from Applicant's package)

5.2 Absorption

A Single Dose Pharmacodynamic Study Comparing PR-171 Administered Intravenously to PR-58825 Administered Both Intravenously and Nasogastrically to Female Cynomolgus Monkeys: Pharmacokinetics of PR-171 and PR-58825 (Study TR-0046-171-PK)

Key Study Findings

- PR-171 clearance follows a biphasic, multi-exponential manner.
- PR-58862 oral bioavailability is very low (0.83%) with high variability.
- PR-58862 clearance follows a one-compartmental model.

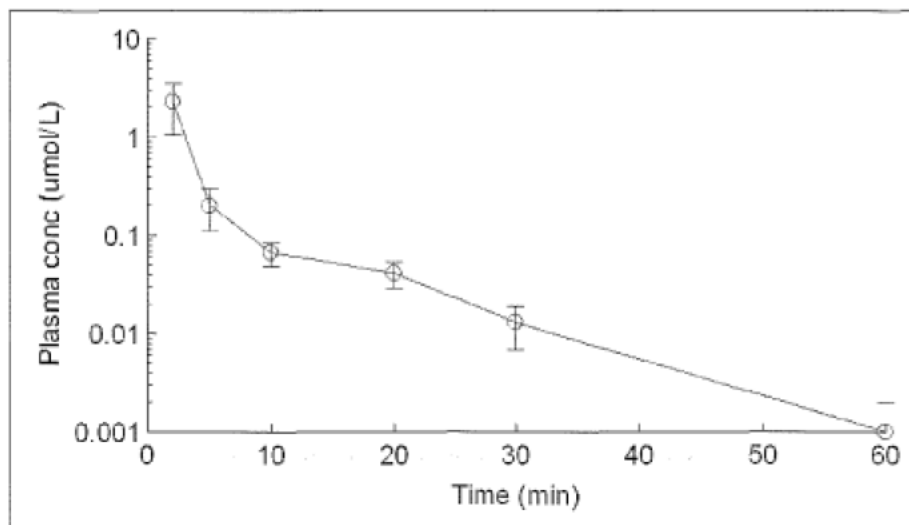
METHODS

Single dose pharmacodynamics and pharmacokinetics were determined in Cynomolgus monkeys after IV bolus administration of 2.5 mg/kg PR-58825 or 2 mg/kg PR-171 and nasogastric (NG) administration of 10 or 20 mg/kg PR-58825. Blood samples were collected at pre-dose, 2, 5, 10, 20, 30, 60, and 120 min post-dose.

RESULTS

PR-171 exposures decline in a biphasic, multi-exponential manner; whereas PR-58825 clearance follows a one-compartmental model. PR-58825 has poor oral availability and high variability, with a bioavailability of 0.83% after NG administration at the highest dose. T_{max} was reached at 35 min after NG administration, compared to being reached immediately after IV administration.

Figure 25: Mean plasma concentration-time profile following an intravenous bolus administration of carfilzomib at 2 mg/kg in female Cynomolgus monkeys



Best Available Copy

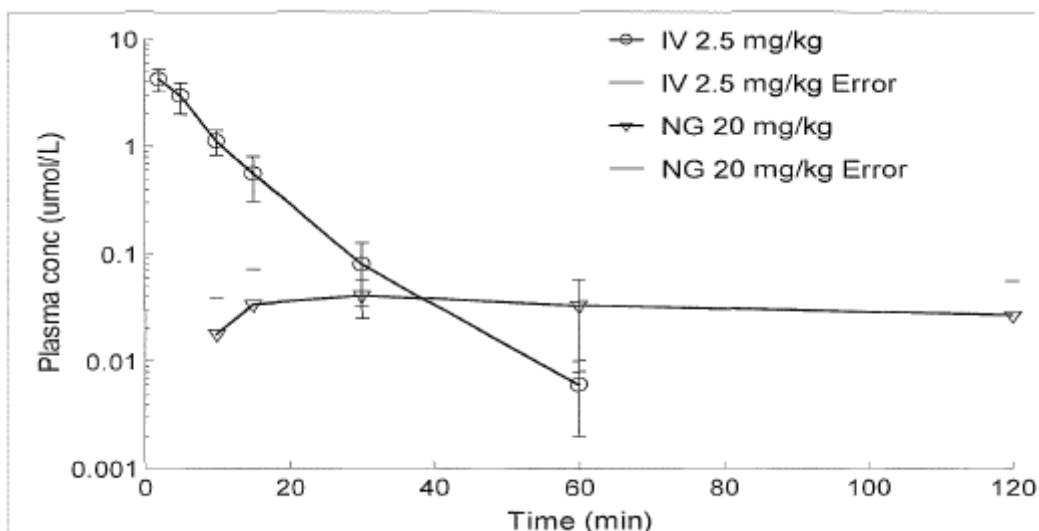
Table 14: Pharmacokinetic parameters of PR-58825 in female Cynomolgus monkeys after intravenous bolus administration at 2.5 mg/kg

Parameter	Units	Monkey number						Mean	SD
		#7	#9	#11	#31	#33	#35		
$T_{1/2}$	min	7.9	7.3	3.9	8.7	6.4	6.8	6.8	1.7
MRT_{INF}	min	6.7	7.9	5.5	6.0	7.6	7.0	6.8	0.9
C_{max}	umol/L	3.75	5.02	3.96	3.52	3.27	5.89	4.23	1.01
AUC_{last}	min*umol/L	34.9	54.9	34.1	29.7	35.6	56.2	40.9	11.5
AUC_{INF}	min*umol/L	35.0	55.0	34.3	29.8	35.6	56.3	41.0	11.5
$AUC_{INF}/Dose$	min*kg*umol/L/umol	6.76	10.6	6.62	5.75	6.87	10.86	7.91	2.22
CL	mL/min/kg	148	94	151	174	145	92	134	33
V_{ss}	L/kg	0.98	0.75	0.82	1.05	1.11	0.64	0.89	0.18

Table 15: Pharmacokinetic parameters of PR-58825 in female Cynomolgus monkeys after nasogastric administration at 20 mg/kg

Parameter	Units	Monkey #13	Monkey #15	Monkey #17	Mean	SD
$T_{1/2}$	min		29	17	23	8
MRT_{INF}	min		58	33	45	17
T_{max}	min	60.0	30	15	35	23
C_{max}	umol/L	0.062	0.047	0.078	0.062	0.016
AUC_{last}	min*umol/L	5.21	2.47	2.41	3.37	1.60
AUC_{INF}	min*umol/L		2.71	2.75	2.73	0.03
$AUC_{INF}/Dose$	min*kg*umol/L/umol		0.065	0.067	0.066	0.001
Bioavailability	%				0.83	

Figure 26: Mean plasma concentration-time profile following administration of PR-58825 via intravenous bolus at 2.5 mg/kg and nasogastric at 20 mg/kg in female Cynomolgus monkeys



(Table and figures excerpted from Applicant's package)

5.3 Distribution

Absorption, Metabolism, and Excretion of ^3H -PR-171 Following Intravenous Administration to Rats (Study TR-0041-171)

Key Study Findings

- ^3H -PR-171 elimination was very slow, wherein it was still present 168 hours postdose.
- ^3H -PR-171 was partially eliminated via the urine (14%) and feces (20%), but was largely eliminated via metabolism followed by incorporation of ^3H -PR-171 byproducts (i.e. ^3H -Phe) into cellular proteins (>44%).
- ^3H -PR-171 was widely distributed to all tissues, more so than blood, except for epididymes and eye.
- Given the proposed high rate of metabolism of ^3H -PR-171 and incorporation of radiolabeled breakdown products into cellular proteins, analysis of proteasome inhibition over time within the broad spectrum of tissues is essential to determine the distribution and retention of the parent compound within the various tissues, in addition to ^3H -PR-171 autoradiography.
- ^3H -PR-171 pharmacokinetics does not mirror PR-171 pharmacokinetics, likely due to contaminating metabolites.

METHODS

Tissue distribution and tissue:plasma concentration ratios of radiolabeled ^3H -PR-171 were determined in Sprague Dawley male rats following a single 2 mg/kg IV administration. For whole-body autoradiography (WBA), animals were perfused 0.5, 6,

24, 72, and 168 hours post-dose. For analysis of excretion and mass balance: urine was collected at 0-8 hrs, 8-24 hrs, and 24-hour intervals up to 168 hrs post-dose; feces were collected at 24-hr intervals up to 168 hrs post-dose. Blood samples were collected at 0.083, 0.25, 0.5, 6, 24, 72, and 168 hrs postdose.

RESULTS

³H-PR-171 was widely distributed in all tissues examined and reached maximum concentrations in tissues at 0.5 (urine, bile, pancreas, bladder, stomach) to 24 (pituitary gland, bone marrow, thyroid, lung, small intestine, spleen, adrenal gland, exorbital lacrimal gland, pineal gland, liver) hours post-dose. Radioactivity was still detected at 168 hours (liver, muscle, skin, small intestine); however, PR-171 is fully cleared from most tissues within 72 hours (study TR-0021-171). Since the radiolabel is present on phenylalanine, its possible that the radioactivity at 168 hours (>44%) is indicative of incorporation of ³H-Phe into cellular proteins, after metabolism of ³H-PR-171, or ³H-Phe-derived compounds or amines (i.e. dopamine). ³H-PR-171-derived activity was present in brain and testis after 168 hrs, but without inhibition of proteasome activity (study TR-0021-171), indicating that inactive PR-171-derived metabolites or ³H-byproducts cross the blood/brain and blood/testis barriers, which is present after longer timepoints. Radioactivity was detected in blood at all time points, indicating that ³H-PR-171 may bind to RBCs and/or plasma. Residual activity in plasma and urine at later timepoints are likely ³H-PR-171 due to the presence of tritiated water after breakdown of ³H-PR-171. Tissue:plasma ratios were greater than 1.0 at all timepoints, except for epididymes and eye, indicating that ³H-PR-171 is readily absorbed into most all tissues and are present more in the tissues than the blood. The highest levels of radioactivity were detected in muscle > liver > skin > intestine. ³H-PR-171 was only partially eliminated via the urine (14%) and feces (20%). Peak radioactivity was seen in plasma at 6 hours instead of 30 min (earliest time point examined), which is what would have been expected based on PR-171 pharmacokinetics in SD rats (Study #TR-0383-171 and #TR-0079-171). This further suggests that the pharmacokinetics of ³H-PR-171 radioactivity studies do not parallel PR-171 pharmacokinetics, likely due to contamination with radiolabeled metabolites.

Figure 27: Concentrations of radioactivity in plasma after a single intravenous dose of ^3H -PR171 to male rats

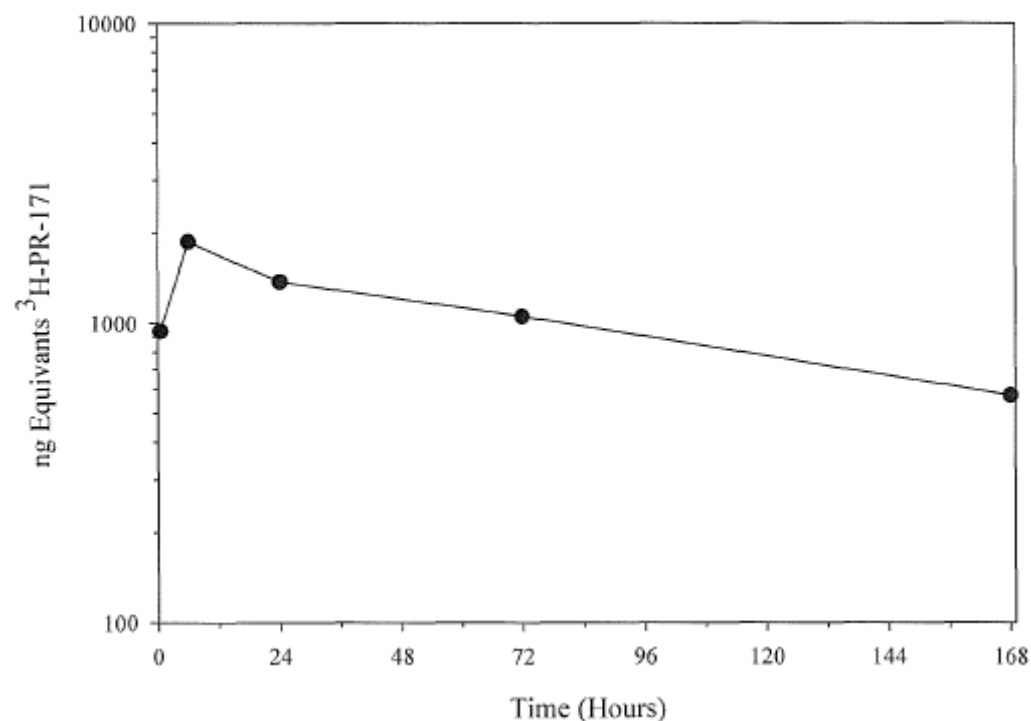


Figure 28: Amount of ^3H -PR-171 in urine and feces (%) after a single intravenous dose in male rats

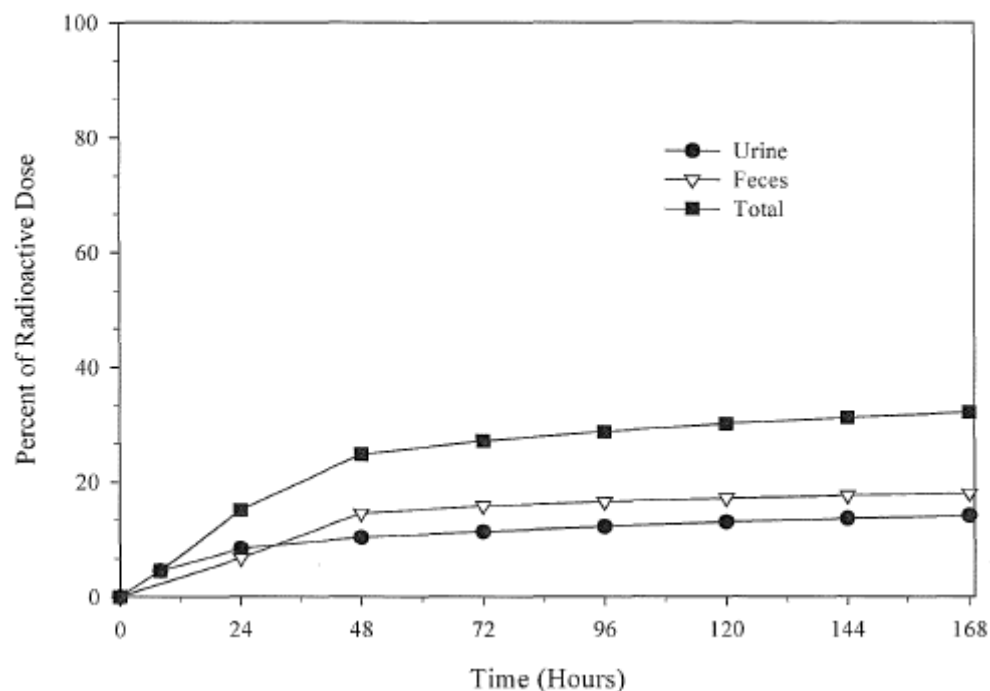


Table 16: Tissue:plasma concentration ratios after a single intravenous administration of ³H-PR-171 to male rats

Tissue	Tissue:Plasma Concentration Ratios				
	Animal Number (Sacrifice Time)				
	C24122 (0.5 Hour)	C24123 (6 Hours)	C24124 (24 Hours)	C24125 (72 Hours)	C24126 (168 Hours)
Adrenal gland	5.34	2.63	4.72	2.48	1.99
Bile	27.6	NA	NA	NA	NA
Blood	NA	NA	NA	NA	NA
Bone	NA	NA	NA	NA	NA
Bone marrow	5.78	4.02	5.69	3.06	1.85
Cecum	4.52	6.68	3.05	2.06	1.82
Cecum contents	NA	8.13	2.13	NA	NA
Cerebellum	0.800	0.515	0.847	1.02	1.78
Cerebrum	0.952	0.482	0.803	0.930	1.74
CSF	3.57	0.947	2.66	NA	NA
Diaphragm	1.99	1.01	1.79	1.58	2.11
Epididymis	0.899	0.567	0.624	0.606	0.864
Esophageal contents	NA	0.459	NA	0.51	NA
Esophagus	1.89	1.02	2.26	2.11	2.34
Exorbital lacrimal gland	7.34	2.76	4.44	2.62	2.55
Eye	0.756	0.248	0.514	0.595	0.96
Eye (lens)	0.418	NA	0.601	0.536	1.25
Fat (abdominal)	NA	NA	NA	0.339	NA
Fat (brown)	1.95	0.904	1.50	1.61	1.44
Harderian gland	1.87	1.12	1.73	1.80	1.90
Intra-orbital lacrimal gland	6.66	2.51	3.03	2.19	NA
Kidney	6.68	3.02	3.91	3.89	3.4
Large intestinal contents	NA	17.1	4.88	0.392	NA
Large intestine	3.53	2.24	2.66	2.31	1.64
Liver	8.30	2.72	4.12	3.40	3.32
Lung	7.28	3.65	5.36	3.43	3.37
Lymph nodes	3.35	1.68	2.13	2.20	1.88
Medulla	0.705	0.437	0.730	0.699	1.45
Muscle	1.60	0.818	1.53	1.37	2.32
Myocardium	3.76	1.83	3.23	2.73	3.91
Nasal turbinates	1.74	1.37	1.28	1.17	1.83
Olfactory lobe	0.896	0.410	0.854	0.971	1.85
Pancreas	16.8	2.04	2.23	1.75	2.08
Periosteum	1.97	1.34	1.28	1.38	1.66
Pineal gland	5.49	2.51	4.32	2.47	NA
Pituitary gland	9.63	4.45	5.71	5.34	5.27

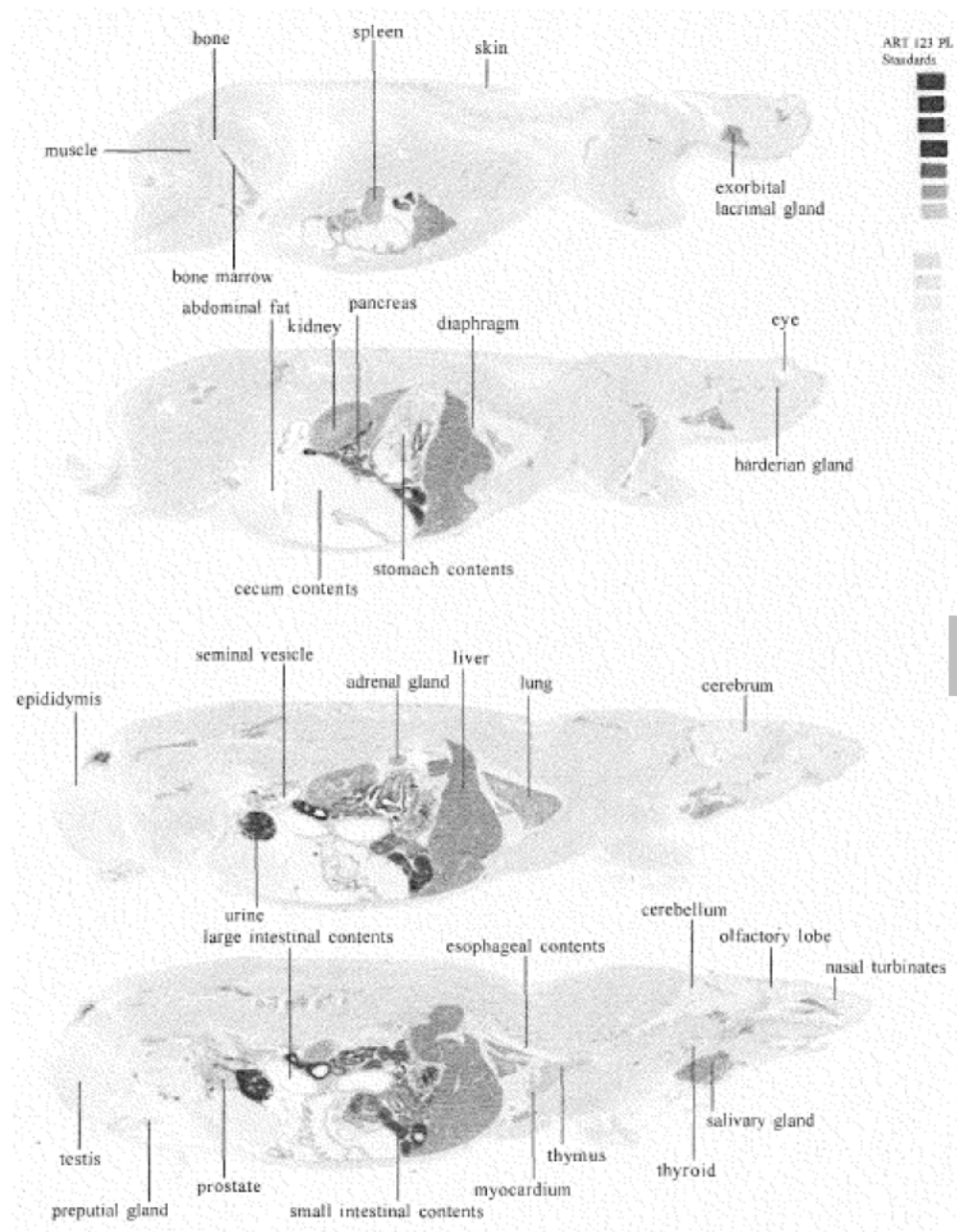
Best Available Copy

Tissue	Tissue:Plasma Concentration Ratios				
	Animal Number (Sacrifice Time)				
	C24122 (0.5 Hour)	C24123 (6 Hours)	C24124 (24 Hours)	C24125 (72 Hours)	C24126 (168 Hours)
Preputial gland	1.17	1.42	1.52	1.23	1.60
Prostate	3.43	1.95	2.74	1.88	2.15
Renal cortex	6.49	2.98	3.91	3.85	3.25
Renal medulla	7.01	3.09	3.79	3.8	3.63
Salivary gland	7.53	2.76	2.81	2.51	2.37
Seminal vesicle	2.48	1.19	3.66	2.83	6.13
Skin	1.21	0.749	0.927	1.00	1.13
Small intestinal contents	13.1	2.86	1.11	NA	NA
Small intestine	9.76	4.13	4.85	2.44	1.65
Spinal cord	0.679	0.230	0.628	0.674	1.25
Spinal cord (gray matter)	1.18	0.387	0.803	0.890	1.55
Spinal cord (white matter)	NA	NA	NA	0.490	0.716
Spleen	5.63	3.25	4.84	4.72	3.12
Stomach	6.61	3.54	3.61	2.50	2.13
Stomach (gastric mucosa)	11.4	4.90	3.99	3.28	2.81
Stomach contents	9.12	0.259	NA	NA	NA
Testis	0.855	0.479	0.796	0.962	1.29
Thymus	3.37	1.69	3.59	2.98	2.93
Thyroid	2.40	3.70	5.40	3.10	4.42
Urinary bladder	13.0	0.509	1.14	1.14	0.890
Urine	43.4	0.957	0.371	0.609	NA

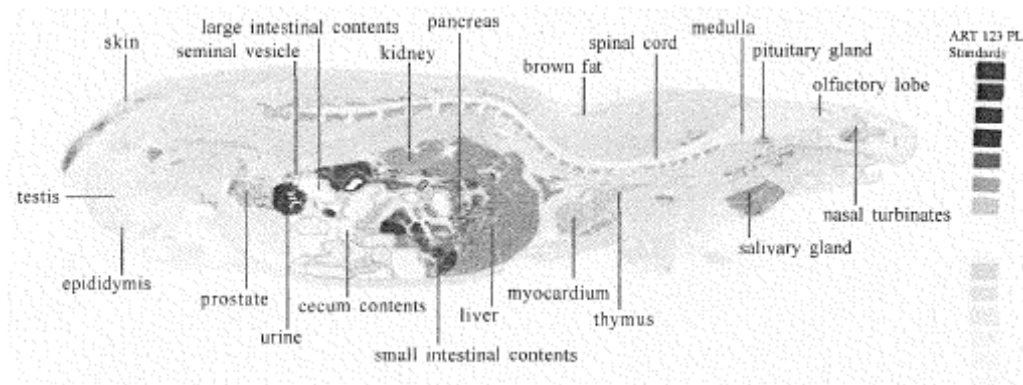
NA Not applicable.

Best Available Copy

Figure 29: Whole-body autoradiograph of male rat (C24122) administered a single intravenous dose of 3H-PR-171

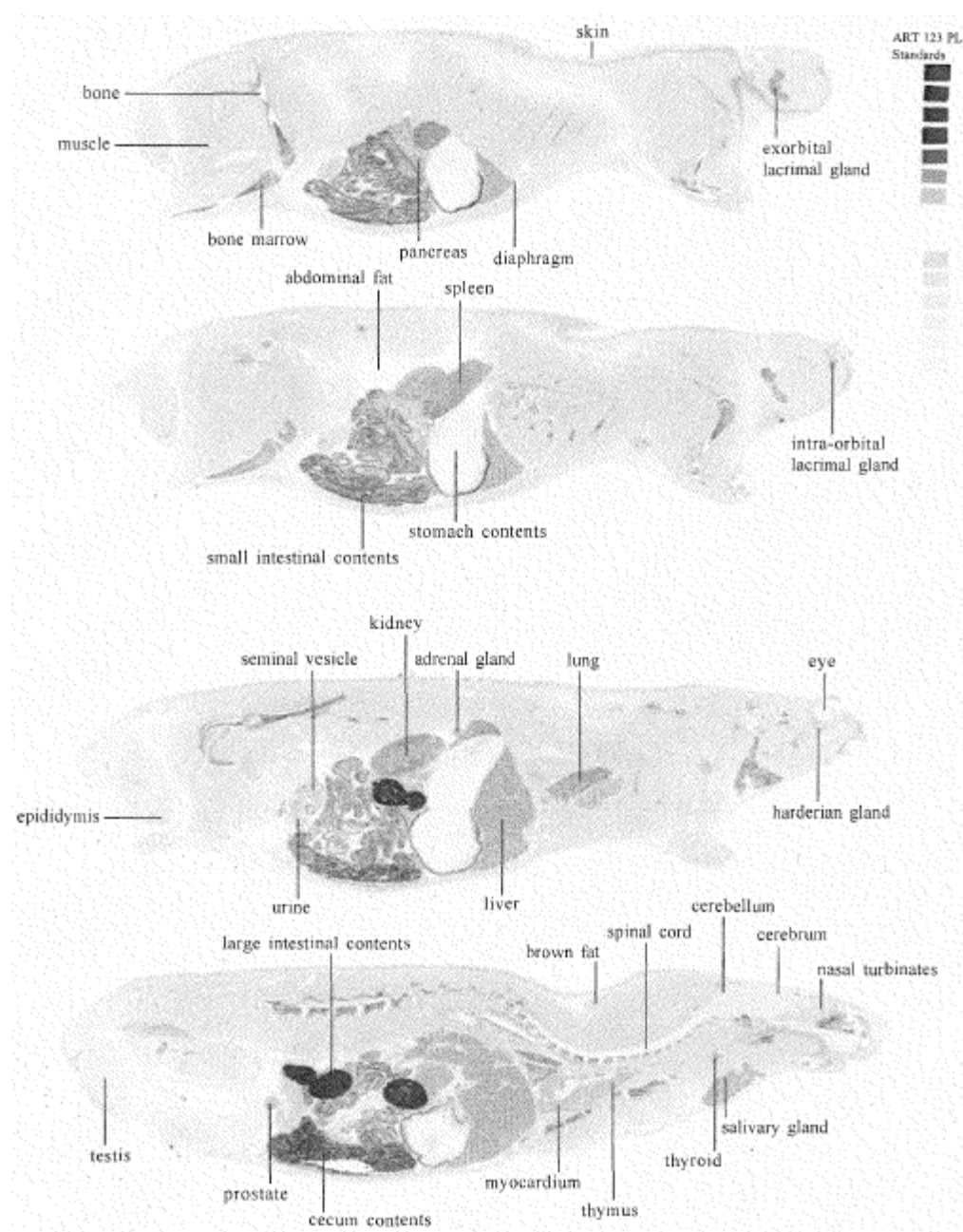


Best Available Copy

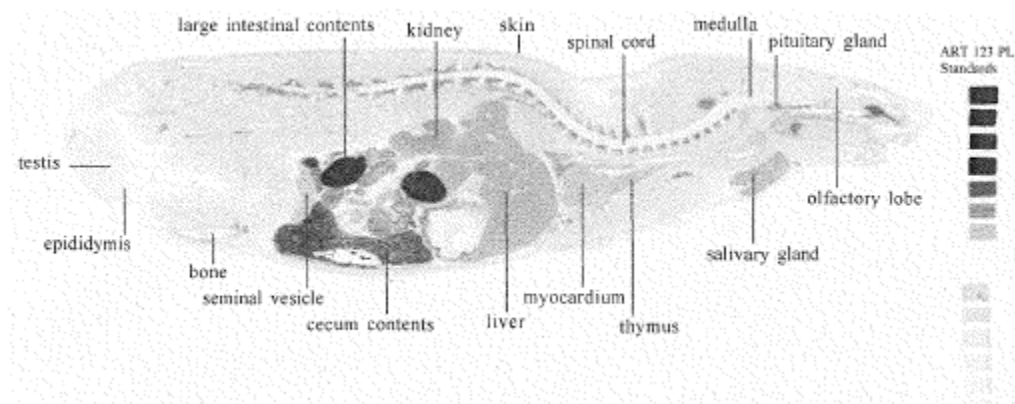


Best Available
Copy

Figure 30: Whole-body autoradiograph of male rat (C24123) administered a single intravenous dose of 3H-PR-171



Best Available Copy



Best Available Copy

(Figures and table excerpted from Applicant's package)

***In vitro* protein binding of PR-171 in human, rat and monkey plasma using equilibrium dialysis (Study TR-0049-171)**

Key Study Findings

- Binding of PR-171 with plasma proteins is high (93-97%) in all 3 species.
- Degradation of PR-171 in plasma is high (39-66%) in all 3 species.
- PR-171 instability could compromise binding studies.

METHODS

Protein binding of 0.5 and 5 μ M PR-171 with rat, monkey, and human plasma was determined *in vitro* following 20 hrs of equilibrium dialysis.

RESULTS

Binding of PR-171 to plasma proteins is similar across all three species between 93% and 97% (Table 1). There were not significant differences in the amount of protein binding at the 2 concentrations tested, suggesting that saturation had been reached. PR-171 degradation over the 20 hr period was significant with 39% in monkey, 42% in human, and 66% in rat. Thus, plasma protein binding data for PR-171 could be compromised by instability and degradation.

Table 17: Percentage of bound carfilzomib in rat, monkey and human plasma

Compound	Species	Concentration (μM)	Bound% (Mean ± S.D.)	Replicates
PR-171	Rat	5	94.6 ± 0.2	3
		0.5	98.6 ± 0.2	3
PR-171	Monkey	5	93.5 ± 0.1	3
		0.5	93.4 ± 0.6	3
PR-171	Human	5	94.0 ± 0.3	3
		0.5	94.8 ± 0.3	3
Propranolol	Human	5	78.4 ± 0.3	3
		0.5	78.8 ± 2.1	3
Warfarin	Human	5	99.38 ± 0.06	3
		0.5	99.42 ± 0.01	3

Table 18: Percentage of remaining carfilzomib after 20 hour incubation in human, monkey and rat plasma

Species	Concentration (μM)	Remaining%
Rat	5	35.4
	0.5	32.0
Monkey	5	62.5
	0.5	59.4
Human	5	60.0
	0.5	56.4

(Tables excerpted from Applicant's package)

***In vitro* Protein Binding of Carfilzomib in Rat, Monkey and Human Plasma Using Rapid Equilibrium Dialysis (RED) Device (Study TR-0457-171)**

Key Study Findings

- Binding of PR-171 with plasma proteins is high (94-97%) in all 3 species.
- PR-171 is stable using the RED device for all 3 species.

METHODS

Protein binding of 0.4 and 4 μM PR-171 with rat, monkey, and human plasma was determined *in vitro* following 6 hrs using a RED device.

RESULTS

Binding of PR-171 to plasma proteins is similar across all three species between 94% and 97% (Table 19). There were no significant differences in the amount of protein binding at the 2 concentrations tested, suggesting that saturation had been reached. PR-171 degradation over the 6 hr period was minor, between 0 and ≤ 10%, and PR-171

is stable under these conditions. Thus, the RED device is optimal for determining plasma protein binding of PR-171.

Table 19: Binding of carfilzomib to plasma proteins of rat, monkeys and human

Compound	Species	Concentration (μM)	Percent (%) Protein Bound ^a
PR-171	Rat	4	96.6 ± 0.1
		0.4	97.6 ± 0.1
PR-171	Monkey	4	94.4 ± 0.6
		0.4	94.4 ± 0.6
PR-171	Human	4	96.9 ± 0.1
		0.4	97.3 ± 0.3
Warfarin	Human	5	99.2 ± 0.0

a. Mean ± SD

Table 20: Stability of carfilzomib in rat, monkey and human plasma

Species	Concentration (μM)	Percent (%) Remaining
Rat	4	95.6
	0.4	89.8
Monkey	4	102.8
	0.4	92.3
Human	4	100.4
	0.4	107.5

(Tables excerpted from Applicant's package)

5.4 Metabolism

***In vitro* metabolic stability of PR-171 in human, monkey, rat and mouse liver microsomes and cytosols (Study TR-0033-171)**

Key Study Findings

- Very high hepatic clearance in human, rat, and mouse.
- High hepatic clearance in monkey.

- The predominant pathway of metabolism in human, rat, mouse, and monkey was P450-mediated. However hydrolysis was also a major pathway of metabolism in monkeys.

METHODS

In Vitro metabolic stability of PR-171 was determined using human, monkey, rat, and mouse liver microsomes. P450-mediated metabolism was determined using liver microsomes with NADPH. Hydrolysis-mediated metabolism was determined using liver cytosol and liver microsomes without NADPH.

RESULTS

The hepatic clearance of PR-171 was high in monkey (82%) and very high in human, rat, and mouse (93-98%). PR-171 is predominantly metabolized by P450 enzymes in all 4 species of liver microsomes (with NADPH). PR-171 is also metabolized by epoxide and/or peptidase hydrolysis in both liver microsomes (without NADPH) and liver cytosol in rat, monkey, and human, but very little in mouse. The major hydrolysis metabolite in monkey liver microsomes is the diol metabolite via epoxide hydrolysis.

Table 21: *In vitro* hepatic extraction ratio from liver microsomal incubation with NADPH

Species	Extraction ratio Re
Human	0.93
Monkey	0.82
Rat	0.94
Mouse	0.98

Table 22: *In vitro* intrinsic clearance (CL_{int} (μL/min/mg proteins)) from liver cytosol incubation and liver microsome incubation with or without NADPH

	Mouse	Rat	Monkey	Human
Liver microsomes with NADPH	989	499	138	302
Liver microsomes without NADPH	0.70	5.6	69	14
Liver cytosol with no co-factor	0.68	3.7	5.3	4.0

(Tables excerpted from Applicant's package)

***In Vitro* Metabolism of [3H]-PR-171 by Rat, Monkey, and Human Hepatocytes (Study TR-0040-171)**

Key Study Findings

- 6 major radiolabeled metabolites were identified in all 3 species.
- Metabolite profiles were similar in rat, monkey, and human.

- Rat: M5 ≈ M4 > M3 ≈ M6 ≈ M2 > M1
- Monkey: M5 > M4 ≈ M6 > M2 > M3 > M1
- Human: M5 > M4 > M2 > M3 ≈ M6 > M1
- No unique human metabolites.
- PR-171 does not alter hepatocyte Phase I and Phase II metabolic activities.

METHODS

In Vitro metabolic activity and metabolism of 3 and 10 μM ^3H -PR-171 were determined using human, monkey, and rat primary hepatocytes. For metabolic activity assessments, integrated Phase I (7-ethoxycoumarin O-deethylase) and Phase II (sulfation and glucuronidation) activities were measured after 0, 30, and 120 min incubation with 7-ethoxycoumarin. For metabolism analysis, PR-171 was incubated with hepatocytes for 0, 30, 60, and 120 min and analyzed by HPLC, LC/MS, and LC/MS/MS.

RESULTS

Phase I and II metabolic activities of primary hepatocytes were reported to be in agreement with historical values despite the presence of PR-171 (Table 4-1), indicating that hepatocyte metabolic activities are not adversely affected by PR-171. The major metabolite in all species was the PR-171 diol (M5), which was highest at 30 min and rapidly metabolizes into M6. The second most abundant metabolite, M4, was not identified but continues to increase at 120 min. Metabolites M1 and M2 are tyrosine and phenylalanine, respectively. There were no human-specific metabolites. Expected metabolites resulting from amide bond cleavage would not be radiolabeled and could not be detected.

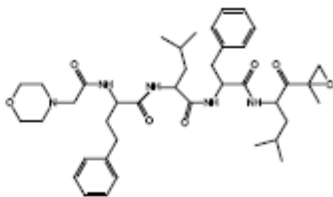
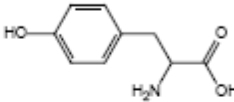
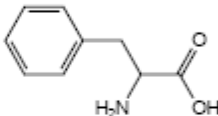
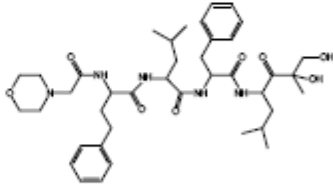
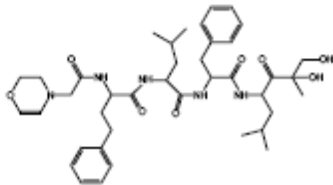
Reviewer note: Metabolite numbering in this study does not correlate with number of metabolites in other nonclinical pharmacokinetic studies. E.g., the major metabolite M5 (diol) is called M16 in other studies.

Table 23: Phase I and II metabolic activities of primary hepatocytes

Incubation Start Time (minute)	7-Hydroxycoumarin	7-Hydroxycoumarin Glucuronide	7-Hydroxycoumarin Sulfate	Total
pmol/minute/1 x 10 ⁶ hepatocytes				
Rat				
0	5.91	28.2	256	290
30	6.99	36.8	312	356
60	6.17	31.2	313	350
120	4.65	17.0	276	298
Monkey				
0	418	885	138	1440
30	383	844	127	1350
60	373	687	103	1160
120	467	815	115	1400
Human				
0	12.8	43.1	18.7	74.6
30	11.0	41.9	20.7	73.6
60	10.3	34.5	18.1	62.9
120	7.97	27.3	18.8	54.1

Note: 7-Ethoxycoumarin was added after 0, 30, 60, and 120 minutes of incubation and incubated another 30 minutes before the reaction was stopped.

Figure 31: Putative structures of metabolites found in hepatocytes

Component ^a	[M + H] ⁺	Putative Structure
Parent (PR-171)	720.5	
M1 (tyrosine)	182	
M2 (phenylalanine)	166	
M3	Undetermined	Structure not determined
M4	319.2	Structure not determined
M5	738.2	
M6	736.4	

a All metabolites were detected in rat, monkey, and human.

Best Available Copy

Table 24: Percentage of carfilzomib metabolites in rat hepatocytes

Incubation Time (Minute)	Percent of Profiled Radioactivity ^a						
	M1	M2	M3	M4	M5	[³ H]-PR-171	M6
	<u>3 μM [³H]-PR-171</u>						
30	ND	7.00	3.18	22.05	22.82	1.67	7.46
60	2.80	9.54	4.52	24.57	6.45	2.47	5.34
120	6.65	10.00	6.94	23.79	6.30	1.85 ^b	1.12 ^b
	<u>10 μM [³H]-PR-171</u>						
30	2.70	6.62	4.97	23.24	31.17	3.63	5.56
60	5.75	9.19	7.30	25.53	23.43	0.70	6.66
120	3.65	7.19	10.45	33.37	8.46	1.09 ^b	4.87

ND: Not detected or below the limit of quantitation (BLQ).

a: N = 2, unless otherwise noted.

b: Single value, second value was ND or BLQ.

Table 25: Percentage of carfilzomib metabolites in monkey hepatocytes

Incubation Time (Minute)	Percent of Profiled Radioactivity ^a						
	M1	M2	M3	M4	M5	[³ H]-PR-171	M6
	<u>3 μM [³H]-PR-171</u>						
30	0.96	4.40	1.40	8.19	34.31	5.35	7.51
60	1.49	7.49	1.62	11.81	14.95	6.42	6.07
120	1.52	10.96	2.37	6.79	4.11	6.95	ND
	<u>10 μM [³H]-PR-171</u>						
30	ND	2.53	1.51	7.44	54.47	6.43	8.22
60	ND	3.11	2.03	8.79	42.29	6.81	10.59
120 ^b	1.20	3.05	1.42	10.25	30.75	9.16	10.25

ND: Not detected or below the limit of quantitation (BLQ).

a: N = 2, unless otherwise noted.

b: Single values, second values were ND or BLQ.

Best Available Copy

Table 26: Percentage of carfilzomib metabolites in human hepatocytes

Table 4
Percent of profiled radioactivity associated with 3 and 10 μ M [³H]-PR-171 and its metabolites after incubation with human hepatocytes for 30, 60, and 120 minutes

Incubation Time (Minute)	Percent of Profiled Radioactivity ^a						
	M1	M2	M3	M4	M5	[³ H]-PR-171	M6
	<u>3 μM [³H]-PR-171</u>						
30	ND	7.94	2.34	6.46	69.33	5.34	2.17
60	1.14	12.96	2.91	10.18	55.29	2.83	2.95
120	1.96	15.73	4.82	18.29	35.02	2.77	5.05
	<u>10 μM [³H]-PR-171</u>						
30	0.90 ^b	9.41	3.11	7.08	68.09	4.91	1.46
60	1.10 ^b	10.99	4.14	12.83	57.96	4.61	1.19 ^b
120	1.77 ^b	13.03	5.90	16.93	48.53	1.67	3.70 ^b

ND: Not detected or below the limit of quantitation (BLQ).

a: N = 2, unless otherwise noted.

b: Single value, second value was ND or BLQ.

(Tables and figure excerpted from Applicant's package)

In Vitro Metabolism of Carfilzomib in Rat Blood and Tissue Homogenates (Study TR-0184-171)

Key Study Findings

- PR-171 is rapidly metabolized via peptidase cleavage in rat blood and liver, lung, and kidney tissue homogenates.

- M14 > M15, no M16

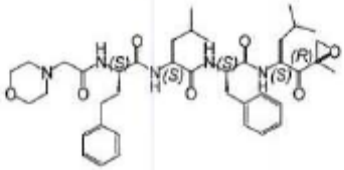
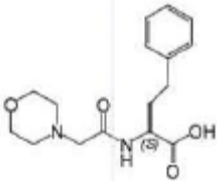
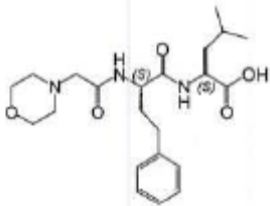
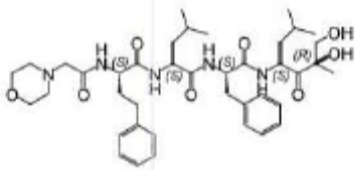
METHODS

In Vitro metabolism of PR-171 (1 µg/mL) was determined in rat blood and liver, lung, and kidney tissue homogenates after 2, 10, 20, 30, 60, and 90 min incubations. LC/MS/MS was used for identification of PR-171 and 3 metabolites (M14, M15, and M16), which are different from the metabolites identified after hepatocytes *in vitro* metabolism (study #TR-0040-171).

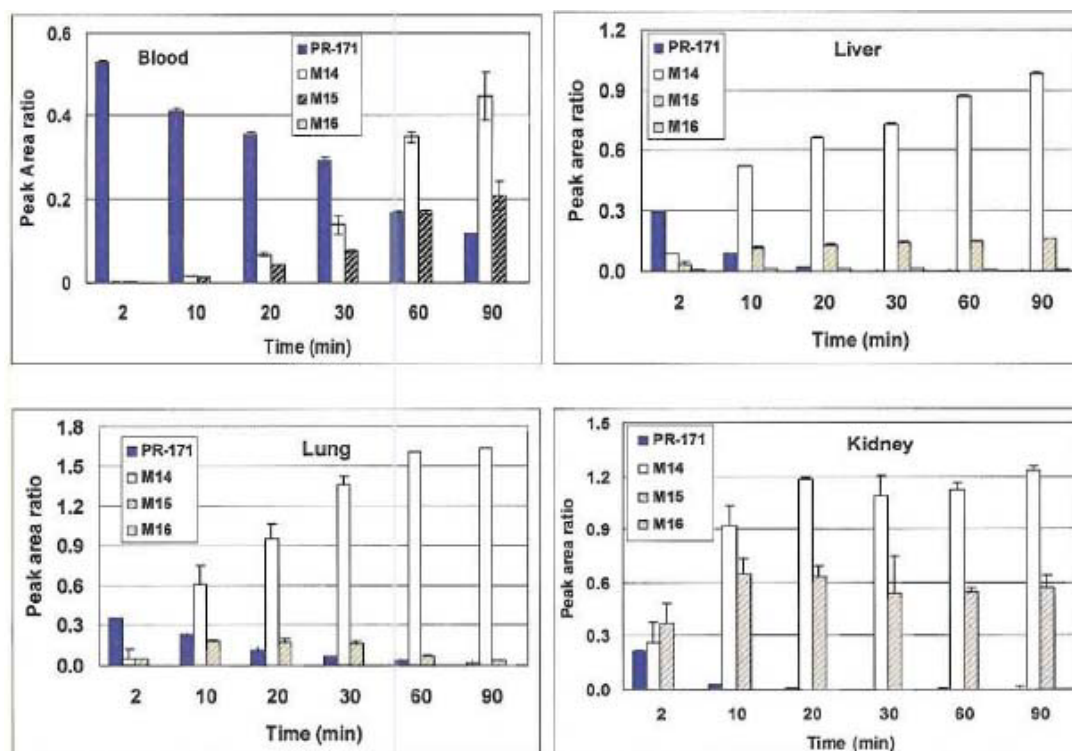
RESULTS

M14 and M15 are due to peptidase cleavage and were identified in all rat blood and tissue homogenates with M14 being more abundant than M15 in all tissues. M16 is a diol resultant from epoxide hydrolysis, but was not detected in this study. PR-171 was rapidly metabolized in liver and kidney ($t_{1/2}$ = 4min), whereas metabolism in blood was 10X slower ($t_{1/2}$ = 39 min). *It is unclear why the predominant metabolites resultant from hepatocytes metabolism, M4 and M5 (study #TR-0040-171), were not monitored in this study despite the investigation of liver homogenates.*

Table 27: Structures of carfilzomib and three metabolites

	Structure	Molecular weight
Carfilzomib	 The structure of Carfilzomib is a complex molecule featuring a central benzyl group connected to a morpholine ring via a carbonyl group. This is further linked to a chain containing several amide bonds, a cyclohexane ring, and a terminal epoxide group. Stereochemical configurations are indicated as (S), (R), and (S).	719.40
M14	 The structure of M14 is a metabolite consisting of a morpholine ring connected to a carbonyl group, which is further linked to a chain containing an amide bond, a benzyl group, and a terminal carboxylic acid group. The stereochemical configuration is indicated as (S).	306.36
M15	 The structure of M15 is a metabolite consisting of a morpholine ring connected to a carbonyl group, which is further linked to a chain containing an amide bond, a benzyl group, and a terminal carboxylic acid group. The stereochemical configurations are indicated as (S) and (S).	419.51
M16	 The structure of M16 is a metabolite consisting of a morpholine ring connected to a carbonyl group, which is further linked to a chain containing several amide bonds, a cyclohexane ring, and a terminal dihydroxy group. Stereochemical configurations are indicated as (S), (S), (S), and (R).	737.93

Best Available Copy

Figure 32: Formation of carfilzomib metabolites in rat blood and tissue homogenates

Best Available Copy

Table 28: Half-lives for *in vitro* metabolism of carfilzomib in rat blood and tissue homogenates

Tissue	Half-life (min)
Blood	39
Liver	4
Lung	18
Kidney	4

(Tables and figure excerpted from Applicant's package)

In Vitro Metabolism of Carfilzomib in Human Hepatocytes (Study TR-0212-171)**Key Study Findings**

- M16, the PR-171 diol resulting from epoxide hydrolysis, is the major metabolite in human hepatocytes.
 - M16 >> M14 > M11 ≈ M15
- PR-171 is not significantly metabolized by human P450 enzymes *in vitro*.

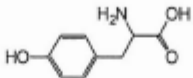
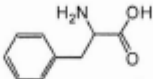
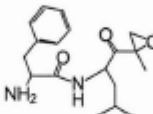
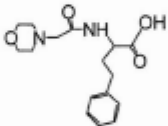
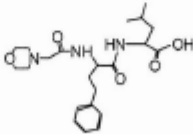
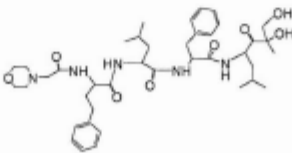
METHODS

In Vitro metabolism of PR-171 (10 μ M) was determined in human hepatocytes after a 2 hr incubation followed by LC/MS/MS. The metabolic stability of PR-171 (1 μ M) in human hepatocytes after 0, 15, 30, 60, and 120 minutes was determined in the absence and presence of 6 cytochrome P450 inhibitors targeting CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A.

RESULTS

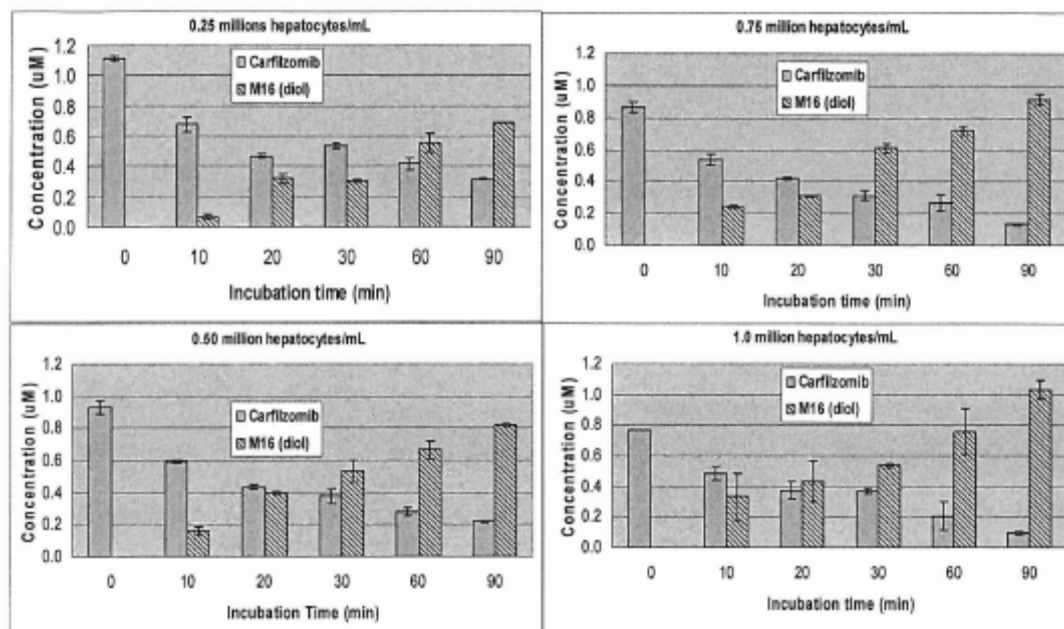
The most prominent metabolite was the diol M16 resultant from epoxide hydrolysis. The peptidase cleavage metabolites M14 and M15 were in low abundance. The phenylalanine moiety metabolite M11 (also designated M4 in study #TR-0040-171) was also identified. P450-dependent metabolites were not identified. The presence of P450 inhibitors did not alter the rate of carfilzomib metabolism, indicating that P450s do not play a significant role.

Table 29: Metabolites of carfilzomib identified from incubation with cryopreserved human hepatocytes

Metabolites	Structure	[M+H] ⁺	Rt (min)
M5		182	2.7
M7		166	4.5
M11		309	18.3
M14		307	9.6
M15		420	13.9
M16		738	20.1

Best Available Copy

Figure 33: Disappearance of carfilzomib and formation of the diol metabolite M16 in cryopreserved human hepatocytes



Best Available Copy

Table 30: Effect of P450 inhibitors on the rate of carfilzomib metabolism

Inhibitor	Inhibition on metabolism of probe substrate			Carfilzomib metabolism rate constant k (mean \pm SD, min ⁻¹)	
	P450 isozyme	Metabolism of probe substrate	Inhibition (%)	Without inhibitor	With inhibitor
Fyrafilline (30 μ M)	CYP1A2	phenacetin-O-deethylation	79	0.015 \pm 0.0003	0.016 \pm 0.002
Montelukast (30 μ M)	CYP2C8	Amodiaquine N-deethylation	83	0.018 \pm 0.001	0.014 \pm 0.001
Sulfaphenazole (10 μ M)	CYP2C9	4'-hydroxy-diclofenac	75	0.0097 \pm 0.0015	0.0073 \pm 0.0012
(+)-N-3-benzylirivanol (10 μ M)	CYP2C19	S-mephenytoin-4'-hydroxylation	78	0.013 \pm 0.0004	0.016 \pm 0.002
Quinidine (10 μ M)	CYP2D6	Dextromethorphan O-demethylation	97	0.0093 \pm 0.0001	0.013 \pm 0.001
Ketoconazole (10 μ M)	CYP3A	Midazolam 1'-hydroxylation	91	0.010 \pm 0.002	0.012 \pm 0.002

(Tables and figure excerpted from Applicant's package)

Searching and Identification of Carfilzomib (PR-171) Metabolites in Rat Plasma, Bile, and Urine and in Monkey Plasma (Study TR-0271-171)

Key Study Findings

- The principle pathway of metabolism for PR-171 is via peptidase cleavage and epoxide hydrolysis.
- The major *in vivo* metabolites in rat and monkey plasma are M14 and M16.

- Rat: M14 > M16
- Monkey: M16 > M14
- The major *in vivo* metabolite in rat urine and bile is M14.
 - Urine: M14 >> M16 > M18 > M12 ≈ M20 > M15 ≈ M10 > M8 > M9 > M11 ≈ M24 ≈ M9 ≈ M2 ≈ M19
 - Bile: M14 > M16 > M18 > M20 > M19 > M24 > M15 > M2 ≈ M8 ≈ M9 ≈ M10 ≈ M12
- Profiles of major PR-171 metabolites in monkey and rat plasma and urine are similar to those of human plasma and urine (see section 2.2.6 Comments on Human Metabolites).

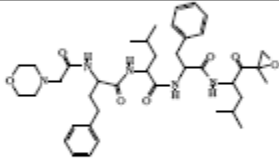
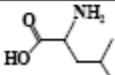
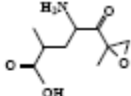
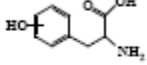
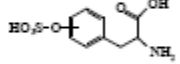
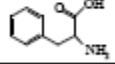
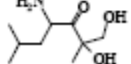
METHODS

In vivo metabolites of PR-171 were identified in monkey plasma (0.5, 1, and 2 mg/kg) and rat plasma (1, 2, and 4 mg/kg), bile (2 mg/kg), and urine (2 mg/kg) using LC/MS and quantified using LC/MS/MS.

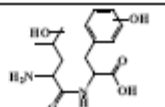
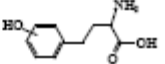
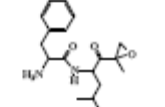
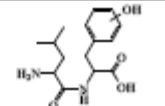
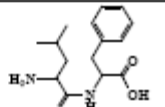
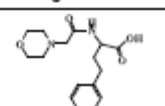
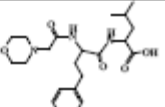
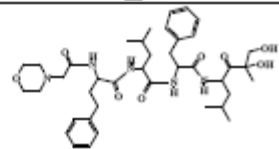
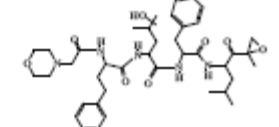
RESULTS

M14 (resultant from peptidase cleavage) was the predominant metabolite in rat urine and bile. In monkey and rat plasma, the predominant metabolites are M14 and M16 (diol resultant from hydrolysis). *These findings are consistent with human plasma and urine (Study #TR-0077-171).*

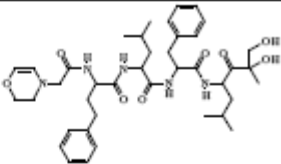
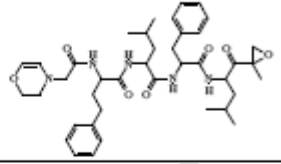
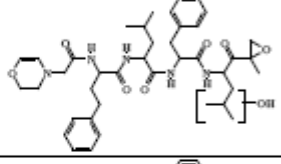
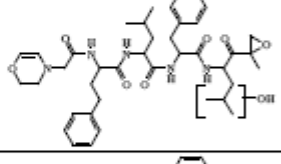
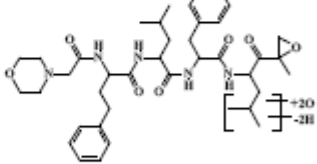
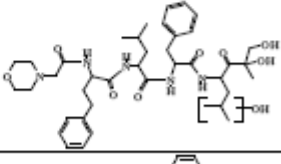
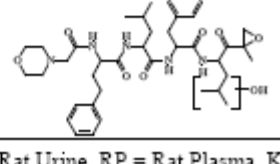
Table 31: Carfilzomib metabolites identified by LC/MS

Metabolite Code	Proposed Structure	MW (Da)	R _t (min)	Sources ^a	Human Sample Sources ^b
Carfilzomib		719	52.3	RP, RU, RB	HU, HP
M1		131	10.4	RP, KP, RU, RB	HU, HP
M2		201	13.1	RP, KP, RU, RB	HU, HP
M5		181	13.1	RP, KP, RU, RB	HU, HP
M6		261	15.9	ND	HU
M7		165	15.9	RP, KP, RU	HU, HP
M8		189	17.2	RP, KP, RU, RB	HU

Best Available Copy

Metabolite Code	Proposed Structure	MW (Da)	R _t (min)	Sources ^a	Human Sample Sources ^b
M9		310	16.7	RP, KP, RB	HU, HP
M10		195	19.0	RU	HU
M11		318	20.2	RP, KP, RU, RB	HU, HP
M12		294	21.2	RP, KP, RU, RB	HU
M13		278	25.1	KP, RB	HP
M14		306	25.9	RP, KP, RU, RB	HU, HP
M15		419	33.0	RP, KP, RU, RB	HU, HP
M16		737	43.8	RP, KP, RU, RB	HU, HP
M17		735	51.1	RP, RU ^c	HU

Best Available Copy

Metabolite Code	Proposed Structure	MW (Da)	R_t (min)	Sources ^a	Human Sample Sources ^b
M18		735	51.1	RU, RB	ND
M19		717	59.3	RB	ND
M20		733	46.2	RU, RB	ND
M21		733	50.1	RU, RB	ND
M22		749	42.5	RB	ND
M23		753	39.1	RB	ND
M24		735	44.4	RU	ND

^a RB=Rat Bile, RU= Rat Urine, RP = Rat Plasma, KP=Monkey Plasma.

^b HU = Human Urine, HP = Human Plasma; data were obtained during the previous study².

(Table excerpted from Applicant's package)

Best Available Copy

5.5 Excretion

Pharmacokinetics and Renal and Biliary Excretion of Carfilzomib (PR-171) and Its Metabolites in Sprague Dawley Rats Following a Single Intravenous Bolus Administration (Study TR-0294-171)

Key Study Findings

- PR-171 is rapidly (majority < 4 hrs) cleared largely extrahepatically, with 30% being cleared via biliary excretion and 30% via the kidneys by 24 hours post-dose, and with the majority being excreted within the first 4 hours.
 - Renal excretion: M14 >> M15 ≈ M16 > PR-171
 - Biliary excretion: M15 ≈ M14 > M16 >> PR-171

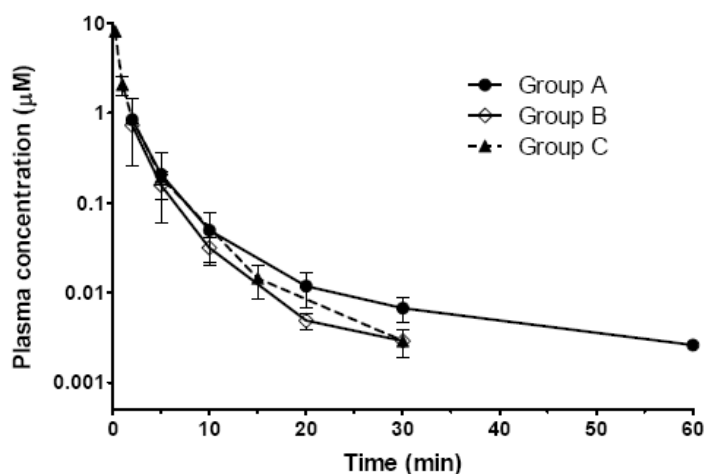
METHODS

Renal and biliary excretion and pharmacokinetics of PR-171 (2 mg/kg IV bolus) and its major metabolites were examined in bile duct-cannulated Sprague Dawley rats. Blood samples were collected at pre-dose, 2, 5, 15, 30, 60, 120, and 240 min post-dose (groups A & B) or pre-dose, 0.2, 1, 2, 5, 15, 30, 60, and 120 min post-dose (group C). Urine samples were collected at 0-4, 4-8, and 8-24 hr post-dose. Cumulative bile samples were collected at 0-4 and 4-8 hr post-dose in cannulated animals.

RESULTS

Plasma Pharmacokinetics: Immediate sampling (Group C) resulted in a 10-fold higher C_{max} , even though the PK profiles are similar, due to the significantly higher exposures before the 2 min time point in Groups A and B. Consequently, plasma clearance is overestimated in Groups A and B. Plasma clearance in Group C is 5-fold higher than rat hepatic blood flow which indicates that PR-171 is largely eliminated extrahepatically.

Figure 34: Plasma concentration-time profiles of carfilzomib after a single intravenous bolus administration of 2 mg/kg in rats



^a First sampling time: 2 min (Groups A and B); 0.2–0.3 min (Group C)

Table 32: Pharmacokinetic parameters of carfilzomib following a single intravenous bolus administration of 2 mg/kg in male rats

Group	A	B	C
First sampling time (min)	2	2	0.2–0.3
T _{1/2} (min)	12 ± 6	4 ± 2	4 ± 2
T _{max} (min)	2.60 ± 1.34	2.00 ± 0.00	0.23 ± 0.06
C _{max} (μM)	0.870 ± 0.559	0.733 ± 0.062	8.11 ± 0.98
C ₀ (μM)	2.85 ± 0.53	2.12 ± 0.46	11.9 ± 2.0
AUC _{last} (min*μmol/L)	6.39 ± 4.10	4.86 ± 0.42	10.8 ± 1.5
AUC _{inf} (min*μmol/L)	6.44 ± 4.12	4.89 ± 0.43	10.8 ± 1.5
AUC _{inf} /D(min*kg*μmol/L/μmol)	2.32 ± 1.48	1.76 ± 0.15	3.89 ± 0.54
CL (mL/min/kg)	567 ± 275	572 ± 49	261 ± 37
V _{ss} (L/kg)	2.20 ± 1.96	1.28 ± 0.29	0.40 ± 0.10

Renal Excretion: The majority was excreted in the first 4 hours. Renal excretion accounted for 26% to 30% of the PR-171 dose. Very little (<1%) was excreted as parent compound, whereas the predominant form was the M14 metabolite.

Table 33: Renal excretion of carfilzomib and its metabolites in rats after an intravenous bolus administration of 2 mg/kg

Analyte	Time (hr)	% Dose					Mean ± SD ^a
		Rat C1	Rat C2	Rat C3	Rat C4	Rat C5	
Carfilzomib	0–4	0.0446	0.0143	0.0596	0.0298	0.00891	0.0315 ± 0.0210
	4–24	0.00257	0.0118	0.0190	0.00419	0.00152	0.00781 ± 0.00743
	0–24	0.0472	0.0262	0.0786	0.0340	0.0104	0.0393 ± 0.0257
PR-389/M14	0–4	30.5	11.2	24.4	22.7	4.20	18.6 ± 10.6
	4–24	5.57	8.14	10.0	7.72	4.28	7.15 ± 2.26
	0–24	36.0	19.4	34.4	30.4	8.48	25.7 ± 11.6
PR-413/M15	0–4	0.292	0.0787	0.311	0.0981	0.0411	0.164 ± 0.127
	4–24	0.0719	0.0491	0.131	0.0578	0.0523	0.0724 ± 0.0338
	0–24	0.364	0.128	0.441	0.156	0.0934	0.236 ± 0.156
PR-519/M16	0–4	0.214	0.0456	0.251	0.0957	0.0282	0.127 ± 0.100
	4–24	0.0158	0.0248	0.117	0.0167	0.00978	0.0367 ± 0.0449
	0–24	0.230	0.0703	0.368	0.112	0.0380	0.164 ± 0.135
Total	0–24	36.6	19.6	35.3	30.7	8.62	26.2 ± 11.9

^a BLQ values were given a value of zero for calculation of mean and standard deviation.

^b BLQ, below limit of quantitation.

Biliary Excretion: The first 4 hours accounted for 98% of what was excreted in the bile. Bile excretion accounted for 30% of the PR-171 dose. Very little (<1%) was excreted as parent compound, whereas the predominant forms were the metabolites M14 and M15.

Table 34: Biliary excretion of carfilzomib and its metabolites in rats after an intravenous bolus administration of 2 mg/kg

Analyte	Time (hr)	% Dose					Mean \pm SD
		Rat C1	Rat C2	Rat C3	Rat C4	Rat C5	
Carfilzomib	0-4	0.00448	0.0490	0.0158	0.0128	0.00295	0.0170 \pm 0.0187
	4-8	BLQ ^a	BLQ	BLQ	BLQ	BLQ	BLQ
	0-8	0.00448	0.0490	0.0158	0.0128	0.00295	0.0170 \pm 0.0187
PR-389/M14	0-4	13.9	9.83	13.5	11.3	15.7	12.9 \pm 2.3
	4-8	0.293	0.254	0.340	0.0580	0.204	0.230 \pm 0.108
	0-8	14.2	10.1	13.8	11.4	15.9	13.1 \pm 2.3
PR-413/M15	0-4	13.4	14.1	14.1	13.3	18.4	14.7 \pm 2.1
	4-8	0.0377	0.0681	0.0595	0.172	0.0401	0.0756 \pm 0.0556
	0-8	13.5	14.2	14.1	13.4	18.4	14.7 \pm 2.1
PR-519/M16	0-4	0.967	2.04	8.07	1.63	0.493	2.64 \pm 3.09
	4-8	0.0102	0.00463	0.0256	0.0214	0.00413	0.0132 \pm 0.0098
	0-8	0.978	2.04	8.09	1.65	0.497	2.65 \pm 3.10
Total	0-8	28.7	26.4	36.1	26.5	34.8	30.5 \pm 4.6

^a BLQ, below limit of quantitation.*(Tables and figure excerpted from Applicant's package)*

5.6 Pharmacokinetic Drug Interactions

Inhibition of cytochrome P4501A2, cytochrome P4502C19, cytochrome P4502D6 and cytochrome P4503A4 catalytic activities by PRX-002 (Study TR-0034-171)

Key Study Findings

- PRX-002 does not inhibit recombinant human CYP1A2, CYP2C9, CYP2C19, and CYP2D6 activities.
- PRX-002 weakly inhibits recombinant human CYP3A activity (IC_{50} = 3 to 4 μ M)

METHODS

In Vitro inhibition of recombinant human cytochrome P450s (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A) by PRX-002 was determined in insect cell microsomes.

RESULTS

PRX-002 weakly inhibits CYP3A activities with an IC_{50} value of 3 to 4 μ M, but does not inhibit any of the other enzymes examined.

Table 35: *In vitro* CYP P450 inhibition by carfilzomib

CYP P450	IC50 (μM)			
	PRX-002		Positive Control*	
1A2	>10	>10	1.093	1.082
2C9	>10	>10	0.269	0.279
2C19	>10	>10	5.525	6.045
2D6	>10	>10	0.006	0.006
3A4 (BFC)	2.95	3.02	0.017	0.017
3A4 (DBF)	3.94	3.46	0.001	0.001

Data represent duplicate determinations and are in units of micromolar.

*- The positive controls used were: furafylline (CYP1A2), sulfaphenazole (CYP2C9), tranylcypromine (CYP2C19), quinidine (CYP2D6) and ketoconazole (CYP3A4).

(Table excerpted from Applicant's package)

The Effect of PR-171 on Hepatic Microsomal Levels of Total Cytochrome P450 and Selected Enzyme Activities in Male and Female Sprague Dawley Rats Following Repeat IV Bolus Administration at Doses of 0, 2, 4, and 6 mg/kg/day for 18 and 19 Days (Study TR-0042-171)

Key Study Findings

- Drug-related decreases in CYP2C activity in both sexes at 1 hr post-dose and in males at 24 hr post-dose, but 2- to 3-fold increase in females at 24 hr post-dose.
- Drug-related decreases in CYP3A activity in both sexes 24 hr post-dose, but not at 1 hr post-dose.
- No drug-related effects on CYP1A and CYP2D activity or on hepatic microsomal protein yield and total cytochrome P450 content.

METHODS

In vivo effects of PR-171 on hepatic microsomal protein and cytochrome P450 enzymes were determined using microsomes isolated on Day 18 (1 hr post-dose) or Day 19 (24 hr post-dose) from SD rats treated with 0, 2, 4, and 6 mg/kg PR-171 IV bolus on Days 0-4 and 14-18. Lethality occurred at 4 and 6 mg/kg, resulting in no Day 18 samples at those doses.

RESULTS

No drug-related effects on hepatic microsomal protein yield or total cytochrome P450 content. PR-171-mediated, dose-dependent decreases in CYP2C activity (↓47-55%, ♂ & ♀) were seen on Day 18 and dose-dependent decreases (up to 94%) in males on Day 19, in contrast to females with up to a 3-fold increase (Day 19). Dose-dependent (up to 84%) decreases in CYP3A activity were seen in males, and to a lesser degree in females, on Day 19. There were no significant changes in CYP3A activity in either sex on Day 18. No drug-related effects on CYP1A and CYP2D activities.

Table 36: CYP2C activities in rats following intravenous bolus administration of carfilzomib

Dose Level [mg/kg/day]	2 α -Hydroxytestosterone Production					
	(nmol/min/mg protein)		(nmol/min/nmol P450)		(nmol/min/g liver)	
	Day 18	Day 19	Day 18	Day 19	Day 18	Day 19
Male						
0	3.38	3.44	3.03	2.67	26.4	25.9
2	1.51 (45%)	1.74 (51%)	1.45 (48%)	1.60 (60%)	15.3 (58%)	18.6 (72%)
4	NA	0.524 (15%)	NA	0.750 (28%)	NA	4.48 (17%)
6	NA	0.194 (6%)	NA	0.237 (9%)	NA	1.27 (5%)
Female						
0	0.073	0.029	0.084	0.036	0.661	0.212
2	0.039 (53%)	0.081 (279%)	0.046 (55%)	0.107 (297%)	0.245 (37%)	0.518 (244%)
4	NA	0.086 (297%)	NA	0.101 (281%)	NA	0.429 (202%)
6	NA	0.025 (86%)	NA	0.036 (100%)	NA	0.117 (55%)

Notes: Values are the mean of two determinations from a single pooled sample. Data in the parentheses are the percentage of the corresponding vehicle control.
NA = Not Applicable.

Table 37: CYP3A inhibition in rats following intravenous bolus administration of carfilzomib

Table 4 Hepatic Microsomal Testosterone 6 β -Hydroxylase Activities (CYP3A) in Male and Female Rats After Repeat IV Bolus Administration of PR-171 at Doses of 0 (control), 2, 4 and 6 mg/kg/day.

Dose Level [mg/kg/day]	6 β -Hydroxytestosterone Production					
	(nmol/min/mg protein)		(nmol/min/nmol P450)		(nmol/min/g liver)	
	Day 18	Day 19	Day 18	Day 19	Day 18	Day 19
Male						
0	1.15	1.41	1.03	1.09	9.03	10.6
2	1.10 (96%)	0.949 (67%)	1.05 (102%)	0.872 (80%)	11.1 (123%)	10.2 (96%)
4	NA	0.580 (41%)	NA	0.831 (76%)	NA	4.96 (47%)
6	NA	0.227 (16%)	NA	0.278 (26%)	NA	1.49 (14%)
Female						
0	0.118	0.143	0.136	0.176	1.07	1.05
2	0.107 (91%)	0.093 (65%)	0.127 (93%)	0.123 (70%)	0.674 (63%)	0.597 (57%)
4	NA	0.105 (73%)	NA	0.124 (70%)	NA	0.526 (50%)
6	NA	0.104 (73%)	NA	0.146 (83%)	NA	0.481 (46%)

Notes: Values are the mean of two determinations from a single pooled sample. Data in the parentheses are the percentage of the corresponding vehicle control.
NA = Not Applicable.

(Tables excerpted from Applicant's package)

The Effect of PR-171 on Hepatic Microsomal Levels of Total Cytochrome P450 and Selected Enzyme Activities in Male and Female Sprague Dawley Rats Following Repeat IV Bolus Administration at Doses of 0, 0.5, 1, and 2 mg/kg/day for 19 and 28 Days (Study TR-0043-171)

Key Study Findings

- Drug-related decreases in CYP1A activity in both sexes (all doses) at 1 day post-dose, but not 10 days after dosing.
- No drug-related effects on CYP3A and CYP2C activity.
- Equivocal effects on hepatic microsomal protein yield and total cytochrome P450 content.

METHODS

In vivo effects of PR-171 on hepatic microsomal protein and cytochrome P450 enzymes were determined using microsomes isolated on Day 19 (1 day post-dose) and Day 28 (10 days post-dose) from Cynomolgus monkeys treated with 0, 0.5, 1, and 2 mg/kg PR-171 IV bolus on Days 0-4 and 14-18. Due to sample pooling, only 2-fold changes were considered significant. Severe toxicity and lethality occurred at 2 mg/kg, resulting in no drug administration on Days 14 – 18 for 2 mg/kg group.

RESULTS

A non-dose-dependent increase in protein yield and drop in total cytochrome P450 activity was observed in males at 1 mg/kg, but with unclear biological significance due to lack of dose-dependency and changes in opposite directions. Decreases in CYP1A activity (↓23-72%) were seen in both sexes (all doses) at 1 day post-dose, but lacked clear dose dependency and were not seen at 10 days post-dose. No drug-related effects on CYP3A and CYP2C activities.

Table 38: Microsomal protein yields and total cytochrome P450 content in Cynomolgus monkeys after intravenous bolus administration of carfilzomib

Dose Level [mg/kg/day]	Protein Yield (mg/g liver weight)		Total Cytochrome P450 (nmol/mg protein)			
	Day 19	Day 28	Day 19	Day 28	Day 19	Day 28
Male						
0	6.33	10.0	2.44	1.36	15.4	13.6
0.5	8.64 (136%)	14.6 (146%)	1.68 (69%)	1.46 (107%)	14.5 (94%)	21.3 (157%)
1	13.4 (212%)	6.43 (64%)	1.17 (48%)	1.36 (100%)	15.6 (101%)	8.80 (65%)
2	NA	11.5 (115%)	NA	1.64 (121%)	NA	18.9 (139%)
Female						
0	8.77	14.3	1.68	1.50	14.7	21.4
0.5	12.3 (140%)	10.6 (74%)	1.31 (78%)	1.24 (83%)	16.1 (110%)	13.2 (62%)
1	9.01 (103%)	8.76 (61%)	1.70 (101%)	1.41 (94%)	15.3 (104%)	12.4 (58%)
2	NA	10.2 (71%)	NA	1.61 (107%)	NA	16.4 (77%)

Notes: Values are the mean of two determinations from a single pool sample. Data in the parentheses are the percentage of the corresponding vehicle control.
NA = Not Applicable.

Table 39: Microsomal CYP1A activity in Cynomolgus monkeys after intravenous bolus administration of carfilzomib

Dose Level [mg/kg/day]	Resorufin Production					
	(nmol/min/mg protein)		(nmol/min/nmol P450)		(nmol/min/g liver)	
	Day 19	Day 28	Day 19	Day 28	Day 19	Day 28
Male						
0	0.974	0.913	0.399	0.670	6.16	9.13
0.5	0.998 (102%)	0.699 (77%)	0.595 (149%)	0.480 (72%)	8.62 (140%)	10.2 (112%)
1	1.40 (144%)	0.252 (28%)	1.20 (301%)	0.185 (28%)	18.8 (305%)	1.62 (18%)
2	NA	0.419 (46%)	NA	0.255 (38%)	NA	4.82 (53%)
Female						
0	0.992	1.69	0.592	1.13	8.70	24.1
0.5	0.721 (73%)	0.755 (45%)	0.551 (93%)	0.608 (54%)	8.87 (102%)	8.00 (33%)
1	1.12 (113%)	0.921 (54%)	0.659 (111%)	0.652 (58%)	10.1 (116%)	8.07 (33%)
2	NA	0.691 (41%)	NA	0.429 (38%)	NA	7.05 (29%)

Notes: Values are the mean of two determinations from a single pooled sample. Data in the parentheses are the percentage of the corresponding vehicle control.

NA = Not Applicable.

(Tables excerpted from Applicant's package)

In Vitro Evaluation of Carfilzomib as an Inhibitor of Human Cytochrome P450 Enzymes (Study TR-0081-171)

Key Study Findings

- PR-171 is a direct, competitive, and irreversible/quasi-irreversible inhibitor of CYP3A4/5 enzymes with relatively moderate potency ($IC_{50} = 0.5\text{-}1.6\ \mu\text{M}$, $K_i = 1.7\ \mu\text{M}$, $K_{inact} = 0.1\ \text{min}^{-1}$).
- PR-171 is also a direct, but weak inhibitor of CYP1A2, CYP2C9, CYP2C19 and CYP2D6 ($IC_{50} > 10\ \mu\text{M}$).
- PR-171 does not inhibit CYP2C8.

METHODS

Direct and time-dependent inhibitory potential of PR-171 on P450 CYP enzyme activities (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5) was evaluated in human liver microsomes. Dependency of PR-171-mediated inhibition on the cofactor NADPH and dilution were also assessed. The ability of PR-171 to form a metabolite inhibitory complex (MIC) was explored with human liver microsomes with high levels of CYP3A4/5 activity.

RESULTS

PR-171 directly and time-dependently inhibits CYP3A4/5 in a competitive manner with $IC_{50} = 0.5\text{-}1.6\ \mu\text{M}$, $K_i = 1.7\ \mu\text{M}$, and $K_{inact} = 0.1\ \text{min}^{-1}$. PR-171 inhibition of CYP3A4/5 is dependent on NADPH and is resistant to dilution, suggesting that it is an irreversible or quasi-irreversible inhibitor. PR-171 also minimally ($IC_{50} > 10\ \mu\text{M}$), but directly, inhibits CYP1A2, CYP2C9, CYP2C19 and CYP2D6. There was no inhibition of CYP2C8 by PR-171. A PR-171-derived MIC was not spectrally visible.

Table 40: *In vitro* evaluation of carfilzomib as an inhibitor of human CYP enzymes

Enzyme	CYP Reaction	Direct inhibition			Time-dependent inhibition		
		Zero-minute preincubation			30-minute preincubation		Potential for time-dependent inhibition ^b
		IC ₅₀ (μM)	Inhibition at 10 μM (%) ^a	K _i (μM)	IC ₅₀ (μM)	Inhibition at 10 μM (%) ^a	
CYP1A2	Phenacetin <i>O</i> -deethylation	>10	12	ND	>10	NA	little or no
CYP2C8	Amodiaquine <i>N</i> -dealkylation	>10	0.74	ND	>10	NA	little or no
CYP2C9	Diclofenac 4'-hydroxylation	>10	16	ND	>10	10	little or no
CYP2C19	<i>S</i> -Mephenytoin 4'-hydroxylation	>10	26	ND	>10	16	little or no
CYP2D6	Dextromethorphan <i>O</i> -demethylation	>10	21	ND	>10	3.2	little or no
CYP3A4/5	Testosterone 6β-hydroxylation	>10	32	ND	0.97	81	yes ^d
CYP3A4/5	Midazolam 1'-hydroxylation	1.6	80	1.7 ^c	0.49	90	yes ^e

Notes: Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC₅₀ values. IC₅₀ values were calculated with XLfit.

a The inhibition (%) was calculated with the following formula and data for the highest concentration of test article evaluated (results are rounded to two significant figures): Inhibition (%) = 100% – Percent of solvent control (Reference Appendix 4 for percent of solvent control).

b Time-dependent inhibition was determined by comparison of IC₅₀ values with and without preincubation, by comparison of the inhibition (%) at 10 μM of carfilzomib with and without preincubation and by visual inspection of the IC₅₀ plot.

c The type of inhibition is competitive.

d Upon further investigation, the increase in inhibition upon preincubation appeared to be dependent on NADPH and resistant to dilution. K_i and K_{inact} determination was performed and the results indicated a K_i value of 11 μM and K_{inact} value of 0.09 min⁻¹.

e Upon further investigation, the increase in inhibition upon preincubation appeared to be dependent on NADPH and resistant to dilution. K_i and K_{inact} determination was performed and the results indicated a K_i value of 11 μM and K_{inact} value of 0.10 min⁻¹.

NA Not applicable. No value was obtained as the rates at the highest concentration of Carfilzomib evaluated (10 μM) were higher than the control rates.

ND Not determined by study design.

(Table excerpted from Applicant's package)

Best
Available
Copy

Evaluation of Induction Potential of Cytochrome P450 Isoforms by Carfilzomib in Cultured Human Hepatocytes (Study TR-0086-171)

Key Study Findings

- PR-171 does not induce CYP1A2 or CYP3A4 activity in primary human hepatocytes.
- PR-171 indirectly decreases CYP1A2 activity at high doses (≥ 2.5 μM).
- PR-171 directly inhibits CYP3A4 protein activity and down-regulates CYP3A4 mRNA expression at all doses examined (≥ 0.1 μM).

METHODS

In Vitro induction of human cytochrome P450s (CYP1A2 and CYP3A) after 3 day exposure to 0, 0.1, 0.5 or 2.5 μM PR-171 was determined in cultured primary hepatocytes using *in situ* probes specific for CYP1A2 and CYP3A substrates and CYP3A4 mRNA expression. Direct enzyme inhibition was examined by treated with PR-171 for 30 min in the presence of a known inducer, followed by *in situ* substrate analysis.

RESULTS

MTT assays confirmed viability of all hepatocyte cultures after drug treatments and there was no PR-171 cytotoxicity. *In situ* experiments showed dose-dependent decreases in CYP3A4 activity at all the concentrations tested, which are possibly due, at least in part, to direct CYP3A4 inhibition. Drug-related effects on CYP1A2 activity

were equivocal at $\leq 0.5 \mu\text{M}$, but clearly resulted in decreased CYP1A2 activity at $2.5 \mu\text{M}$ that were not likely due to a direct inhibition of CYP1A2. CYP3A4 mRNA expression decreased dose-dependently at all doses in 2/3 cultures and at $\geq 0.5 \mu\text{M}$ in 1/3 cultures, indicating that the larger degree of PR-171-mediated CYP3A4 inhibition after 3 days of exposure may be associated with down-regulation of CYP3A4 mRNA expression. PR-171 is not a CYP1A2 or CYP3A4 inducer.

Table 41: *In vitro* CYP1A2 activity in human primary hepatocytes treated with carfilzomib

Donor	Treatment (μM)	Activity (pmol/mg/min)			Fold induction			Induction as % of positive control*
HH247	0 (DMSO)	5.0	±	0.44	-		-	
	0.1	7.1	±	0.42	1.4	± 0.080	2.7	
	0.5	5.7	±	1.7	1.1	± 0.34	0.91	
	2.5	<LLOQ			-		-	
	Positive control							
	20 μM BNF	82	±	4.5	16	± 0.89	-	
HH249	0 (DMSO)	5.7	±	0.62	-		-	
	0.1	4.2	±	0.77	0.73	± 0.13	NA	
	0.5	3.3	±	0.69	0.57	± 0.12	NA	
	2.5	<LLOQ			-		-	
	Positive control							
	20 μM BNF	52	±	10	9.2	± 1.8	-	
HH255	0 (DMSO)	8.6	±	1.3	-		-	
	0.1	8.6	±	0.20	0.99	± 0.023	NA	
	0.5	8.5	±	0.77	0.99	± 0.090	NA	
	2.5	6.6	±	0.32	0.76	± 0.037	NA	
	Positive control							
	20 μM BNF	410	±	37	47	± 4.2	-	

Data are the mean from three separate wells in each group.

* % of positive control = (Activity of test article-Activity of DMSO)/(Activity of BNF-Activity of DMSO) x100. This was not applicable if the enzyme activity in test article-treated cells was lower than that from solvent vehicle control-treated cells.

NA: Not applicable

LLOQ: low limit of quantification ($0.076 \mu\text{M}$, corresponding to 0.84 pmol/mg/min activity, assuming that average protein content is 0.3 mg/well .)

Table 42: *In vitro* CYP3A4 activity in human primary hepatocytes treated with carfilzomib

Donor	Treatment (μM)	Activity (pmol/mg/min)			Fold induction			Induction as % of positive control*
HH241	0 (DMSO)	28	±	1.4	-	-	-	-
	0.1	25	±	3.0	0.87	±	0.10	NA
	0.5	24	±	0.65	0.85	±	0.023	NA
	2.5	16	±	4.5	0.55	±	0.16	NA
	Positive control							
	20 μM RIF	1407	±	194	50	±	6.8	-
HH247	0 (DMSO)	69	±	21	-	-	-	-
	0.1	37	±	15	0.53	±	0.22	NA
	0.5	13	±	1.8	0.19	±	0.026	NA
	2.5	2.7	±	0.42	0.039	±	0.0061	NA
	Positive control							
	20 μM RIF	1206	±	129	17	±	1.9	-
HH249	0 (DMSO)	55	±	6.8	-	-	-	-
	0.1	45	±	4.9	0.81	±	0.089	NA
	0.5	13	±	1.4	0.23	±	0.025	NA
	2.5	3.8	±	0.52	0.068	±	0.0094	NA
	Positive control							
	20 μM RIF	777	±	117	14	±	2.1	-

Data are the mean from three separate wells in each group ± SD from two replicates.

* % of positive control = (Activity of test article-Activity of DMSO)/ (Activity of RIF-Activity of DMSO) x100. This was not applicable if the enzyme activity in test article-treated cells was lower than that from solvent vehicle control-treated cells.

NA: Not applicable

Table 43: Effect of carfilzomib on CYP3A4 mRNA expression in primary human hepatocytes

Donor	Treatment (μM)	Fold induction			Induction as % of positive control*
HH241	0 (DMSO)	-	-	-	-
	0.1	1.7	±	1.2	0.18
	0.5	0.44	±	0.099	NA
	2.5	0.045	±	0.0055	NA
	Positive control				
	20 μM RIF	397	±	59	-
HH247	0 (DMSO)	-	-	-	-
	0.1	0.58	±	0.079	NA
	0.5	0.13	±	0.047	NA
	2.5	0.034	±	0.009	NA
	Positive control				
	20 μM RIF	16	±	10	-
HH249	0 (DMSO)	-	-	-	-
	0.1	0.50	±	0.10	NA
	0.5	0.072	±	0.0033	NA
	2.5	0.0040	±	0.0015	NA
	Positive control				
	20 μM RIF	13	±	4.1	-

Data are the mean from three separate wells in each group ± SD from two replicates.

* % of positive control = (Response of test article-1)/ (Response of RIF- 1) x100. This was not applicable if the response in test article-treated cells was lower than that from solvent vehicle control-treated cells.

NA: Not applicable

(Tables excerpted from Applicant's package)

Assessment of Carfilzomib as Substrate and Inhibitor of P-glycoproteins in a Caco-2 Monolayer System (Study TR-0087-171)

Key Study Findings

- PR-171 is a P-glycoproteins (P-gp) substrate at all concentrations examined (≥ 0.1 μM).
- PR-171 weakly inhibits P-gp transport at 3 μM. Dose-dependent inhibition was not assessed.

METHODS

Bi-directional permeability across a Caco-2 monolayer was used to assess if PR-171 is a substrate (0.1, 1, and 3 μM PR-171) or inhibitor (3 μM only) of P-gp.

RESULTS

Efflux ratios of PR-171 ranged from 6.3 to 14.5 in Caco-2 cells, suggesting that efflux proteins transport PR-171. Furthermore, P-gp inhibitors (ketoconazole and cyclosporine A) decreased PR-171 efflux ratios, further indicating the PR-171 is a P-gp substrate (≥ 0.1 μM). PR-171 (3 μM) inhibited the transport of Digoxin by 25%, suggesting that PR-171 is a weak inhibitor of P-gp transport.

Ex-Vivo Evaluation of Cytochrome P450 Expression in Sprague-Dawley Rats Treated with Carfilzomib (Study TR-0196-171)**Key Study Findings**

- PR-171 decreases CYP2B, CYP2C, and CYP3A expression and activity in male rats, but not females.
- PR-171 may increase CYP4A mRNA expression and activity in male rats, but not females.
- PR-171 does not have a clear effect on CYP1A due to equivocal mRNA expression and enzyme activity data.
- PR-171 decreases total hepatic CYP content in males at all doses and in females at 4 mg/kg.

METHODS

In vivo effects of PR-171 on hepatic CYP enzyme (CYP1A, CYP2B, CYP2C, CYP3A, and CYP4A) activity and mRNA expression in Sprague Dawley rats from the 6-month toxicity study #TR-0074-171, wherein animals received IV bolus administration of 0, 1, 2, or 4 mg/kg PR-171 for 2 consecutive days per week for 3 weeks on a 28-day cycle for a total of 6 months. Microsomes were prepared from liver samples were harvested 24 hr post-dose at the end of the final cycle (Cycle 6) on Day 157. CYP enzyme activity was measured by quantifying metabolite formation and qRT-PCR was used to assess mRNA expression. Total hepatic CYP content was determined via CYP-carbon monoxide complex absorbance using spectrophotometry.

RESULTS

CYP1A mRNA expression was dose-dependently increased in males, but not females and was not associated with a clear increase in activity. CYP2B mRNA expression (↓80%) and enzyme activity (↓40%) were both reduced in males at 4 mg/kg, but there was lack of dose-dependency or correlation of expression and activity data at lower doses and in females. CYP2C activity (↓27-72%) and mRNA expression (↓61-89%, dose-related) were decreased at all doses in males, but not in females. CYP3A4 mRNA expression (↓52-89%, dose-related) at ≥2 mg/kg and enzyme activity (↓81%) at 4 mg/kg were both reduced in males, but there was lack of dose-dependency or correlation of expression and activity data in females. Increases in CYP4A1 activity (23-35%) and mRNA expression (↑18.8-fold) were observed in males at 4 mg/kg, but lacked dose-dependency or correlation of expression and activity data at lower doses and in females. Total CYP-carbon monoxide complex absorbance was decreased in males (↓21-67%) at all doses and females (↓36%) at 4 mg/kg.

Ex-Vivo Evaluation of Cytochrome P450 Expression in Cynomolgus Monkeys Treated with Carfilzomib (Study TR-0197-171)**Key Study Findings**

- PR-171 decreases CYP1A expression and activity in females, but male findings were equivocal.

- PR-171 may increase CYP2C mRNA expression and activity in males, but not females.
- PR-171 does not have a clear effect on male or female CYP3A enzyme expression and activity, due to non-correlative findings.
- PR-171 decreases total hepatic CYP content in females at all doses, but not males.

METHODS

In vivo effects of PR-171 on hepatic CYP enzyme (CYP1A, CYP2C, and CYP3A) activity and mRNA expression (CYP1A1 and CYP3A4) in Cynomolgus monkeys from the 9-month toxicity study #TR-0073-171, wherein animals received IV bolus administration of 0, 0.5, 1, or 2 mg/kg PR-171 for 2 consecutive days per week for 3 weeks on a 28-day cycle for a total of 9 months (9 cycles). Microsomes were prepared from liver samples that were harvested 24 hr post-dose at the end of the final cycle (Cycle 9) on Day 241. CYP enzyme activity was measured by quantifying metabolite formation and qRT-PCR was used to assess mRNA expression. Total hepatic CYP content was determined via CYP-carbon monoxide complex absorbance using spectrophotometry.

RESULTS

A dose-dependent decrease in CYP1A activity ($\downarrow 61\%$) with a non-significant trend for decreased mRNA expression was observed in females at ≥ 1 mg/kg. Although males had significantly decreased CYP1A mRNA expression ($\downarrow 71\%$) at 4 mg/kg, there were no differences in male CYP1A activity. Non-dose-dependent increases in CYP2C activity ($\uparrow 42\%$) were seen at 1 mg/kg, but mRNA expression was not determined. CYP3A activity was dose-dependently increased in males (≥ 1 mg/kg, $\uparrow 18-37\%$) and non-dose-dependently decreased in females (0.5 and 1 mg/kg only); whereas, CYP3A mRNA expression was dose-dependently decreased ($\downarrow 76-85\%$), reaching significance at 2 mg/kg, in males and females. Since CYP3A activity changes do not necessarily correlate with the direction of mRNA expression changes, these findings suggest that PR-171 does not necessarily alter CYP3A. PR-171 dose-dependently decreased total hepatic CYP content in females ($\downarrow 16-29\%$) at all doses, but only at 1 mg/kg in males ($\downarrow 19\%$).

5.7 Toxicokinetics

Toxicokinetics of Carfilzomib in an Intravenous Embryo-Fetal Toxicity Study in Rats (GLP) (Study TR-0250-171)

Key Study Findings

- Metabolite exposure levels: PR-389 (M14) > PR-519 (M16) > PR-413 (M15)
- The half life increases with dose from 9 to 71 min at the highest dose
- The half life of PR-389 is up to 4 times higher than the parent compound.

- Reduction in exposures to PR-171 at HD and PR-519 on GD17 may be due to peptidase cleavage and epoxide hydrolysis, rather than changes in hepatic P450 metabolism.

METHODS

Toxicokinetics of PR-171 and its metabolites (PR-389, PR-413, and PR-519) were evaluated in an embryo-fetal rat toxicity study. Pregnant Sprague Dawley rats received daily IV injections of PR-171 (0.5, 1 or 2 mg/kg) from Gestation Day (GD) 6 to 17. On the first and last day of dosing, plasma samples were collected at 5, 15, 30, and 60 min post-dose.

RESULTS

A dose-proportional increase in exposure to PR-171 was seen between 0.5 and 1 mg/kg, but more than dose-proportionally (>2-fold) on GD6 and less than dose-proportional increases on GD 17 at 2 mg/kg. PR-389 is the most abundant metabolite, followed by PR-519, than PR-413. Exposure to all 3 metabolites increases dose-proportionally. Exposures for PR-519 and 2 mg/kg PR-171 were lower on GD17 compared to GD6. The sponsor proposes that decreased exposures on GD 17 are likely due to peptidase cleavage and epoxide hydrolysis, rather than drug-related changes in hepatic P450 metabolism. Plasma clearance is higher than rat hepatic blood flow at all doses. T_{max} was reached at 5 min for all 4 compounds. The half-life of PR171 increased from 9 min to 14 - 71 min (GD6) with increased dosing. The half-life of the predominate metabolite, PR-389, is up to 4 times longer (25-43 min) than the parent compound at doses ≤ 1 mg/kg.

Table 44: Pharmacokinetic parameters of carfilzomib and its metabolites at 0.5 mg/kg in pregnant rats

Parameters	carfilzomib		PR-389		PR-413		PR-519	
	GD 6	GD 17	GD 6	GD 17	GD 6	GD 17	GD 6	GD 17
C ₀ (ng/mL)	393	381						
C _{max} (ng/mL)	95.1	85.5	95.6	103	28.0	26.7	59.8	40.9
T _{max} (min)	5	5	5	5	5	5	5	5
AUC _{last} (ng*min/mL)	1778	1657	2945	3723	356	442	689	446
AUC _{inf} (ng*min/mL)	1799	1675	4875	4911	372	455	723	471
Cl (mL/min/kg)	278	298						
V _{ss} (L/kg)	0.9	0.9						
t _{1/2} (min)	9	9	43	28	16	12	17	6

Table 45: Pharmacokinetic parameters of carfilzomib and its metabolites at 1 mg/kg in pregnant rats

Parameters	carfilzomib		PR-389		PR-413		PR-519	
	GD 6	GD 17	GD 6	GD 17	GD 6	GD 17	GD 6	GD 17
C ₀ (ng/mL)	502	639						
C _{max} (ng/mL)	170	173	183	188	52.4	50.7	107	60.0
T _{max} (min)	5	5	5	15	5	5	5	5
AUC _{last} (ng*min/mL)	2860	3141	5847	7177	735	919	1381	829
AUC _{inf} (ng*min/mL)	2892	3157	8862	10231	777	949	1430	839
Cl (mL/min/kg)	346	317						
V _{ss} (L/kg)	1.9	1.3						
t _{1/2} (min)	14	12	39	31	15	11	11	9

Table 46: Pharmacokinetic parameters of carfilzomib and its metabolites at 2 mg/kg in pregnant rats

Parameters	carfilzomib		PR-389		PR-413		PR-519	
	GD 6	GD 17	GD 6	GD 17	GD 6	GD 17	GD 6	GD 17
C ₀ (ng/mL)	3122	888						
C _{max} (ng/mL)	536	246	347	464	112	109	227	119
T _{max} (min)	5	5	5	5	5	5	5	5
AUC _{last} (ng*min/mL)	12296	4474	11677	14495	1476	1671	2538	1432
AUC _{inf} (ng*min/mL)	13200	4509	14755	18064	1521	1696	2603	1442
Cl (mL/min/kg)	152	444						
V _{ss} (L/kg)	2.1	2.1						
t _{1/2} (min)	71	14	27	25	14	10	14	9

(Tables excerpted from Applicant's package)

Toxicokinetics of Carfilzomib in a 3/6-Month Intravenous Toxicity Study in Rats (GLP) (Study TR-0251-171)

Key Study Findings

- Exposures of PR-171 and 3 inactive metabolites [PR-389 (M14), PR-413 (M15), and PR-519 (M16)], increase dose-proportionally.
- Elimination of PR-171 and the metabolites decreases with repeat dosing.

METHODS

Toxicokinetics of PR-171 and its metabolites (PR-389, PR-413, and PR-519) were evaluated in satellite groups as part of a 9-month toxicity study (study # 07-2026/TR-0072-171). Sprague Dawley rats received IV injections of PR-171 (1, 2, or 4 mg/kg) on Days 1, 2, 8, 9, 15, and 16 of a 28-day cycle for a total of 6 months. On the first day of dosing for cycle 1 (Day 1), cycle 3 (Day 57), and cycle 6 (Day 142), plasma samples were collected predose and at 5, 15, 30, and 60 min post-dose.

RESULTS

Mortality occurred at 4 mg/kg. Exposures increased dose-proportionally. C_{max} and AUC exposures were 2-fold higher on Day 57 and 142 than on Day 1, indicating accumulation at all doses. Exposures to the parent compound at ≥ 2 mg/kg were lower in females than in males on Day 57 and 142, but comparable on Day 1, indicating that

the parent compound is less efficiently degraded at high doses in females than in males. Plasma clearance values were higher than hepatic blood flow at all doses, suggesting that PR-171 is largely eliminated extrahepatically. Since the half-life is very short, the increase in exposures with time is likely due to decreased elimination rather than drug accumulation.

Metabolite exposures increased dose-proportionally and showed signs of decreased elimination after repeated dosing, similarly to the parent compound in both males and females.

Table 47: Pharmacokinetic parameters of carfilzomib in rats

Male									
	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142
Dose (mg/kg)	1			2			4		
C₀ (ng/mL)	527	528	966	1319	3050	2301	3024	4847	1921
C_{max} (ng/mL)	146	173	258	341	678	519	809	1169	729
T_{max} (min)	5	5	5	5	5	5	5	5	5
AUC_{last} (ng*min/mL)	2637	2980	4741	6360	13350	10215	14728	22277	12262
AUC_{inf} (ng*min/mL)	2646	3004	4770	6376	13375	10250	14817	22365	12393
Cl (mL/min/kg)	378	333	210	314	150	195	270	179	323
V_{ss} (L/kg)	1.57	1.82	0.92	1.24	0.46	0.67	1.12	0.65	2.11
t_{1/2} (min)	11	12	13	10	11	12	13	12	12
Female									
	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142
Dose (mg/kg)	1			2			4		
C₀ (ng/mL)	489	953	908	1081	2069	2116	2915	3244	2171
C_{max} (ng/mL)	128	212	204	278	471	466	721	981	636
T_{max} (min)	5	5	5	5	5	5	5	5	5
AUC_{last} (ng*min/mL)	2311	4211	4027	5178	9230	9281	13599	17041	11318
AUC_{inf} (ng*min/mL)	2342	4229	4045	5202	9259	9311	13647	17377	11414
Cl (mL/min/kg)	427	236	247	384	216	215	293	230	350
V_{ss} (L/kg)	1.51	0.84	0.88	1.57	0.74	0.72	1.09	1.4	1.75
t_{1/2} (min)	8	13	13	12	12	12	12	31	13

Table 48: Pharmacokinetic parameters of the carfilzomib metabolite, PR-389, in rats

Male									
	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142
Dose (mg/kg)	1			2			4		
C _{max} (ng/mL)	157	183	213	336	434	432	792	893	939
T _{max} (min)	15	15	5	15	5	15	15	5	15
AUC _{last} (ng*min/mL)	5812	8135	8183	13448	17910	18218	32393	36955	41760
AUC _{inf} (ng*min/mL)	7992	11693	11186	18215	24436	24417	51720	48039	61789
t _{1/2} (min)	29	33	30	29	30	29	39	27	35
Female									
	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142
Dose (mg/kg)	1			2			4		
C _{max} (ng/mL)	180	219	218	371	448	479	810	774	867
T _{max} (min)	5	5	5	5	5	5	5	15	5
AUC _{last} (ng*min/mL)	5827	7830	7600	13628	17513	16960	28730	31133	33148
AUC _{inf} (ng*min/mL)	7150	10499	9611	19230	23927	22267	38194	41734	44524
t _{1/2} (min)	24	29	26	32	30	28	29	28	29

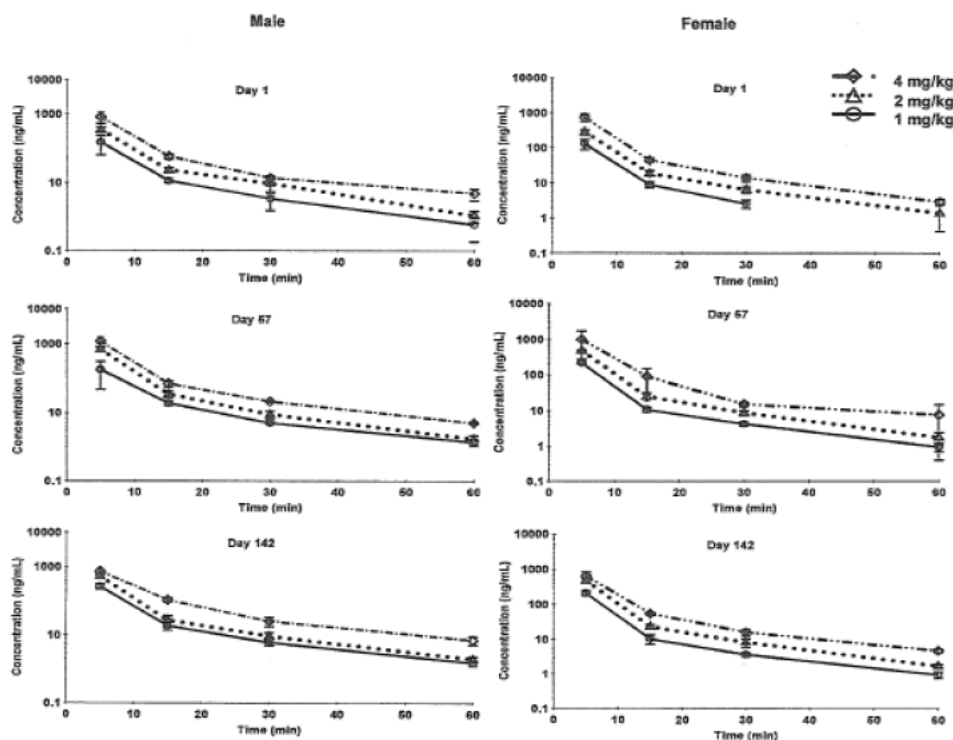
Table 49: Pharmacokinetic parameters of the carfilzomib metabolite, PR-413, in rats

Male									
	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142
Dose (mg/kg)	1			2			4		
C _{max} (ng/mL)	55.5	66.5	108	129	217	196	309	393	412
T _{max} (min)	5	5	5	5	5	5	5	5	5
AUC _{last} (ng*min/mL)	999	1580	1942	2493	4206	3903	6310	9187	8664
AUC _{inf} (ng*min/mL)	1011	1627	1988	2528	4297	4004	6447	9646	8857
t _{1/2} (min)	9	11	11	9	10	11	10	13	11
Female									
	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142
Dose (mg/kg)	1			2			4		
C _{max} (ng/mL)	44.1	57.8	55.6	96.8	116	132	260	193	243
T _{max} (min)	5	5	5	5	5	5	5	5	5
AUC _{last} (ng*min/mL)	569	965	943	1651	2179	2283	4247	3908	4957
AUC _{inf} (ng*min/mL)	621	985	964	1685	2244	2340	4300	3989	5062
t _{1/2} (min)	8	11	11	11	12	11	9	10	10

Table 50: Pharmacokinetic parameters of the carfilzomib metabolite, PR-519, in rats

Male									
	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142
Dose (mg/kg)	1			2			4		
C_{max} (ng/mL)	62.2	119	156	133	301	282	271	549	437
T_{max} (min)	5	5	5	5	5	5	5	5	5
AUC_{last} (ng*min/mL)	821	1807	2203	2001	4213	4003	4005	7539	7080
AUC_{inf} (ng*min/mL)	862	1831	2231	2012	4244	4047	4038	7582	7183
$t_{1/2}$ (min)	6	10	10	8	9	9	8	8	10

Female									
	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142
Dose (mg/kg)	1			2			4		
C_{max} (ng/mL)	77.5	132	120	151	257	273	319	443	339
T_{max} (min)	5	5	5	5	5	5	5	5	5
AUC_{last} (ng*min/mL)	854	1688	1487	2042	3448	3426	4310	5572	4619
AUC_{inf} (ng*min/mL)	910	1704	1505	2060	3490	3460	4332	5618	4660
$t_{1/2}$ (min)	8	9	10	9	10	9	8	9	9

Table 51: Plasma concentration-time profile for carfilzomib in rats

(Tables excerpted from Applicant's package)

Best Available Copy

Toxicokinetics of Carfilzomib in a 9-Month Intravenous Toxicity Study in Cynomolgus Monkeys (Study RE-0252-171)

Key Study Findings

- Exposures of carfilzomib increased less than dose-proportionally, likely due to increased clearance.
- Exposures of the 3 major inactive metabolites [PR-389 (M14), PR-413 (M15), and PR-519 (M16)], increased dose-proportionally.
- The metabolite PR-519 had the highest C_{max} value, but both PR-519 and PR-389 had the highest metabolite exposure levels.
- The half-life of PR-389 was longer (30-60 min) than that of carfilzomib (11-15 min), PR-519 (10 min), and PR-413 (20 min).

METHODS

Cynomolgus monkeys (4-6/sex/group) were administered IV bolus of 0, 1, 2, or 4 mg/kg carfilzomib for 9 cycles. For each cycle, monkeys were dosed the 2 consecutive days per week for 3 weeks, followed by a 12-day washout period. Plasma concentrations of carfilzomib and its metabolites (PR-389, PR-413, and PR-519) were determined on the first day of dosing for cycle 1 (Day1), 3 (Day 57), 6 (Day 141), and 9 (Day 225) at Predose, 5, 15, 30, and 60 minutes post-dose.

RESULTS

The average half-life of carfilzomib in both males and females was 11 to 15 minutes. Plasma clearance was markedly (>10-fold) higher than hepatic blood flow, indicating extrahepatic elimination. There were no indications of accumulation or gender effects in carfilzomib or metabolite exposure levels. Dose-dependent increases in plasma clearance were likely responsible for the observed less than dose-proportional increase in carfilzomib exposures. However, metabolite exposures increased dose-proportionally. Metabolites formed rapidly, reaching T_{max} ranging from 5 to 19 minutes post-dose. The metabolite with the highest C_{max} was PR-519, but it also had the shortest metabolite terminal half-life of 10 minutes. Since PR-389 had the longest half-life (30-60 min), total exposure (AUC_{last}) levels were similar to PR-519. PR-413 had an intermediate half-life of 20 min and the lowest exposure levels.

Table 52: Pharmacokinetic parameters of carfilzomib in Cynomolgus monkeys

Gender	Dose (mg/kg)	Day	t _{1/2} (min)	t _{max} (min)	C _{max} (ng/mL)	C ₀ (ng/mL)	AUC _{last} (min*ng/mL)	AUC _{inf} (min*ng/mL)	AUC _{inf/D} (min*kg*ng/mL/mg)	AUC _{0-24h} (min*ng/mL)	Cl (mL/min/kg)	V _{ss} (L/kg)
Male	0.5	1	11 ± 1	5 ± 0	41.2 ± 5.0	70.1 ± 9.2	785 ± 90	800 ± 93	1599 ± 186	800 ± 93	632 ± 79	7.4 ± 0.9
		57	11 ± 1	5 ± 0	41.4 ± 5.9	70.3 ± 14.5	779 ± 89	792 ± 87	1584 ± 173	792 ± 87	637 ± 74	7.3 ± 1.5
		141	12 ± 0	5 ± 1	41.1 ± 7.3	68.4 ± 15.2	830 ± 153	848 ± 158	1696 ± 315	848 ± 158	603 ± 98	7.6 ± 1.2
		225	11 ± 0	5 ± 0	43.1 ± 4.6	74.5 ± 5.7	804 ± 99	820 ± 101	1640 ± 202	820 ± 101	616 ± 70	6.9 ± 0.6
	1	1	12 ± 0	5 ± 0	62.3 ± 8.4	111 ± 18	1163 ± 134	1186 ± 134	1186 ± 134	1186 ± 134	851 ± 93	9.8 ± 1.6
		57	12 ± 0	5 ± 0	58.9 ± 9.6	102 ± 17	1122 ± 183	1146 ± 185	1146 ± 185	1146 ± 185	889 ± 133	10.7 ± 1.8
		141	13 ± 1	6 ± 1	61.4 ± 8.7	112 ± 14	1241 ± 129	1277 ± 128	1277 ± 128	1277 ± 128	789 ± 85	10.2 ± 1.7
		225	14 ± 1	5 ± 0	66.8 ± 12.2	133 ± 36	1237 ± 199	1273 ± 203	1273 ± 203	1273 ± 203	801 ± 128	9.6 ± 2.2
	2	1	12 ± 1	5 ± 1	103 ± 37	202 ± 74	1934 ± 555	1974 ± 565	987 ± 282	1974 ± 565	1085 ± 317	12.2 ± 4.0
		57	13 ± 1	5 ± 0	87.1 ± 15.4	165 ± 41	1651 ± 245	1653 ± 273	827 ± 136	1653 ± 273	1242 ± 239	14.8 ± 3.0
		141	15 ± 1	6 ± 1	76.7 ± 22.7	143 ± 35	1572 ± 323	1644 ± 349	822 ± 175	1644 ± 349	1268 ± 295	18.0 ± 3.8
		225	15 ± 1	5 ± 0	85.0 ± 7.1	158 ± 16	1624 ± 180	1694 ± 186	847 ± 93	1693 ± 186	1192 ± 131	16.2 ± 1.8
Female	0.5	1	12 ± 2	5 ± 1	45.7 ± 5.3	80.3 ± 5.8	890 ± 74	912 ± 75	1824 ± 149	912 ± 75	551 ± 44	6.7 ± 1.0
		57	11 ± 1	5 ± 0	48.5 ± 4.2	82.8 ± 10.0	918 ± 69	937 ± 75	1873 ± 150	937 ± 75	536 ± 42	6.3 ± 0.6
		141	13 ± 2	5 ± 0	46.8 ± 7.2	74.5 ± 15.6	942 ± 136	975 ± 153	1949 ± 307	975 ± 153	522 ± 77	7.1 ± 1.1
		225	11 ± 1	5 ± 0	47.5 ± 9.4	83.4 ± 24.4	886 ± 148	903 ± 149	1806 ± 298	903 ± 149	566 ± 96	6.5 ± 1.5
	1	1	13 ± 1	6 ± 1	51.6 ± 5.6	92.0 ± 5.9	1047 ± 41	1074 ± 41	1074 ± 41	1074 ± 41	933 ± 37	12.0 ± 0.7
		57	12 ± 1	5 ± 0	52.3 ± 7.8	87.4 ± 15.7	1009 ± 123	1033 ± 122	1033 ± 122	1033 ± 122	979 ± 121	12.3 ± 2.4
		141	14 ± 2	6 ± 2	60.9 ± 14.2	106 ± 22	1236 ± 132	1281 ± 124	1281 ± 124	1281 ± 124	786 ± 73	10.8 ± 2.8
		225	12 ± 1	5 ± 0	69.1 ± 8.6	110 ± 23	1449 ± 453	1485 ± 470	1485 ± 470	1485 ± 470	716 ± 181	8.7 ± 1.3
	2	1	14 ± 2	5 ± 0	86.9 ± 24.1	151 ± 57	1692 ± 380	1755 ± 380	877 ± 190	1755 ± 380	1181 ± 230	16.7 ± 4.9
		57	15 ± 3	5 ± 0	107 ± 34	221 ± 126	2015 ± 515	2081 ± 526	1041 ± 263	2081 ± 526	1010 ± 234	12.9 ± 5.0
		141	15 ± 2	6 ± 2	81.6 ± 29.3	142 ± 53	1741 ± 376	1827 ± 392	914 ± 196	1827 ± 392	1135 ± 239	17.7 ± 4.7
		225	14 ± 2	5 ± 0	114 ± 26	209 ± 58	2129 ± 414	2202 ± 419	1101 ± 209	2202 ± 419	935 ± 177	12.0 ± 3.4

Table 53: Pharmacokinetic parameters of PR-389 in Cynomolgus monkeys

Gender	Dose (mg/kg)	Day	t _{1/2} (min)	t _{max} (min)	C _{max} (ng/mL)	AUC _{last} (ng*min/mL)	AUC _{inf} (ng*min/mL)	AUC _{inf/D} (min*kg*ng/mL/mg)	AUC _{0-24h} (ng*min/mL)
Male	0.5	1	43 ± 13	15 ± 0	82.1 ± 17.1	3648 ± 362	5993 ± 560	11986 ± 1120	5995 ± 562
		57	35 ± 8	15 ± 0	108 ± 13	4387 ± 405	6614 ± 331	13227 ± 662	6614 ± 331
		141	38 ± 5	15 ± 1	98.3 ± 13.2	4184 ± 432	6565 ± 846	13131 ± 1692	6569 ± 851
		225	62 ± 32	14 ± 1	113 ± 20	4943 ± 613	10621 ± 2707	21241 ± 5415	10622 ± 2708
	1	1	31 ± 6	15 ± 0	170 ± 11	7231 ± 553	10092 ± 1109	10092 ± 1109	10096 ± 1111
		57	36 ± 8	19 ± 7	186 ± 13	7959 ± 766	12067 ± 2170	12067 ± 2170	12073 ± 2175
		141	45 ± 15	15 ± 1	166 ± 34	7212 ± 912	12382 ± 1702	12382 ± 1702	12387 ± 1704
		225	53 ± 16	14 ± 1	175 ± 15	7769 ± 1165	15016 ± 4173	15016 ± 4173	15009 ± 4166
	2	1	34 ± 6	15 ± 1	343 ± 80	14213 ± 2999	20780 ± 4814	10390 ± 2407	20785 ± 4818
		57	37 ± 4	15 ± 0	375 ± 40	14988 ± 1382	23476 ± 2693	11738 ± 1346	23477 ± 2690
		141	49 ± 18	17 ± 6	395 ± 72	17253 ± 3754	32240 ± 13876	16120 ± 6938	32261 ± 13913
		225	45 ± 9	14 ± 1	456 ± 87	19454 ± 4138	34537 ± 10093	17268 ± 5046	34544 ± 10099
Female	0.5	1	37 ± 4	19 ± 8	72.3 ± 15.9	3133 ± 796	4817 ± 1250	9633 ± 2500	4818 ± 1253
		57	34 ± 6	15 ± 0	97.5 ± 24.6	4289 ± 1010	6369 ± 2055	12738 ± 4109	6372 ± 2056
		141	36 ± 9	15 ± 2	103 ± 31	4225 ± 1227	6580 ± 2121	13159 ± 4243	6579 ± 2122
		225	42 ± 10	13 ± 1	98.9 ± 18.5	3957 ± 907	6757 ± 1750	13515 ± 3499	6756 ± 1752
	1	1	35 ± 8	17 ± 10	129 ± 2	5497 ± 812	8075 ± 1964	8075 ± 1964	8077 ± 1967
		57	29 ± 2	15 ± 0	180 ± 25	7285 ± 1071	9873 ± 1535	9873 ± 1535	9874 ± 1536
		141	45 ± 11	16 ± 2	158 ± 13	6600 ± 167	11778 ± 1793	11778 ± 1793	11777 ± 1792
		225	38 ± 6	14 ± 1	183 ± 35	7305 ± 1249	11694 ± 2851	11694 ± 2851	11690 ± 2846
	2	1	43 ± 15	15 ± 0	305 ± 51	13867 ± 3121	24002 ± 10329	12001 ± 5165	24013 ± 10338
		57	41 ± 23	16 ± 1	420 ± 80	17424 ± 4752	31298 ± 21487	15649 ± 10744	31306 ± 21507
		141	46 ± 6	14 ± 3	364 ± 112	16245 ± 5530	28400 ± 9104	14200 ± 4552	28405 ± 9113
		225	43 ± 10	15 ± 1	417 ± 111	18119 ± 6078	31864 ± 15275	15932 ± 7637	31873 ± 15302

Best
Available
Copy

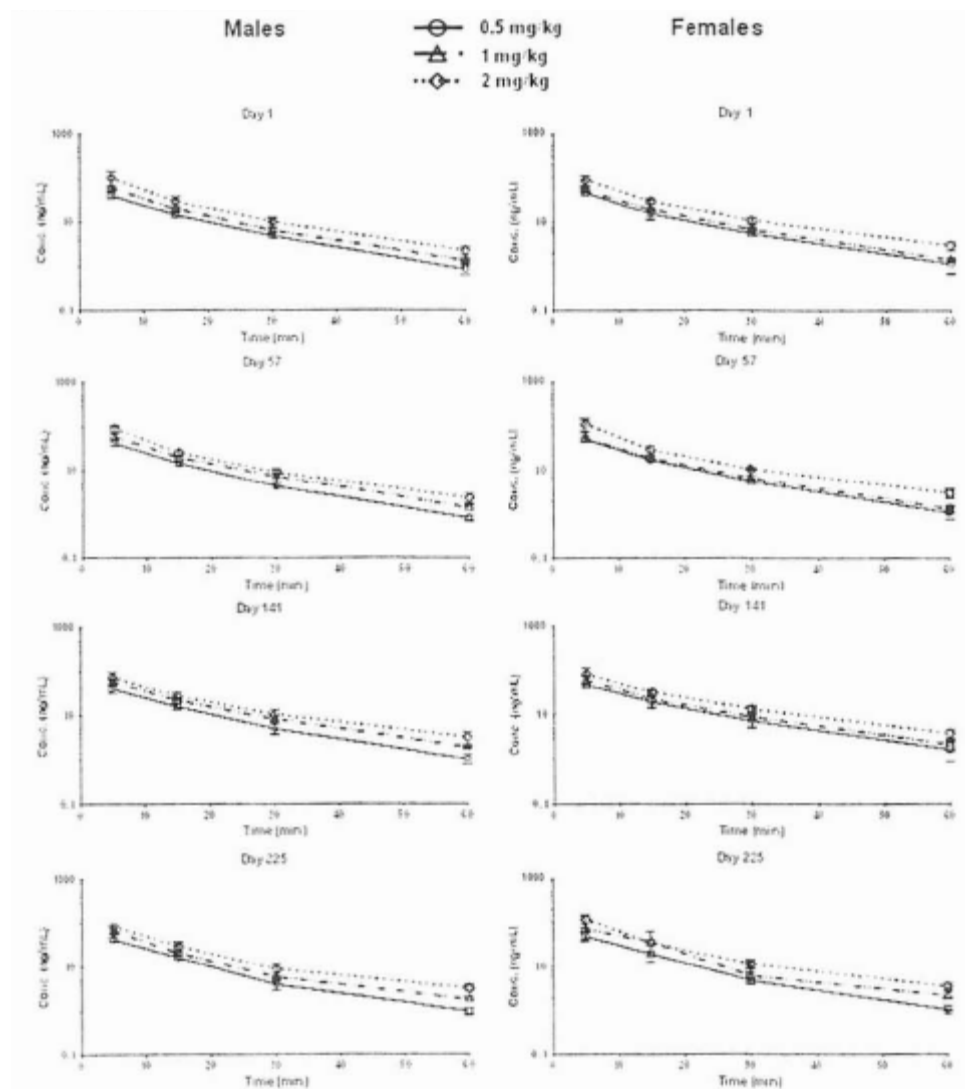
Table 54: Pharmacokinetic parameters of PR-413 in Cynomolgus monkeys

Gender	Dose (mg/kg)	Day	$t_{1/2}$ (min)	t_{max} (min)	C_{max} (ng/mL)	AUC_{last} (ng*min/mL)	AUC_{inf} (ng*min/mL)	AUC_{inf}/D (min*kg*ng/mL/mg)	AUC_{0-24h} (ng*min/mL)
Male	0.5	1	21 ± 3	8 ± 5	29.1 ± 6.6	942 ± 229	1095 ± 241	2190 ± 481	1095 ± 241
		57	19 ± 2	8 ± 5	31.6 ± 5.6	1071 ± 221	1217 ± 230	2435 ± 461	1217 ± 230
		141	21 ± 2	8 ± 5	31.2 ± 3.0	1085 ± 69	1268 ± 76	2537 ± 153	1269 ± 77
		225	26 ± 8	10 ± 6	35.1 ± 9.0	1216 ± 281	1555 ± 210	3109 ± 420	1554 ± 210
	1	1	17 ± 3	10 ± 6	42.9 ± 5.7	1537 ± 256	1714 ± 306	1714 ± 306	1714 ± 306
		57	17 ± 4	5 ± 0	47.7 ± 3.6	1660 ± 276	1853 ± 402	1853 ± 402	1853 ± 402
		141	19 ± 3	6 ± 1	48.3 ± 9.6	1591 ± 368	1830 ± 405	1830 ± 405	1830 ± 405
		225	22 ± 4	9 ± 5	45.9 ± 5.8	1623 ± 353	1962 ± 540	1962 ± 540	1962 ± 540
	2	1	17 ± 3	11 ± 5	87.4 ± 15.0	3030 ± 731	3410 ± 988	1705 ± 494	3410 ± 988
		57	16 ± 2	8 ± 5	83.6 ± 17.0	2781 ± 595	3099 ± 726	1549 ± 363	3099 ± 726
		141	21 ± 4	6 ± 1	102 ± 25	3392 ± 1509	4080 ± 2024	2040 ± 1012	4080 ± 2025
		225	20 ± 2	12 ± 4	106 ± 19	3603 ± 811	4194 ± 1081	2097 ± 540	4194 ± 1081
Female	0.5	1	19 ± 1	8 ± 5	19.5 ± 5.1	647 ± 172	739 ± 204	1478 ± 408	739 ± 204
		57	17 ± 2	8 ± 5	25.6 ± 3.1	859 ± 157	950 ± 195	1900 ± 390	950 ± 195
		141	18 ± 2	12 ± 5	27.9 ± 7.6	912 ± 314	1043 ± 374	2086 ± 749	1043 ± 374
		225	20 ± 2	7 ± 4	26.0 ± 2.5	778 ± 163	911 ± 194	1823 ± 388	911 ± 194
	1	1	21 ± 6	12 ± 12	31.9 ± 6.0	1121 ± 203	1311 ± 288	1311 ± 288	1311 ± 288
		57	15 ± 1	5 ± 0	39.5 ± 6.7	1391 ± 232	1511 ± 269	1511 ± 269	1511 ± 269
		141	23 ± 5	13 ± 6	34.6 ± 5.5	1246 ± 257	1550 ± 276	1550 ± 276	1550 ± 277
		225	20 ± 2	8 ± 6	42.4 ± 10.3	1375 ± 359	1616 ± 450	1616 ± 450	1615 ± 450
	2	1	20 ± 5	14 ± 4	70.1 ± 11.6	2556 ± 462	2985 ± 532	1492 ± 266	2985 ± 533
		57	17 ± 5	8 ± 5	83.6 ± 16.5	2800 ± 287	3148 ± 440	1574 ± 220	3148 ± 440
		141	21 ± 1	10 ± 5	91.3 ± 24.4	2934 ± 756	3468 ± 919	1734 ± 460	3468 ± 919
		225	19 ± 2	9 ± 6	88.5 ± 14.8	3006 ± 700	3473 ± 905	1736 ± 453	3473 ± 905

Table 55: Pharmacokinetic parameters of PR-519 in Cynomolgus monkeys

Gender	Dose (mg/kg)	Day	$t_{1/2}$ (min)	t_{max} (min)	C_{max} (ng/mL)	AUC_{last} (ng*min/mL)	AUC_{inf} (ng*min/mL)	AUC_{inf}/D (min*kg*ng/mL/mg)	AUC_{0-24h} (ng*min/mL)
Male	0.5	1	11 ± 1	5 ± 0	210 ± 35	3168 ± 465	3239 ± 492	6478 ± 984	3239 ± 492
		57	10 ± 0	5 ± 0	218 ± 19	3124 ± 347	3173 ± 356	6346 ± 712	3173 ± 356
		141	11 ± 1	5 ± 1	223 ± 58	3487 ± 877	3566 ± 907	7132 ± 1814	3566 ± 907
		225	12 ± 1	5 ± 0	276 ± 76	4020 ± 1316	4148 ± 1381	8296 ± 2763	4148 ± 1381
	1	1	10 ± 0	5 ± 0	396 ± 52	6016 ± 892	6111 ± 922	6111 ± 922	6111 ± 922
		57	10 ± 1	5 ± 0	418 ± 46	6145 ± 1037	6243 ± 1104	6243 ± 1104	6243 ± 1104
		141	11 ± 1	6 ± 1	384 ± 51	5957 ± 1056	6095 ± 1094	6095 ± 1094	6095 ± 1094
		225	11 ± 1	5 ± 0	507 ± 87	7185 ± 1551	7339 ± 1632	7339 ± 1632	7339 ± 1632
	2	1	10 ± 1	5 ± 1	807 ± 218	12593 ± 3489	12816 ± 3647	6408 ± 1824	12816 ± 3647
		57	9 ± 1	5 ± 0	777 ± 204	11589 ± 3580	11765 ± 3684	5883 ± 1842	11765 ± 3684
		141	11 ± 1	6 ± 1	706 ± 275	11709 ± 5638	12077 ± 5949	6039 ± 2975	12077 ± 5949
		225	11 ± 1	5 ± 0	849 ± 194	13161 ± 4340	13471 ± 4583	6736 ± 2292	13471 ± 4583
Female	0.5	1	11 ± 1	5 ± 1	178 ± 19	2742 ± 375	2809 ± 400	5618 ± 800	2809 ± 400
		57	9 ± 0	5 ± 0	195 ± 28	2898 ± 434	2938 ± 449	5876 ± 898	2938 ± 449
		141	11 ± 0	5 ± 0	187 ± 55	3133 ± 855	3212 ± 888	6424 ± 1776	3212 ± 888
		225	11 ± 1	5 ± 0	207 ± 37	2882 ± 405	2942 ± 424	5885 ± 849	2943 ± 424
	1	1	11 ± 2	6 ± 1	322 ± 76	5137 ± 1117	5253 ± 1165	5253 ± 1165	5253 ± 1165
		57	9 ± 1	5 ± 0	366 ± 62	5738 ± 1006	5816 ± 1031	5816 ± 1031	5816 ± 1031
		141	11 ± 1	6 ± 2	348 ± 124	5582 ± 1436	5729 ± 1418	5729 ± 1418	5729 ± 1418
		225	11 ± 1	5 ± 0	464 ± 65	6788 ± 1316	6940 ± 1383	6940 ± 1383	6940 ± 1383
	2	1	10 ± 1	5 ± 0	682 ± 59	10771 ± 1492	10971 ± 1552	5485 ± 776	10971 ± 1553
		57	9 ± 0	5 ± 0	788 ± 154	11604 ± 1945	11732 ± 1971	5866 ± 985	11732 ± 1971
		141	11 ± 1	6 ± 2	620 ± 200	10119 ± 2748	10400 ± 2849	5200 ± 1424	10400 ± 2849
		225	10 ± 1	5 ± 0	781 ± 110	12051 ± 1955	12285 ± 2051	6143 ± 1026	12285 ± 2051

Best
Available
Copy

Figure 35: Plasma concentration-time profile for carfilzomib in Cynomolgus monkeys

(Tables and figure excerpted from Applicant's package)

Best Available Copy

6 General Toxicology

6.1 Single-Dose Toxicity

The Applicant submitted 4 single dose toxicity reports (3 rat, 1 nonhuman primate). The single dose IV bolus administration of carfilzomib in rats resulted in significant toxicity at ≥ 7 mg/kg, which included lethargy and piloerection. Chymotrypsin-like proteasome activity inhibition was observed in whole blood, kidney, liver, heart, lung and adrenal at ≥ 1 mg/kg, with reversibility noted in tissues, but not blood. Signs of hepatotoxicity and nephrotoxicity based on gross pathology, hematology and clinical chemistry were

observed. Infusion of carfilzomib resulted in reduced toxicity compared to bolus administration.

Study Report #TR-0006-171

Single bolus intravenous doses ranging from 0.5 to 9 mg/kg were administered to male Sprague-Dawley rats in two separate studies (#012: 0.5, 1.5, 4.5, and 7 mg/kg (n=15/group); #015: 1, 3, and 9 mg/kg (n=12/group)). There was significantly decreased body weight at 9 mg/kg and two deaths between 24 and 48 hrs post-dose. In both studies, animals at ≥ 7 mg/kg exhibited lethargy and piloerection at 1 and 4 hrs post-dosing. Dose-dependent, near-complete, irreversible blood cell chymotrypsin-like proteasome activity inhibition was observed in Study #012 (maximal effect at 7 mg/kg). In most tissues, inhibition ranged from 40-80% within 1 hr of dosing; the most sensitive tissues were blood, heart, lung and adrenal glands. There was no effect noted in the brain. Minor, inconsistent changes were noted in hematology and clinical chemistry values on Day 1 with recovery by Day 7. Reticulocytes (absolute and %) were significantly increased at ≥ 4.5 mg/kg in Study# 012, but no change was noted in Study #015. Platelets were significantly decreased at 9 mg/kg in #015, but not in #012. Serum phosphorus at 7 mg/kg and albumin at ≥ 4.5 mg/kg were significantly decreased in Study# 012, but not in Study# 015.

Study Report #TR-0010-171

Single bolus intravenous doses ranging from 2 to 25 mg/kg were administered to male Sprague-Dawley rats (n=12/group) in two separate parts (A: 10, 15, and 25 mg/kg; B: 2, 4.5, 9 mg/kg). Mortality was observed in 2/12 animals at 10 mg/kg and all animals at ≥ 15 mg/kg in Part A. At 10 mg/kg, surviving animals displayed trouble breathing, rough coats and lethargy during the first hour following dosing with transient weight loss that was fully recovered by Day 10. Albumin, albumin:globin ratio, and ALP were significantly decreased on Days 1-2, and calcium and glucose were significantly reduced on Day 1 at all doses. AST, BUN, and creatinine were significantly increased on Day 1. At all doses, inhibition of chymotryptic-like proteasome activity in tissues was observed, with adrenal, lung, heart, whole blood (primarily erythrocytes), PBMC, spleen, and bone marrow showing $>90\%$; liver and kidney demonstrated reduced inhibitions. Inhibition was absent in whole blood at Day 7 in the 10 mg/kg group. In Part B, PR-171 demonstrated dose-proportional linear AUC exposure with a very short half-life of 15 minutes. All at doses, inhibition of whole blood chymotryptic-like proteasome activity was $>85\%$ and $>90\%$ at 1 and 2 hr post-dose, respectively.

Study Report #TR-0356-171

Single bolus or infusion intravenous doses of 8 mg/kg carfilzomib were administered to male Sprague-Dawley rats (n=3-10/group) in 7 separate parts (SN311, SN314, SN317, SN320, SN328, SN329, and SN331 (8, 10, and 12 mg/kg as a 10 or 30 min infusion)). Overall, mortality was noted in 14/32 animals receiving bolus compared to 0/24 and 1/8 receiving 30 min or 10 min infusion, respectively. At 10 and 12 mg/kg there were 1/6 and 4/6 deaths following a 30 min infusion. Most deaths occurred between 12 and 24 hr post-dose and were preceded by clinical signs of ruffled fur, dyspnea, lethargy, and pale ears; infusion reduced incidence and severity of findings. Serum BUN, creatinine

and ALT at 8 mg/kg bolus were increased. Infusion of 8 mg/kg resulted in reduced increases compared to bolus but had a dose-dependent increase; recovery by 72 hr was noted for both 8 mg/kg bolus and infusion. Cardiac biomarkers myoglobin and troponin I were not affected. Bolus administration of carfilzomib and bortezomib resulted in enlarged and impacted stomachs, pale livers, and congested adrenals, intestines, kidneys and lungs. Necropsy findings were impacted stomach and pale liver in animals receiving the infusion of 8 mg/kg carfilzomib. Potent (>80%) inhibition of proteasome activity was seen in all examined tissues at 8 mg/kg carfilzomib. Bortezomib at 0.5 mg/kg (IV bolus) resulted in 4/6 animal deaths with comparable pharmacodynamics and toxicities to carfilzomib.

Study Report #TR-0008-171

Single bolus intravenous doses of 1, 1.16, 2 and 4 mg/kg or 1 mg/kg for 5 daily doses were administered to male Cynomolgus monkeys (n=3/group). Two animals (one at 1 mg/kg and one at 4 mg/kg) were found dead on Day 1 and Day 2 following dosing, respectively. Hunched posture and emesis were observed in 2/4 animals at 4 mg/kg, including the animal that died. Both animals had fluid in the pericardial cavity; the animal at 1 mg/kg had fluid in the thoracic cavity; and the 4 mg/kg animal had congestion of the liver and kidney and discoloration of the GI tract. Serum BUN and creatinine at 4 mg/kg, and monocytes at ≥ 1.16 mg/kg were elevated and platelets at 1 and 4 mg/kg were decreased. Proteasome activity in whole blood and PBMCs was inhibited >80% at ≥ 1 mg/kg with a single dose and at 1 mg/kg for 5 days when evaluated 1 hr post-dose. Recovery of activity was observed by 9 days, except in erythrocytes.

6.2 Repeat-Dose Toxicity

The Applicant submitted study reports from 3 dose range-finding toxicity studies, each of ≤ 2 weeks total duration (2 rat, 1 nonhuman primate).

Study Report #TR-0009-171

Male rats were administered bolus intravenous doses of 4 or 5 mg/kg daily for five days then monitored for another seven days (n=4/group). Significant body weight loss occurred in the treated groups, and weight gain partially recovered compared to controls after the dosing period. No treatment-related clinical signs were observed. On Day 5, there were significant increases %neutrophils and serum cholesterol at 5 mg/kg and monocytes at 4 mg/kg, and decreased %lymphocytes at 5 mg/kg. There was significantly decreased albumin, calcium and potassium at ≥ 4 mg/kg. On Day 12, there was a significant increase in %reticulocytes at ≥ 4 mg/kg and decreased glucose and calcium at 5 mg/kg. One hour after a 4 mg/kg single dose, proteasome activity in whole blood and adrenal was reduced >90% and was reduced by 45% in the liver.

Study Report #TR-0020-171

Male rats were administered bolus intravenous doses of 1, 2 or 4.5 mg/kg once daily for five days (QDx5) then monitored another seven days or 2, 4.5, or 9 mg/kg twice daily two times per week on Days 1, 4, 8, and 11 (BIWx2) (n=4/group). Piloerection was

observed in all but the lowest dose groups for each regimen around the third dose. Rapid respiration was observed on Day 11 at 9 mg/kg, BIWx2. No body weight differences from control were observed. Both regimens caused changes in the same direction for neutrophils, lymphocytes, monocytes and hematocrit, hemoglobin, albumin, globin and ALP with some variation based on dose and/or timing. Absolute and % neutrophils at ≥ 4.5 mg/kg, absolute monocytes at 4.5 mg/kg only on Day 5 were significantly increased. Hemoglobin on Day 5 at 4.5 mg/kg QDx5, hematocrit on Days 5 at 4.5 mg/kg QDx5 and Day 12 at 9 mg/kg BIWx2, % lymphocytes on Day 5 at 4.5 mg/kg QIDx5 and ≥ 4.5 mg/kg BIWx2, albumin on Day 5 at 4.5 mg/kg QDx5 and at all doses BIWx2 (and p mg/kg on Day 12), globin on Day 5 at all doses QDx5 and 4.5 mg/kg BIWx2, and ALP at ≥ 2 mg/kg QDx5 and 9 mg/kg BIWx2 on both days were significantly decreased. Reticulocytes, LDH, and AST at 4.5 mg/kg QDx5 on Day 12, leukocytes at 4.5 mg/kg BIWx2 on Day 5, BUN and creatinine at 9 mg/kg BIWx2 on Day 5 were significantly increased. Bilirubin at ≥ 2 mg/kg QDx5 and calcium at 9 mg/kg BIWx2 were significantly decreased on Day 5. Proteasome activity in whole blood was abolished at all doses on Day 5 of both regimens; there was a partial recovery noted on Day with the QDx5 regimen.

A Two Dose, 7-Day Intravenous Toxicity Study of Carfilzomib in Cynomolgus Monkeys

Study no.:	TR-0157-171
Study report location:	eCTD: 4.2.3.2.1.TR-0577-171
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 3, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PR-171, #PCF001, 100%

Key Study Findings

- Elevated serum C-reactive protein (CRP), BUN, creatinine, AST, fibrinogen, troponin I, WBCs, neutrophils, and monocytes and decreased lymphocytes, platelets, reticulocytes, total protein, albumin and phosphorus noted at 2 mg/kg compared to controls.
- There were 3/6 monkeys with minimal to moderate mononuclear cell infiltration and 4/6 with minimal to mild increased mesangial matrix in the kidney.
- One animal had myocardial degeneration correlated with high troponin I and CRP levels that did not recover.

Methods

Doses: 2 mg/kg
Frequency of dosing: Days 1 and 2
Route of administration: IV bolus
Dose volume: 1 mL/kg
Formulation/Vehicle: 10% HPBCD (Hydroxypropyl- β -cyclodextrin)/ 50 mM sodium citrate, pH 3.5
Species/Strain: Monkey/Cynomolgus
Number/Sex/Group: 6♂/group
Age: 2.8-6.0 years
Weight: 3.4-4.9 kg
Satellite groups: none
Unique study design: none
Deviation from study protocol: Minor deviations that did not affect the conduct or evaluation of the study.

Observations and Results**Mortality**

None

Clinical Signs

One treated animal (#2002) showed slight tremors at 2 hr post dose on Day 2. This animal had elevated serum troponin I and C-reactive protein (CRP) levels with macro and microscopic correlates of left ventricle discoloration with vascular congestion and myocardial degeneration. Decreased activity, scratching feet and constant movement were each noted in 1/6 treated animals.

Body Weights, Feed Consumption Ophthalmoscopy, Organ Weights

Not remarkable

Hematology

There were increased WBCs, neutrophils, and monocytes and decreased %lymphocytes and platelets in treated animals compared to control, and to pre-study levels. The effects were noted on Days 2 to 4, and tended to fully recover, except for monocytes. Other changes noted by the sponsor tended to have high variability or the trends were similar when treated compared to control, without a clear drug-relationship.

Parameter	Change on Day 2 or 4 Compared to:	
	Control	Pre-Study
	Day 2	
Absolute White blood cells	2.7X	1.5X
% Lymphocytes	-3.4X	-2.5X
Absolute Neutrophils	5.7X	2.3X
Absolute Monocytes	2.1X	1.5X
	Day 4	
Absolute Platelets	-5.4X	-6.2X
Absolute Reticulocyte	-2.4X	-33%

Clinical Chemistry

There were increases in serum AST, BUN, creatinine and fibrinogen, and decreases in serum calcium, phosphorus, total protein, and albumin in treated animals compared to control, and to pre-study levels. The effects were noted on Days 2 to 4 and the largest difference observed is in the Table below; the effects tended to fully recover.

Parameter	Control	Pre-Study	Day
AST	2.2X	1.8X	2
BUN	1.8X	1.8X	2
Creatinine	+71%	+20%	2
Calcium	-7%	-6%	3
Phosphorus*	-31%	-35%	4
Total Protein	-13%	-14%	2
Albumin	-19%	-35%	3
Fibrinogen	+85%	+99%	4

*, Large SD

Urinalysis

Specific gravity was higher than 1.017 in 4/6 treated animals on Day 2 correlating with elevated serum BUN.

Special Evaluation- CRP

Treated animals had peak mean CRP levels on Day 3 that was 102X controls, and 204X over pre-study levels. (Control animals on Day 3 had a 3X increase over pre-study.) By Day 7, CRP levels recovered except to control levels except for Animal #2002.

Special Evaluation- Troponin I

Four treated animals had increased serum troponin I levels compared to 0/6 control animals. One animal (#2002 again) did not recover by Day 7.

Special Evaluation- Bone Marrow

Treated animals all demonstrated >90% proteasome activity in whole blood.

Special Evaluation- Pharmacodynamics

Treated animals showed a difference in erythroid:lymphoid (E:L) ratios with a mean of 1:0.2 compared to 1:0.5 for controls.

Gross Pathology

As mentioned under clinical signs, animal #2002 had red discoloration of the left ventricle; otherwise, unremarkable.

Histopathology

Adequate Battery- No: heart, lung, kidney, and bone marrow ONLY

Peer Review- none

Histological Findings-

Heart- Animal #2002 had moderate myocardial degeneration with mild vascular congestion in the left ventricle. This corresponded with a series of other clinical signs.

Kidney-There were 3/6 monkeys with minimal to moderate mononuclear cell infiltration and 4/6 with minimal to mild increased mesangial matrix (including #2002). The sponsor states in the table below that there were four animals with mononuclear cell infiltration, but the individual tabular data only indicated three (one missing animal was a minimal). The kidney findings were observed in the same animals.

Table 56: Histopathological Findings of Note in Male Monkeys Given 2 mg/kg Intravenous PR-171 for Two Days

Group Dose (mg/kg) No. animals examined	Males	
	1	2
	0	6
	6	6
Heart		
Degeneration, myocardium	(0) ^a	(1)
Moderate	0	1
Kidney		
Infiltrate, mononuclear cell	(0)	(4)
Minimal	0	3
Moderate	0	1
Mesangial matrix, increased	(1)	(4)
Minimal	0	1
Mild	1	3

^a Number in parentheses is the total incidence of all severity grades

(Table pg. 281 (pg. 11 of Pathology Report) excerpted from Applicant's package)

Toxicokinetics

Not reported.

Dosing Formulation Analysis

Test article concentration was confirmed (106.6%) and was stable (107.2%) at RT.

Study title: Three Week Daily Dose Range-Finding Toxicology Study of PR-171 in Male Sprague Dawley Rats

Study no.: TR-0007-171
Study report location: eCTD 4 2 3 2 1 TR-0007-171
Conducting laboratory and location: (b) (4)
Date of study initiation: Not provided
GLP compliance: No
QA statement: Not provided
Drug, lot #, and % purity: PR-171, #1315, not provided

Key Study Findings

- Mortality was observed in one animal at 6 mg/kg on day 2 of dosing preceded by weight loss.
- Treatment-related findings in the heart, kidney, liver, lung, and intestinal fat with little or no dose-dependency to incidence and severity. There were no significant effects on clinical chemistries.
- Necrosis of varying severity was noted in heart (≥ 4 mg/kg), liver (≥ 2 mg/kg), and pancreas (2 and 4 mg/kg).
- Decreased body weight gain was observed in all treated groups with neutrophilic mononuclear infiltration of the large intestine with hyperplasia of mucosal epithelium at 6 mg/kg.
- Mild to moderate lung histopathological findings suggestive of pneumonia were noted in all treated animals.
- All treated groups had protease inhibition.
- Decreased WBCs, RBCs and hematocrit and increased reticulocytes were noted at ≥ 4 mg/kg with neutrophilic infiltration in liver, lung, fat and intestine at all doses.

Methods

Doses: 2, 4, 6 mg/kg
Frequency of dosing: Days 1-5, 8-12, and 15-19
Route of administration: IV bolus
Dose volume: 2 mL/kg
Formulation/Vehicle: 10% HPBCD (Hydroxypropyl- β -cyclodextrin)/ 50 mM sodium citrate, pH 3.5
Species/Strain: Rat/Sprague-Dawley
Number/Sex/Group: 4♂/group
Age: Not provided
Weight: 200-225g
Satellite groups: none
Unique study design: Due to a death in 1/4 animals receiving 6 mg/kg on Day 2, animals in this group received PR-171 on Days 1-3, 8,9,15 and 16.
Deviation from study protocol: Blood was not drawn on Day 5 as planned for clinical pathology as per original protocol was due to a technical oversight.

Observations and Results**Mortality**

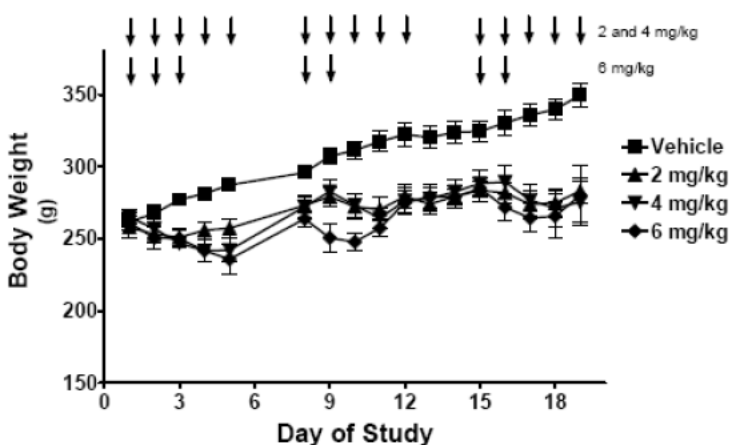
One animal (#15) at 6 mg/kg died on Day 2. Death was preceded by weight loss; no discussion of cause was provided.

Clinical Signs

At 4 mg/kg, piloerection and transient dyspnea immediately following dosing was observed. Rough coats and slightly pale complexion were observed following the 3rd dose. At 6 mg/kg, lethargy, piloerection, dehydration and pale complexion following the first dose were observed. The clinical signs persisted during treatment period.

Body Weights

All treated groups demonstrated a comparable significant decrease in body weight gain of ~22% compared to controls with marginal absolute body weight gains at Day 21. A decrease in Body Weight compared to control was noted during the periods of dosing that recovered during the periods where no treatment was given, with an attenuation of effect on sequential dosing periods.

Figure 36: Body weights in male rats given multiple doses of PR-171

Rats received bolus IV administration of 2, 4 or 6 mg/kg on days indicated by the arrows. Mean body weights (+ SEM; N = 4 for Vehicle, 2 and 4 mg/kg; N = 3 for 6 mg/kg from days 3 – 19).

(Figure 1, pg 10 excerpted from Applicant's package)

Feed Consumption, Urinalysis, Ophthalmoscopy, Organ Weights, Gross Pathology, Toxicokinetics

Not reported.

Hematology and Clinical Chemistry

Significant decreases in WBC and lymphocytes, platelets, and total RBCs with corresponding hematocrit decrease were noted at ≥ 4 mg/kg on Day 19. Most of these changes were dose-dependent but reticulocytes and platelets showed a decreased magnitude of change at 6 mg/kg. BUN increased and albumin and the corresponding A/G ratio decreased, albeit not significantly due to high variation in the values. Clinical chemistry values are shaded blue.

Parameter	Comments
White blood cells	↓ Day 19 at ≥ 4 mg/kg
Lymphocytes	↓ Day 19 at ≥ 4 mg/kg
Total red blood cells	↓ Days 12 and 19 ≥ 4 mg/kg
% red blood cells	↑ Day 19 at ≥ 4 mg/kg
Reticulocytes	↑ Day 19 at 2 and 4 mg/kg, and Day 12 for 4 mg/kg
Hematocrit	↓ Days 12 and 19 ≥ 4 mg/kg
Platelets	↓ Day 19 all doses, and Day 12 at 2 and 4 mg/kg
BUN	↑ Day 19 all doses
Albumin	↓ Day 19 all doses
A/G ratio	↓ Day 19 all doses

Histopathology

Adequate Battery- No: heart, lung, kidney, liver, spleen, small intestine, large intestine, testes, sciatic nerve and spinal cord ONLY

Peer Review- none

Histological Findings-

There were significant treatment-related findings in the heart, kidney, liver, lung, and intestinal fat. In general, there was little or no dose-dependency for incidence and severity; findings at 6 mg/kg tended to be no more severe than the 2 and 4 mg/kg groups; the 2x (6 mg/kg) vs. 5x (2 and 4 mg/kg) weekly dosing schedule was adopted after the first 3 doses. Animals with findings tended to have findings in multiple organs or tissues. Injection site findings were noted for all animals. No NOAEL was set due to findings in the lung and heart.

Lung- In all treated animals, there were findings of mild to moderate alveolar histiocytosis, neutrophilic, interstitial, subpleural mononuclear infiltration, intraalveolar edema, and hypertrophy of interstitial, bronchial epithelial and mesothelial, alveolar, and medial artery cells. Applicant reports these signs are indicative of pneumonia.

Heart- In 2/4 animals/treated group, there was mild to moderate myocardial hypercellularity with myofiber atrophy; and mild to moderate necrosis at ≥ 4 mg/kg was noted in some of these animals (2/2 at 4 mg/kg and 1/2 at 6 mg/kg).

Intestinal fat- Minimal to mild perivascular neutrophilic mononuclear infiltration was noted at ≥ 2 mg/kg with incidence and severity showing a reverse dose-dependency. Minimal to mild histiocytic neutrophilic mononuclear infiltration with adipocyte degeneration was noted in 2/4 animals at ≥ 4 mg/kg. Most of the findings were considered to be in the mesenteric fat.

Kidney- There was a slight increase in mild to moderate tubular vacuolation incidence and severity at ≥ 2 mg/kg. One animal at 6 mg/kg had tubular casts with epithelial necrosis and hydronephrosis.

Liver- Minimal single cell hepatocellular necrosis was noted in all treated groups with the incidence highest at 2 mg/kg (3 animals, vs 1 at 4 and 6 mg/kg). Subacute, multifocal to coalescing necrosis with neutrophilic mononuclear infiltrates, mineralization, and necrotizing arteritis and fibrosis was noted in one animal at 6 mg/kg.

Pancreas- Single acinar cell necrosis was noted in 1/4 (minimal) and 4/4 animals (minimal to mild) was noted at 2 and 4 mg/kg, respectively.

Large Intestine- One animal at 6 mg/kg had fibrosis, glandular dilatation, and mucosal epithelial hyperplasia and two animals had mild neutrophilic mononuclear infiltration.

Table 57: Histopathological Findings of Note with Severity* in Rats Administered PR-171 by Intravenous Bolus (n=3 or 4/group)

Organ	Finding	Dose (n)			
		0 mg/kg (4)	2 mg/kg (4)	4 mg/kg (4)	6 mg/kg (3)
Fat (mesenteric)	Mononuclear infiltration-neutrophilic, perivascular		1-minimal 1-mild	1-mild	1-minimal
	Histiocytic mononuclear infiltration-neutrophilic, w/ adipocyte degeneration			1-minimal 1-mild	1-minimal 1-mild
Heart	Myocardial hypercellularity w/ myofiber atrophy		1-mild 1-moderate	2-mild	2-mild
	As above, with necrosis			1-mild 1-moderate	1-moderate
Kidney	Tubular vacuolation		1-minimal 1-mild	1-minimal 1-mild	2-minimal 1-moderate
	Tubular casts w/ epithelial necrosis				1-mild
	Hydronephrosis				1-moderate
Large Intestine	Hyperplasia-mucosal epithelial				1-moderate
	Glandular dilatation				1-moderate
	Fibrosis-interstitial				1-moderate
	Mononuclear infiltration-neutrophilic				2-mild
Liver	Hepatopathy-vacuolar nondiscrete	1-mild 1-moderate	2-moderate	1-moderate	2-moderate 1-mild
	Necrosis-single cell hepatocellular		3-minimal	1-minimal	1-minimal
	Mononuclear infiltration-neutrophilic, multifocal with necrotizing arteritis and fibrosis				1-moderate
	Necrosis- subacute, multifocal to coalescing, neutrophilic-mononuclear infiltrates and mineralization				1-moderate
Lung	Hemorrhage- acute	1-mild			
	Mononuclear infiltration-neutrophilic, interstitial, subpleural		3-mild 1-moderate	1-mild 3-moderate	2-mild 1-moderate
	Hypertrophy/hyperplasia-epithelial		3-mild 1-moderate	1-mild 3-moderate	2-mild 1-moderate
	Histiocytosis-alveolar		3-mild 1-moderate	1-mild 3-moderate	2-mild 1-moderate
	Hypertrophy-mesothelial		3-mild 1-moderate	1-mild 3-moderate	2-mild 1-moderate
	Hypertrophy-interstitial cell		3-mild 1-moderate	1-mild 3-moderate	2-mild 1-moderate
	Hypertrophy- medial artery		3-mild 1-moderate	1-mild 3-moderate	2-mild 1-moderate
	Edema-intraalveolar		2-mild 2-moderate	1-mild 3-moderate	2-mild 1-moderate
	Neutrophilic infiltration in bronchioles			2-moderate	
	Mineralization-pulmonary artery		1-mild		
Pancreas	Necrosis single acinar cell		1-minimal	2-minimal 2-mild	
Spleen	Extramedullary hematopoiesis	1-mild	3-moderate	1-mild 2-moderate	2-moderate 1-severe
	Lymphoid atrophy	1-moderate	1-mild	2-mild	1-mild

*Pathologist often gave a range of severity; only most severe designation is included.

Study title: A 4 Week Intravenous Injection Neurotoxicity Study of Bortezomib and Carfilzomib in Male Sprague Dawley Rats with a 4 Week Recovery Period

Study no.: TR-00297-171
 Study report location: eCTD: 4.2.3.2.1.TR-00297-171
 Conducting laboratory and location: (b) (4)
 Date of study initiation: April 27, 2007
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: PR-171, T08RD02, 99.3%
 Bortezomib, 106677, not supplied

Key Study Findings

- Study was inadequate due to lack of positive control for assays, explanation of results, and no units used in Tables.
- Neurotoxicity (functional or histopathological findings) was not clearly evident at the doses used for either drug. Since bortezomib is reportedly neurotoxic, the study is not considered useful in assessing neurotoxicity of carfilzomib.

Methods

Doses: 2 mg/kg (0.2 mg/kg bortezomib)
 Frequency of dosing: PR-171: Days 1,2,8,9,15,16,22, and 23
 Ctrl and bortezomib: Days 1,4,8,11,15,18,22, 25
 Route of administration: IV bolus
 Dose volume: 5 mL/kg (2 mg/mL)
 Formulation/Vehicle: 10% HPBCD (Hydroxypropyl- β -cyclodextrin)/ 50 mM sodium citrate, pH 3.5
 Species/Strain: Rat/Sprague-Dawley (CrI:CD® (SD)IGS BR)
 Number/Sex/Group: 20 males/group with 10 for main and 10 for recovery
 Age: 8 weeks
 Weight: ♂230-260g
 Satellite groups: None
 Unique study design: Neurological examinations (Functional Observational Battery, Von frey, Hot Plate, Tailflip) were conducted twice before, weekly during and during weeks 1 and 4 of recovery. Nerve electrophysiological measurements were conducted twice pretreatment, on Days 15 and 27 of treatment period and Day 41 of recovery period.
 Deviation from study protocol: None reported

Observations and Results
Mortality

None

Clinical Signs

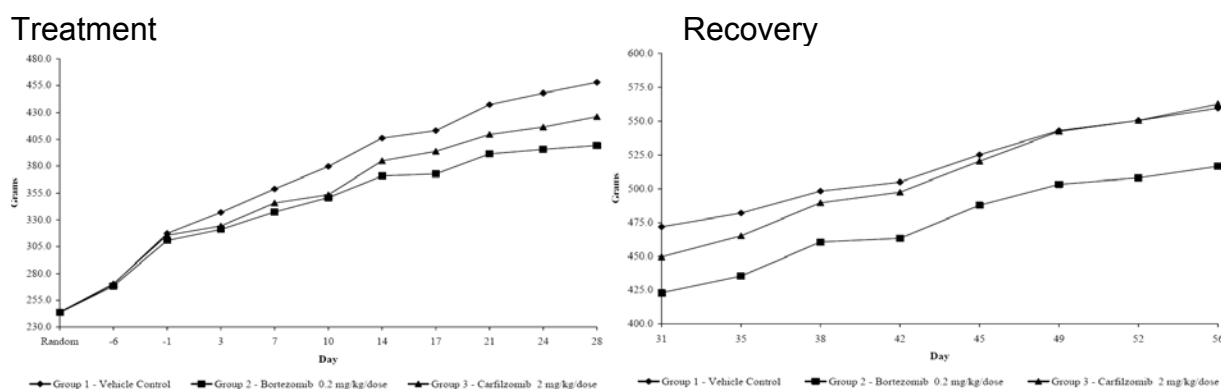
Not remarkable.

Body Weights

There was a significant decrease in mean body weight by 7% and body weight gain by 8% in animals treated with bortezomib compared to control. Carfilzomib treated animals and showed no differences during recovery period.

There was a significant decrease in mean body weight by 13% and body weight gain by 8% in animals treated with bortezomib compared to control. After the recovery period, body weight gains increased by 8%, but were still weighed 8% less compared to control.

Figure 37: Group Mean Body Weights of Male Rats Treated with Carfilzomib or Bortezomib



(Figure excerpted from Applicant's package)

Feed Consumption

Consumption was consistent with observed body weight differences. There was a 13% decrease for bortezomib and an 8% decrease for carfilzomib compared to controls. Food consumption increased for both treated groups comparable with control during recovery period.

Ophthalmoscopy

Not remarkable.

Hematology, Clinical Chemistry, Urinalysis, Organ Weights, Ophthalmoscopy, Toxicokinetics

Not performed.

Gross Pathology

Not remarkable.

Histopathology

Adequate Battery- Only Peripheral and Central Nervous System (PNS and CNS) tissues were examined.

PNS

Sciatic nerve (mid-thigh region) - longitudinal and cross-sections

Sciatic nerve (at sciatic notch) - longitudinal and cross-sections

Sural nerve (at knee) - longitudinal and cross-sections

Peroneal nerve (mid portion) - longitudinal and cross-sections

Digital nerve (toe) - common digital nerve, cross section

CNS

Lumbar dorsal root ganglion (L4) - cross section

Lumbar dorsal root (L4) - cross section

Lumbar ventral root (L4) - cross section

Cervical dorsal root ganglion (C5) - cross section

Cervical dorsal root (C5) - cross section

Cervical ventral root (C5) - cross section

Grossly abnormal central and peripheral nervous system tissues

Peer Review- No

Histological Findings- Not remarkable for bortezomib treated animals, so sponsor did not perform for carfilzomib.

Special Evaluation- Functional Observational Battery

Animals treated with carfilzomib had increased incidences of flaccid body tone (Weeks 1-3), rapid tail pinch reflex (Weeks 1-3), and piloerection (Week 4) and decreased pinpoint pupil size (Weeks 2-3) compared to control. No significant or remarkable differences were noted during pre-treatment period or recovery compared to control. There were some differences between carfilzomib and bortezomib, but carfilzomib was not different from control.

Table 58: Notable Findings in FOB in Male Rats Administered Intravenous 2 mg/kg Carfilzomib or 0.2 mg/kg Bortezomib for 4 Weeks (n=20)

Observation	Week	Control	Bortezomib	Carfilzomib
Flaccid body tone	1	1	3	9**
	2	1	5	10**
	3	3	2	7
Rapid Tail Pinch	1	2	2	6
	2	4	5	7
	3	1	8*	5
Partially Closed Eyes	2	1	5	2
	3	2	5	0
Pinpoint Pupil Size (small)	2	5	3	1
	3	5	11	3&
Abnormal Arousal (total)	3	9	13	7&
Piloerection	4	2	2	5

*, P<0.05 compared to control; **, P<0.01 compared to control; &, P<0.05 compared to Bortezomib

Special Evaluation- Tail Flick Test

Not remarkable. The changes observed in test article-treated groups were inconsistent in direction and magnitude compared to pre-treatment and/or control values.

Special Evaluation- Hot Plate Response

There were comparable response time decreases of 10-40% and 11-37% noted for bortezomib and carfilzomib, respectively, compared to control values and/or pre-treatment test values over weeks 1, 2, and 4. The changes were not statistically significant. At the end of recovery, animals treated with carfilzomib still had a 19% decrease in response time, while bortezomib completely recovered.

Special Evaluation- Mechanical (Tactile) Allodynia (Von Frey Test)

Not remarkable. The changes observed in test article-treated groups were inconsistent in direction and magnitude compared to pre-treatment and/or control values.

Special Evaluation- Electrophysiology (Nerve Conduction Velocity)

There was no significant effect on electrophysiologic measures of peripheral nervous system activity observed with treatment of carfilzomib. There was a significant decrease of 15% in caudal amplitude on Day 27 only when animals were treated with bortezomib.

Study title: A 3/6 Month Intravenous Toxicity Study of Carfilzomib in Rats with an 8-week Recovery Period

Study no.: TR-0072-171
Study report location: eCTD: 4.2.3.2.1.TR-0072-171
Conducting laboratory and location: (b) (4)
Date of study initiation: August 21, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: PR-171, 050307, 93.33% and
PR-171, PC F001, 95.8%

Key Study Findings

- Dose-dependent morbidity/mortality was noted at ≥ 2 mg/kg in both sexes preceded by a number of clinical signs, including signs of stress. Causes of deaths were attributed to GI tract hemorrhage/necrosis, cardiac, or kidney failure.
- Males had decreases in body weights at all doses with decreased food consumption.
- Increased neutrophils, monocytes, and fibrinogen at all doses with decreased albumin at 4 mg/kg were noted suggesting acute phase response in both sexes at termination.
- Kidney toxicity in both sexes was noted, indicated by increased BUN, creatinine, and phosphorus with enlarged kidneys, and microscopic findings of chronic progressive nephropathy and glomerulonephropathy.
- Microscopic findings consistent with significant cardiac fibrosis and cardiac failure were noted in unscheduled necropsy. Milder findings were noted in scheduled sacrifice necropsy including
- Findings of liver toxicity were noted in females at 3 and 6 months with increased liver weight and triglycerides, decreased ALT, AST and cholesterol with centrilobular hepatocellular hypertrophy and periportal hepatic fatty vacuolation at all doses; ALP was increased at 4 mg/kg. Males at 4 mg/kg had increased liver weights and decreased triglycerides and AST at 6 months. Males at 4 mg/kg had comparable microscopic findings as females at 3 months, but these were not found at 6 months. Decreased total hepatic CYP protein content was observed in both sexes at 4 mg/kg.
- Increased neutrophils, monocytes, reticulocytes, WBCs and leukocytes and decreased RBCs were noted with microscopic correlates in organs related to hematopoiesis such as pigmented Kupfer cells in the liver, bone marrow hypercellularity (increased reticulocytes), gross and microscopic mesenteric lymph node findings (increased mast cells), and gross and microscopic spleen findings (decreased marginal zone width and/or cellularity), including increased spleen weight.

Methods

Doses: 1, 2, 4 mg/kg
 Frequency of dosing: Three or six dosing cycles of 28 day: Days 1-2, 8-9, and 15-16 of the 28 day cycle
 Route of administration: IV bolus
 Dose volume: 2 mL/kg
 Formulation/Vehicle: 10% HPBCD (hydroxypropyl- β -cyclodextrin)/ 50 mM sodium citrate, pH 3.5
 Species/Strain: Rat/Sprague-Dawley Crl:CD® (SD)IGS BR
 Number/Sex/Group: 25/sex/group
 Age: 8 weeks
 Weight: ♂:254-357g, ♀:163-234g
 Satellite groups: TK: 8/sex/treatment group - blood was drawn pre-dose, 5, 15, and 60 min post-dose on Days 1, 57, 142
 Unique study design: 10/sex/group will be sacrificed 12 days after last dose of Cycle 3 (Day 85), 1 day after last dose of Cycle 6 (Day 158), and 5/sex/group 8 weeks after end of Cycle 6 (Day 224). Additionally, a functional observational battery was performed on all main study animals with pretest, Days 81 - 84 prior to interim necropsy, Days 154 -155 prior to terminal necropsy and Recovery Days 53 - 54 prior to recovery necropsy. Liver samples were frozen and subjected to CYP analysis.
 Deviation from study protocol: Two significant deviations were reported: due to adverse clinical or physical signs, the following animals were not dosed at the indicated cycles/days:

Animal No.	Cycle(s)	Dose Administration	Day(s)
4016, 4023, 4513, 4518, 4523, 4525	3	Second	58
4523, 4525	3	Fourth	65
4019	3, 5	Second	72, 114, 121

Animal #1026 replaced Animal #1003 after the second week's dosing in Cycle 1, and therefore was not dosed on Days 1, 2, 8 and 9. Four minor deviations that did not affect study conduct and interpretation.

Observations and Results

Mortality

Dose-dependent treatment-related mortality was observed in the main group at ≥ 2 mg/kg; there was mortality observed in the 4 mg/kg TK group. These animals were found dead or humanely euthanized as moribund. There were 1, 3, and 16 unscheduled deaths at 1, 2, and 4 mg/kg, respectively, with 0/1, 2/3, and 15/16 were determined to be test article-related, regardless if the cause of death was identified. The one death at 1 mg/kg was not related to treatment, and most probably due to dosing error. Overall, the deaths appeared to occur sooner in the dosing period at 4

mg/kg compared to 2 mg/kg. The identified main causes of death were cardiac fibrosis (4), GI hemorrhage/ necrosis (3), cardiac arrest (2), renal pathology (2), septic thrombus (1) and experimental (1). Glomerulonephropathy was observed in 4 males at 4 mg/kg and considered the causing or contributing to the death of 2 males at 2 mg/kg. Seven deaths were undetermined, but the Applicant states the condition and clinical signs of the animals were consistent with known causes. Mortality was higher in males than females with 4 of 4 total animals at 2 mg/kg and 10/16 at 4 mg/kg. However females tended to die sooner in the dosing period including 2 cardiac failure deaths in the first cycle.

Findings that only occurred in the unscheduled deaths were related to the GI tract and adipose tissue which were observed in shorter duration studies. There were 2 males at 4 mg/kg with red discoloration of the GI tract (stomach through cecum) necrosis, hemorrhage/congestion and erosion/ulceration. In 2 females at 4 mg/kg that died of cardiac failure, there was yellow colored adipose tissue suggestive of jaundice.

Table 59: Probable Causes of Unscheduled Deaths in Main Study Based on Histopathology / Sacrifices Occurring during 6 Cycles of Dosing with PR-171

mg/kg	Animal No.	M/H/FD	Day	Cause of death
1	2007M	H	77	Fractured palate
2	3002M	FD	44	undetermined
	3022M	FD	133	Glomerulonephropathy
	3025M	M	165	Cardiac Fibrosis and glomerulonephropathy
4	4005M	FD	43	Septic thrombus
	4011M	M	61	Gastric hemorrhage/necrosis
	4012M	M	116	GI hemorrhage/necrosis
	4014M	FD	58	Gastric hemorrhage/necrosis
	4015M	FD	58	undetermined
	4019M	FD	122	undetermined
	4020M	FD	94	undetermined
	4021M	FD	129	Cardiac Fibrosis
	4023M	FD	101	Cardiac Fibrosis
	4025M	M	145	undetermined
	4501F	FD	6	Cardiac failure
	4505F	FD	59	undetermined
	4506F	FD	58	Renal tubular necrosis
	4512F	FD	5	Cardiac failure
	4521F	FD	129	undetermined
	4525F	FD	87	Cardiac fibrosis

M = suffix for animal number (male); F = suffix for animal number (female);

FD = found dead; M = moribund; H = Humane

(Table 3.2-1, pg. 44 excerpted from Applicant's package)

Clinical Signs

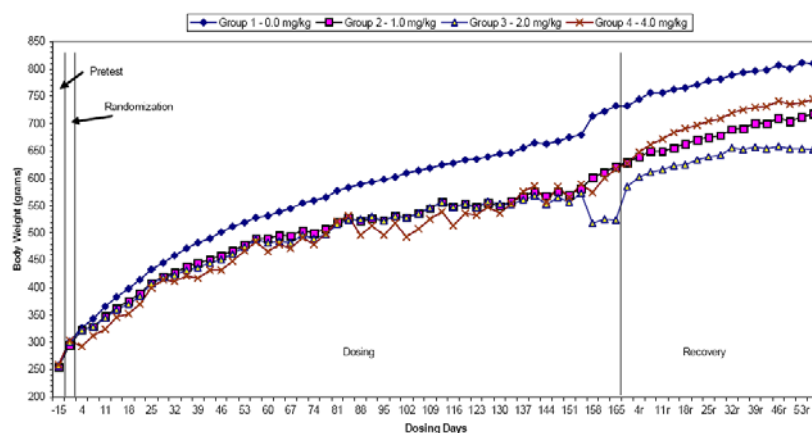
Increased clinical signs consistent with declining physical condition were observed at 4 mg/kg, including decreased food consumption and fecal volume, fur staining (anogenital, snout), red tears, hunched appearance, pallor, lethargy, decreased activity, and/or labored breathing. Piloerection was observed at ≥ 2 mg/kg with the observation occurring earlier in the dosing cycles. No treatment-related findings were observed during the recovery period.

Body Weights

Males had significantly decreased body weights and body weight gains at all doses at 12 and 22 weeks compared to control animals. There was no dose-dependency, but body weight appeared to be reduced during the periods of dosing rather than the 12 days between each cycle. Significant weight decreases began as early as Day 4 at 4 mg/kg and Day 11 for 1 and 2 mg/kg and maintained through the dosing period. The reduced number of animals at 4 mg/kg may have skewed the values from demonstrating a dose relationship since the sicker and lighter animals will have died off. The recovery was partial with the difference rising from -13-14% to -9-10% and an increased rate of weight gain at all dose compared to control.

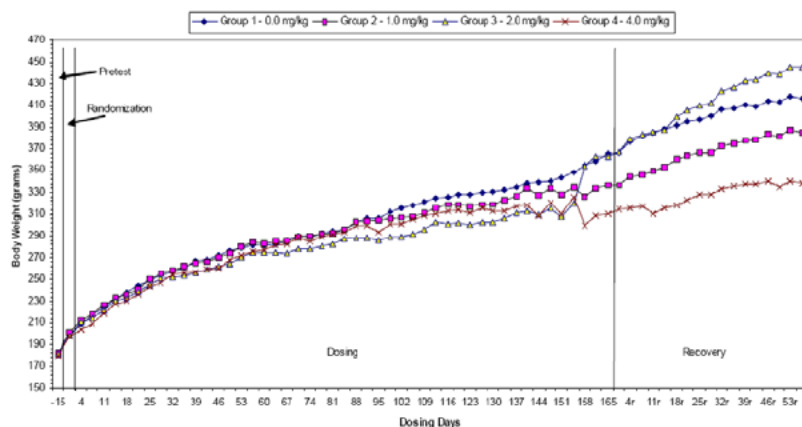
For females, no significant effects on body weight and body weight gain were noted. There was a non-significant 4-8% decrease in body weights that lack a dose relationship at Week 22 compared to control.

Figure 38: Body Weights in Male Rats Given 6 Months of Intravenous Doses of PR-171



Best Available Copy

(Figure 1, pg. 80 excerpted from Applicant's package)

Figure 39: Body Weights in Female Rats Given 6 Months of Intravenous Doses of PR-171

Best Available Copy

(Figure 2, pg. 81 excerpted from Applicant's package)

Table 60: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) in Rats Administered Intravenous PR-171 for 12 or 22 Weeks

Males	Week -1 BWt (g)	Week 12 BWt (g)	% Gain	% Δ	N	Week 22 BWt (g)	% Gain	% Δ	N
0 mg/kg	302.2	583.2	93	--	25	680.0	125	--	15
1 mg/kg	295.7	528.4**	79	-9	24	581.7**	97	-14	15
2 mg/kg	299.9	523.6**	75	-10	24	572.3**	91	-16	14
4 mg/kg	303.3	530.8**	75	-9	21	588.6**	94	-13	6
Females									
0 mg/kg	199.0	295.9	48	--	25	348.2	75	--	15
1 mg/kg	202.0	295.9	47	0	25	335.1	66	-4	11
2 mg/kg	198.0	287.5	45	-3	25	319.4	61	-8	11
4 mg/kg	197.4	293.7	49	-1	21	325.5	65	-6	11

**, P<0.01 v. control

Table 61: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) Following 10 Weeks of Recovery

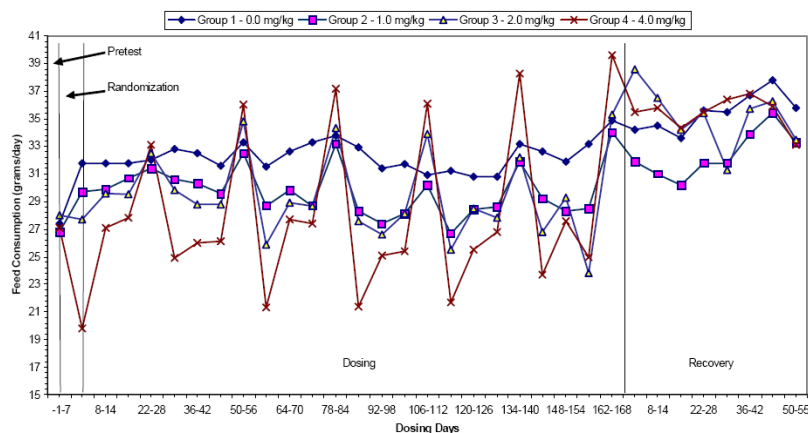
Males (mg/kg)	Week 23 BWt (g)	Week 30 BWt (g)	% Gain	% Δ	N
0	713.7	809.8	13	--	5
1	600.8	717.8	19	-11	5
2	517.9	652.9	26	-19	3
4	574.3	744.2	30	-8	3
Females					
0	355.7	416.2	17	--	5
1	326.1	385.3	18	-31	5
2	354.3	445.7	26	7	5
4	299.6	338.8	13	-19	3

Feed Consumption

Overall, males showed a dose-dependent decrease in food consumption consistent with the observed body weight decreases. Consumption in the treated groups followed a

pattern that showed decreases during the week of dosing and increases between each cycle. Moreover, the control group showed little or no cyclicity. This cyclical pattern was not evident in the recovery period.

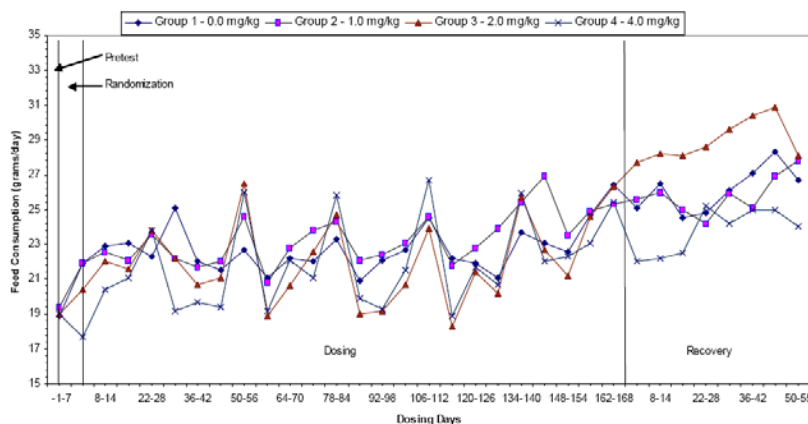
Figure 40: Mean Feed Consumption in Males Given 6 Months of Intravenous Doses of PR-171



(Figure 3, Table pg. 82 excerpted from Applicant's package)

Females had a dose-dependent decrease in food consumption at ≥ 2 mg/kg that ranged from 7-19%, compared to control. Like males, consumption in the treated groups followed a pattern that showed decreases during the week of dosing and increases between each cycle. This cyclical pattern was not evident in the recovery period.

Figure 41: Mean Feed Consumption in Females Given 6 Months of Intravenous Doses of PR-171



(Figure 4, Table pg. 83 excerpted from Applicant's package)

Hematology

A number of hematological parameters were altered by the administration of PR-171 in both sexes; this is due to the expected effect of the drug product.

Interim: In males, there were dose-dependent (significant) increases in reticulocytes (all), MCV (≥ 2 mg/kg), and MCH (4 mg/kg), significantly increased leukocytes and WBCs at 4 mg/kg; there were dose-dependent (significant) decreases in MCHC (≥ 2 mg/kg) and PT (≥ 2 mg/kg) and decreased RBCs significant at 4 mg/kg. Females had a similar profile, with the following exceptions: reticulocytes and WBCs at all doses were increased in a non-dose-dependent manner and no significant change was noted for leukocytes.

Termination: The findings tended to be significant at lower doses and additional parameters were affected compared to the interim. There were dose-dependent increases (significant) in reticulocytes (≥ 2 mg/kg), fibrin (≥ 2 mg/kg), MCV (all), MCH (all), neutrophils (all), monocytes (all), and leukocytes (2 mg/kg only) and increased WBCs and PT at 4 mg/kg. There were dose-dependent decreases in MCHC (all) and decreased RBCs at 4 mg/kg. Additionally, hematocrit was increased at 1 and 2 mg/kg and platelets were significantly decreased at all doses. Females were comparable to males with only minimal differences.

Recovery: All parameters recovered in males, except PT at 4 mg/kg ($\downarrow 7\%$). RBCs (all doses, $\uparrow 6\text{--}10\%$), hematocrit (all doses, $\uparrow 3\text{--}7\%$) and neutrophils (4 mg/kg, $\uparrow 76\%$), did not recover and reticulocytes (all doses, $\downarrow 30\text{--}35\%$) decreased, and hemoglobin (all doses, $\uparrow 4\text{--}9\%$) increased in females.

Table 62: Percent or Fold (X) Change in Selected Mean Hematology Parameters at Termination Compared to Control in Rats Given 6 Months of Intravenous Doses of PR-171

Parameter	Males			Females		
	Dose (mg/kg)					
	1	2	4	1	2	4
Neutrophils (10 ³ /μL)	+ 102**	+ 76**	↑5.2X	+ 85**	+ 75**	↑3.0X**
Monocytes (10 ³ /μL)	+ 84**	↑3.1X**	↑3.4X**	+102**	↑2.7X**	↑4.0X**
Leukocytes (10 ³ /μL)		↑3.4X**	↑13.1X	↑2.6X**	↑3.7X**	↑6.8X**
Reticulocytes (10 ⁹ /L)		+ 30**	+ 53**			+ 70**
WBCs (10 ³ /μL)			+ 65**			+ 42*
RBCs (10 ⁶ /μL)			- 16**			- 8*
Platelets (10 ³ /L)	- 28**	- 20**	- 52**	- 18*	- 43**	- 48**
MCV (fL)	+ 5*	+11**	+ 18**	+ 3*	+ 6**	+ 14**
MCH (pg)	+ 3*	+ 6**	13**			+ 9**
MCHC (g/dL)	- 1*	- 2**	- 2**	- 3**	- 4**	- 5**
Ht (%)	+ 8**	+ 11*				+ 5*
Hb (g/dL)	+ 6*	+ 6*				
Fibrin (mg/dL)	+ 36**	+ 62**	+ 112**		+ 43**	+ 109**
Prothrombin Time (sec)			+ 9**			

*, $P < 0.05$; **, $P < 0.01$

Clinical Chemistry

Interim: Males had significantly decreased AST at all doses, ALT at 4 mg/kg, and glucose (dose-dependent at ≥ 2 mg/kg). Bilirubin and phosphorus showed dose-dependent increased that were significant at 4 mg/kg. Females had similar decreases

to males for AST and increases for bilirubin and phosphorus. There was a significant decrease in total protein, albumin and creatinine at 4 mg/kg.

Termination: In general, the effects were similar to the interim with new parameters affected. At 4 mg/kg, significant decreases were noted in AST, total protein, globulin and potassium and significant increases were noted in BUN and creatinine. There were significant dose-dependent decreases in albumin at ≥ 2 mg/kg and dose-dependent increase in phosphorus that was significant at 4 mg/kg. For females at 4 mg/kg, there were significant increases in ALP, phosphorus, BUN, and triglycerides and decreases in total protein, albumin, and potassium. AST and ALT showed a significant decrease at all doses. Cholesterol was significantly increased but not dose-related.

Recovery: All parameters recovered except a partial recovery for total protein ($\downarrow 6$ -10%) in males at ≥ 2 mg/kg. ALT and AST (increased by 5.3X and 9.1X, respectively), and bilirubin (increased by 66%) did not recover in females at 4 mg/kg.

Table 63: Percent or Fold (X) Change in Selected Clinical Chemistry Parameters at Termination Compared to Control in Rats Given 6 Months of Intravenous Doses of PR-171

Parameter	Males			Females		
	Dose (mg/kg)					
	1	2	4	1	2	4
ALP (U/L)						+ 54*
ALT (U/L)				- 42**	- 63**	- 43**
AST (U/L)			- 44**	- 32*	- 51**	- 51**
BUN (mg/dL)			↑6.1X**			+ 38**
CREAT (mg/dL)			↑2.3X**			
TPROT (g/dL)			- 21**			- 20**
ALB (g/dL)		- 8**	- 22**			- 21**
GLOB (g/dL)			- 19**			- 19**
Phos (mmol/L)			+ 33**			+ 16**
K (mmol/L)			- 24**			- 12*
TRIG (mg/dL)			↓2.6X**			↑ 6.7X**
CHOL (mg/dL)				- 34*	-31*	-24*

*, $P < 0.05$; **, $P < 0.01$

Urinalysis

Not remarkable.

Ophthalmoscopy

Evaluation performed on Day 149 and Recovery Day 53 by Lionel F. Rubin, VMD. Not remarkable.

Organ Weights

Interim: Both sexes at 4 mg/kg had significant increases in heart weights. Spleen weights were significantly increased in females at ≥ 2 mg/kg and in males at 4 mg/kg. Kidney weights were increased in males only at 4 mg/kg.

Terminal: The noted effects of >10% on organ weights were greater than at the interim. Due to the BW differences noted in males, the organ weight/BW is the more appropriate comparison. In males at all doses, there were dose-dependent increases in heart and kidney and nondose-dependent increases for prostate; all were significant except at 4 mg/kg for kidney and prostate due to few animals. There were significant dose-dependent increases in liver and spleen weights at ≥ 2 mg/kg for both sexes. The adrenal had a significant increase at 4 mg/kg. The females had no significant BW changes, so both absolute and organ weight/BW comparisons are appropriate. There were significant increases in liver (dose-dependent) and kidney (nondose-dependent) at all doses. The heart and spleen showed dose-dependent increases at ≥ 2 mg/kg. The thymus and ovary were increased and the uterus was decreased at 4 mg/kg. The thymus was significantly increased at 2 mg/kg, but was elevated at the other doses as well.

Table 64: Percent Changes in Terminal Organ Weight Values Compared to Control Following 6 Months of Intravenous Administration of PR-171 to Rats

Organ	Weight Change	Males			Females		
		Dose (mg/kg)					
		1	2	4	1	2	4
Adrenal	Absolute			+ 23			
	% BW			+ 39*			
Heart	Absolute	- 4	+ 10	+ 35**		+ 2	+ 20**
	% BW	+ 13*	+ 26**	+ 53**		+ 20**	+ 28**
Kidney	Absolute	- 7	+ 10	+ 67	+ 18	+ 5	+ 19**
	% BW	+ 10*	+ 26**	+ 88	+ 20**	+ 22**	+ 26**
Liver	Absolute			+ 26*	+ 25*	+ 20**	+ 58**
	% BW		+ 16**	+ 44**	+ 29**	+ 41**	+ 67**
Spleen	Absolute		+ 16	+ 44		+ 22*	+ 56**
	% BW		+ 33**	+ 65**		+ 44**	+ 65**
Thymus	Absolute					+ 13	
	% BW					+ 33*	
Ovary	Absolute						+ 41**
	% BW						+ 53**
Uterus	Absolute						- 44**
	% BW						- 41*
Prostate	Absolute	+ 7	+ 12	+ 10			
	% BW	+ 27*	+ 29*	+ 27			

*, P<0.05; **, P<0.01

Recovery: In males, there was little or no recovery in kidney ($\uparrow 24$ -66%, significant at all doses), heart ($\uparrow 11$ -24%, significant at 2 mg/kg) and spleen weights ($\uparrow 21$ -41%, significant at 1 and 2 mg/kg) based on organ weight/BW at all doses.

Gross Pathology

Almost all significant gross pathology occurred in the unscheduled deaths and is described above.

Interim: In males at 4 mg/kg, there were 1/9 and 2/9 with enlarged or discolored kidneys, respectively. One of 10 females at 2 mg/kg had a discolored liver and 1/8 had a dilated kidney pelvis at 4 mg/kg.

Terminal: In males at 4 mg/kg, there were 1/3 with enlarged kidneys and 1/3 with enlarged spleens. There were 2/10 and 1/3 at 2 and 4 mg/kg, respectively with discolored mesenteric lymph nodes (LN) and 1/10 at 2 mg/kg with enlarged LN. Enlarged spleens were noted in 1 each at 2 and 4 mg/kg. In females, there were 3/8 at 4 mg/kg with discolored mesenteric LN, and 1/10 and 2/8 with enlarged LN at 2 and 4 mg/kg, respectively. There were 1/8 and 1/8, respectively, with enlarged liver and spleen at 4 mg/kg.

Histopathology

Adequate Battery- Yes, plus sciatic, sural and peroneal nerves were also examined in all animals and timepoints at 1 and 2 mg/kg.

Peer Review- none

Histological Findings-

Overall, in the animals that survived to schedule sacrifice, there were few significant findings. The target organs were similar between sexes and included the bone marrow, heart, kidney, mesenteric lymph nodes (LN), and spleen. Findings at 4 mg/kg tended to be no more severe than at ≤ 2 mg/kg, and except for a few instances, were minimal or slight in severity. The findings were noted at both the interim and terminal sacrifice in these organs. There were findings in the liver of both sexes at interim, but findings were not noted in males at termination.

The findings in unscheduled deaths are described under mortality. Consistent with scheduled sacrifice, causes of test article-related unscheduled deaths based on histopathology were heart, kidney and/or liver failure, except two cases of GI hemorrhage/necrosis. This is markedly different- but expected- from the generally mild findings in schedule necropsies.

Kidney- There were 2 males with slight and moderate glomerulonephropathy noted at 2 mg/kg (interim) and 4 mg/kg (termination), respectively. Minimal to moderate chronic progressive nephropathy (CPN) was present in all groups, both sexes, and both timepoints with dose-dependent increases in incidence. Females appeared to be more sensitive and with increased severity noted; males at termination showed no change in severity. Dose-dependent increases in minimal to moderate tubular vacuolation were noted in males at termination. Comparable CPN was found in all recovery groups, suggesting that this finding may be an age-related background finding in rats.

Liver- Notable findings above background based on incidence and/or severity were noted at ≥ 2 mg/kg. Slight hepatocellular hypertrophy was noted at 4 mg/kg in interim females and minimal to slight was noted in termination females at ≥ 2 mg/kg. There were minimal to slight pigmented Kupfer cells in both sexes at 4 mg/kg at interim and in termination female at 4 mg/kg. There was an increased incidence in periportal fatty vacuolation in both sexes at 4 mg/kg (interim) and at ≥ 2 mg/kg (termination). Although

the finding was present in animals at ≤ 1 mg/kg at interim, no animals at termination had this finding. The findings in the liver did not recover.

Lymph Node (Mesenteric)- Notable findings above background based on incidence and/or severity were noted at ≥ 1 mg/kg. Minimal to slight increased mast cells were observed in males at ≥ 2 mg/kg at all timepoints, except 4 mg/kg recovery, and were minimal in severity in females at ≥ 1 mg/kg at all timepoints except 4 mg/kg recovery. Minimal to slight increased lymphocytes were noted in interim males at ≥ 1 mg/kg, and moderate increased lymphocytes were noted in two females at both interim and recovery, but not at termination. Intrasinusoidal erythrocytes were present at ≥ 2 mg/kg in both sexes at termination. Minimal findings of brown pigment were noted at ≥ 2 mg/kg in both sexes at termination. Findings present at termination did not recover.

Spleen- The most significant finding was minimal to moderate decreased marginal zone width and/or cellularity in both sexes at ≥ 2 mg/kg at interim and ≥ 1 mg/kg at termination, with a dose-dependent effect on incidence (based on % of animals with finding). Slight increased histiocytes were noted in one termination male at 2 and 4 mg/kg. Partial recovery was noted with generally minimal findings at ≥ 1 mg/kg in both sexes.

Bone Marrow- Minimal to slight hypercellularity was noted in both sexes at both treatment evaluations with increased incidence at ≥ 2 mg/kg. Females had incidences at all doses, while males only had findings at ≥ 2 mg/kg. This is an expected response to the increased blood cells noted in the hematology.

Heart- The most significant findings were minimal myofiber degeneration/necrosis in two termination males at 2 mg/kg and a single male with moderate myocyte hypertrophy. Findings of minimal fibrosis at 4 mg/kg in 1 interim male and 1 of each sex at termination, minimal congestion/hemorrhage in one termination male at 1 mg/kg, and cardiomyopathy in both sexes at all doses and both were also noted.

The following organs had some minor findings that warranted documentation based on other findings or observations from other studies.

Adrenal- Minimal to slight cortical vacuolation was noted only in interim males at 4 mg/kg.

Lung- In termination females at 4 mg/kg, there was 1/8 animal with minimal perivascular mixed inflammatory cell infiltrate and 3/8 with minimal eosinophilic alveolar material.

Pancreas- At termination only at 4 mg/kg, there was one animal per sex with minimal apoptosis and one female with minimal and one with slight acinar atrophy.

Table 65: Histopathological Findings of Note with Incidence and Severity in Rats Given 3 Months of Intravenous Doses of PR-171 (Interim)

Organ	Finding	Sex	Dose (n)			
			0 mg/kg (10♂, 10♀)	1 mg/kg (9♂, 10♀)	2 mg/kg (9♂, 10♀)	4 mg/kg (9♂, 8♀)
Adrenal	Cortical vacuolation	M				2-minimal 2-slight
Bone Marrow	Hypercellularity	M			4-minimal 1-slight	6-minimal 3-slight
		F	2-minimal 2-slight	7-minimal 1-slight	2-minimal 7-slight	2-minimal 5-slight
Heart	Fibrosis	M				1-minimal
	Cardiomyopathy	M	3-minimal		1-minimal	1-minimal 1-slight
		F	1-minimal		3-minimal	2-minimal
Kidney	Glomerulonephropathy	M			2-slight	
	Chronic progressive nephropathy	M	2-minimal	2-minimal	3-minimal	3-minimal 1-mild
		F	1-minimal	1-minimal	2-minimal 1-slight	2-minimal
	Tubular vacuolation	M	1-slight	1-minimal		2-minimal
	Dilated pelvis	F				1-slight
Liver	Hepatocellular hypertrophy	F				5-slight
	Pigmented Kupfer cells	M				2-minimal
		F				6-minimal 2-slight
	Periportal fatty vacuolation	M	1-minimal 1-slight	3-minimal 1-slight	1-slight	7-minimal 1-slight
		F	1-minimal		1-minimal	4-minimal 1-slight
	Periportal mixed inflammatory cell infiltrate	M	1-slight			1-minimal 1-slight
		F	2-minimal			1-slight
Mesenteric Lymph Node	Increased Mast cells	M			1-minimal 1-slight	3-minimal
		F		1-minimal	1-minimal	2-minimal
	Increased lymphocytes	M		1-slight		2-minimal
		F			1-minimal	1-moderate
	Chronic inflammatory cell infiltrate	F				1-slight
Spleen	Congestion	M				1-slight
	Decreased marginal zone width and/or cellularity	M			3-minimal 1-slight	1-minimal 1-slight
		F			2-minimal	3-minimal 2-slight

Table 66: Histopathological Findings of Note with Incidence and Severity in Rats Given 6 Months of Intravenous Doses of PR-171 (Termination)

Organ	Finding	Sex	Dose (n)			
			0 mg/kg (10♂, 10♀)	1 mg/kg (10♂, 10♀)	2 mg/kg (10♂, 10♀)	4 mg/kg (3♂, 8♀)
Bone Marrow	Hypercellularity	M		1-minimal	5-minimal	2-minimal
		F	4-minimal	5-minimal	5-minimal 3-slight	3-minimal 5-slight
Heart	Fibrosis	M				1-minimal
		F			1-minimal	1-minimal
	Myofiber degeneration/necrosis	M			2-minimal	
	Myocyte hypertrophy	M				1-moderate
	Congestion/hemorrhage	M		1-minimal		
	Cardiomyopathy	M	4-minimal			
		F			1-minimal	
Kidney	Glomerulonephropathy	M				2-moderate
	Chronic progressive nephropathy	M	1-minimal	3-minimal	4-minimal	
		F	2-minimal	2-minimal 2-moderate	3-minimal 1-slight 2-moderate	2-minimal 2-slight
	Tubular vacuolation	M			1-minimal	1-minimal 1-slight 1-moderate
		F	1-minimal			
	Cyst	M		1-minimal		
		F			1-slight	
Liver	Hepatocellular hypertrophy	F			8-minimal	6-minimal 2-slight
	Pigmented Kupfer cells	M	1-minimal	1-minimal		1-minimal
		F				4-minimal 4-slight
	Periportal fatty vacuolation	M	1-minimal	3-minimal	3-minimal	2-minimal
		F			3-minimal 2-slight	5-minimal 1-slight
	Periportal mixed inflammatory cell infiltrate	M			1-minimal	
Lung	Perivascular mixed inflammatory cell infiltrate	M				1-minimal
	Eosinic alveolar material	F				3-minimal
Mesenteric Lymph Node	Increased Mast cells	M			1-minimal 1-slight	3-minimal
		F		1-minimal	1-minimal	2-minimal
	Histiocytic aggregates	M			1-minimal 3-slight	1-slight
		F	2-minimal 1-slight		4-minimal 4-slight	1-slight
	Intrasinusoidal erythrocytes	M			9-Present	3-Present
		F	1-Present		5-Present	8-Present
	Brown pigment	M			4-minimal	
		F			2-minimal	2-minimal
Pancreas	Apoptosis	M				1-minimal
		F				1-minimal
	Acinar atrophy	F				1-minimal 1-slight
Spleen	Decreased marginal zone width and/or cellularity	M		1-minimal	1-slight	1-slight
		F		1-minimal 3-slight	1-minimal 4-slight	3-minimal 4-slight 1-moderate
	Prominent brown pigment	M				1-Present
		F	2-Present	1-Present	1-Present	4-Present
	Increased histiocytes	M		1-minimal	1-slight	1-slight
		F				

Table 67: Histopathological Findings of Note with Incidence and Severity in Rats Given 6 Months of Intravenous Doses of PR-171 (Recovery)

Organ	Finding	Sex	Dose (n)			
			0 mg/kg (5♂, 5♀)	1 mg/kg (5♂, 5♀)	2 mg/kg (5♂, 5♀)	4 mg/kg (3♂, 5♀)
Kidney	Chronic progressive nephropathy	M	2-minimal	2-minimal 1-slight	1-slight	3-minimal 1-slight
		F	2-minimal 1-moderate	1-slight	2-minimal 3-slight	2-minimal 1-moderate
	Tubular vacuolation					1-minimal
Liver	Hepatocellular hypertrophy	F			5-slight	2-slight 1-moderate
	Pigmented Kupfer cells	M				2-minimal
		F				6-minimal 2-slight
	Periportal fatty vacuolation	M	2-minimal	3-minimal	1-slight	1-minimal 1-moderate
		F		2-slight		
Mesenteric Lymph Node	Increased Mast cells	M			3-minimal	
		F		1-minimal	1-minimal 1-slight	
	Increased lymphocytes	F				2-moderate
	Histocytic aggregates	M	2-minimal	1-minimal 2-slight	2-minimal	2-minimal
		F	1-minimal 1-slight	1-minimal 2-slight	3-minimal 1-slight	1-minimal
	Intrasinusoidal erythrocytes	F			1-Present	
	Brown pigment	M			1-minimal	1-minimal
		F		2-minimal	1-minimal	
Pancreas	Chronic inflammatory cell infiltrate	M	1-minimal			1-minimal
		F				2-minimal
Spleen	Decreased lymphocytes	F				2-minimal
	Decreased marginal zone width and/or cellularity	M		2-minimal		3-minimal
		F		1-minimal	1-minimal 2-slight	2-minimal
	Prominent brown pigment	F		2-Present	1-Present	1-Present

Special Evaluation- Functional Observational Battery

Not remarkable.

Special Evaluation- Pharmacodynamics

At all dose levels and times, PR-171 induced >85% inhibition of proteasome activity in whole blood after the first dose. There was minimal recovery following the washout periods at all doses and times.

Special Evaluation- CYP Evaluation (ex vivo)

The analysis was conducted by (b) (4) on portions of frozen liver obtained at necropsy. Samples were collected 24 hrs after last dose of the 3- and 6- month cycle and recovery (n=5/sex/group). Liver microsomes were prepared for enzymatic evaluation and used with appropriate positive controls for each CYP, and expression was determined by qRT-PCR on another sample.

Decreased total hepatic CYP protein content was observed in both sexes at 4 mg/kg. CYP2B, CYP2C, and CYP3A enzyme activity were decreased in both sexes, but slightly increased CYP4A enzyme expression in males at 4 mg/kg. The expression of CYPs was not consistent with activity. CYP1A1 and CYP4A1 expression increased in both sexes and CYP2B1/2 increased in females; CYP3A1 in both sexes and CYP2C11 in males were decreased. Except for the CYP4A1 increase, males showed the effects at all doses. Females were decreased at all doses for CYP2B1/2 and CYP3A1, and increased at ≥ 2 mg/kg for CYP1A1 and CYP4A1.

Table 68: Ex vivo Evaluation of CYP Activity in Rats Administered Intravenous PR-171

Summary of Enzyme Activity in Male Sprague-Dawley Rats (Group Means)

Treatment	CYP1A (pmol/min/mg)	CYP2B (pmol/min/mg)	CYP2C (pmol/min/mg)	CYP3A (pmol/min/mg)	CYP4A (nmol/min/mg)
0 mg/kg Carfilzomib	113 ± 7	12.5 ± 0.7	2770 ± 180	2630 ± 280	1.40 ± 0.07
1 mg/kg Carfilzomib	85.6 ± 5.2	10.6 ± 0.5	1250 ± 90	1930 ± 170	1.22 ± 0.08
2 mg/kg Carfilzomib	117 ± 8	15.2 ± 1.0	2020 ± 110	3210 ± 290	1.90 ± 0.08
4 mg/kg Carfilzomib	78.5 ± 2.9	7.45 ± 0.33	785 ± 186	500 ± 137	1.73 ± 0.10

Summary of Enzyme Activity in Female Sprague-Dawley Rats (Group Means)

Treatment	CYP1A (pmol/min/mg)	CYP2B (pmol/min/mg)	CYP2C (pmol/min/mg)	CYP3A (pmol/min/mg)	CYP4A (nmol/min/mg)
0 mg/kg Carfilzomib	102 ± 3	7.26 ± 0.21	132 ± 8	64.2 ± 4.4	1.05 ± 0.03
1 mg/kg Carfilzomib	117 ± 3	7.67 ± 0.20	178 ± 15	66.3 ± 7.2	1.16 ± 0.06
2 mg/kg Carfilzomib	109 ± 4	7.85 ± 0.15	144 ± 5	82.6 ± 6.9	1.25 ± 0.10
4 mg/kg Carfilzomib	98.1 ± 6.2	6.83 ± 0.36	101 ± 10	62.0 ± 5.8	1.02 ± 0.05

(Table, pg 2723 excerpted from Applicant's package)

Table 69: Ex vivo Evaluation of CYP Expression in Rats Administered Intravenous PR-171

Best Available
Copy

Summary of mRNA Content in Male Sprague-Dawley Rats (Group Means)

Treatment	CYP1A1 (Relative Fold)	CYP2B1/2 (Relative Fold)	CYP2C11 (Relative Fold)	CYP3A1 (Relative Fold)	CYP4A1 (Relative Fold)
0 mg/kg Carfilzomib	1.40 ± 0.47	1.29 ± 0.23	1.16 ± 0.17	1.03 ± 0.08	1.09 ± 0.14
1 mg/kg Carfilzomib	3.27 ± 0.93	0.210 ± 0.068	0.450 ± 0.078	0.571 ± 0.028	0.690 ± 0.098
2 mg/kg Carfilzomib	4.60 ± 1.50	0.335 ± 0.099	0.305 ± 0.053	0.494 ± 0.189	0.266 ± 0.013
4 mg/kg Carfilzomib	7.07 ± 1.98	0.254 ± 0.067	0.128 ± 0.045	0.111 ± 0.025	18.8 ± 0.7

Summary of mRNA Content in Female Sprague-Dawley Rats (Group Means)

Treatment	CYP1A1 (Relative Fold)	CYP2B1/2 (Relative Fold)	CYP2C11 (Relative Fold)	CYP3A1 (Relative Fold)	CYP4A1 (Relative Fold)
0 mg/kg Carfilzomib	1.15 ± 0.19	1.40 ± 0.26	1.32 ± 0.27	1.37 ± 0.26	1.04 ± 0.07
1 mg/kg Carfilzomib	1.14 ± 0.34	2.82 ± 0.80	2.56 ± 0.88	0.479 ± 0.152	0.699 ± 0.143
2 mg/kg Carfilzomib	8.67 ± 3.91	5.78 ± 1.52	1.85 ± 0.43	0.806 ± 0.023	26.1 ± 2.9
4 mg/kg Carfilzomib	4.53 ± 0.87	2.80 ± 0.60	1.55 ± 0.20	0.470 ± 0.073	15.5 ± 1.0

(Table, pg 2724 excerpted from Applicant's package)

Toxicokinetics

As expected for an intravenous drug, T_{max} was 5 minutes at all times and doses; PR-171 was rapidly eliminated and the half-life was 8-13 minutes, except on Day 57 females at 4 mg/kg when it was 31 minutes. Exposure measured by C_{max} and AUC was comparable between the sexes, with males having slightly higher exposure levels. The

exposure was dose-linear proportional for both sexes from 1 mg/kg to 2 mg/kg. In males at 4 mg/kg, the exposure peaked on Day 57 dose-linear compared to 2 mg/kg, but declined on Day 142 back to Day 1 levels that were lower than 2 mg/kg. For females at 4 mg/kg, the same trend occurred but the terminal levels were higher than 2 mg/kg, but not dose linear in proportion.

Table 70: TK Parameters of PR-171 in Rats

Male									
	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142
Dose (mg/kg)	1			2			4		
C ₀ (ng/mL)	527	528	966	1319	3050	2301	3024	4847	1921
C _{max} (ng/mL)	146	173	258	341	678	519	809	1169	729
T _{max} (min)	5	5	5	5	5	5	5	5	5
AUC _{last} (ng*min/mL)	2637	2980	4741	6360	13350	10215	14728	22277	12262
AUC _{Inf} (ng*min/mL)	2646	3004	4770	6376	13375	10250	14817	22365	12393
Cl (mL/min/kg)	378	333	210	314	150	195	270	179	323
Vss (L/kg)	1.57	1.82	0.92	1.24	0.46	0.67	1.12	0.65	2.11
t _{1/2} (min)	11	12	13	10	11	12	13	12	12

Female									
	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142	Day 1	Day 57	Day 142
Dose (mg/kg)	1			2			4		
C ₀ (ng/mL)	489	953	908	1081	2069	2116	2915	3244	2171
C _{max} (ng/mL)	128	212	204	278	471	466	721	981	636
T _{max} (min)	5	5	5	5	5	5	5	5	5
AUC _{last} (ng*min/mL)	2311	4211	4027	5178	9230	9281	13599	17041	11318
AUC _{Inf} (ng*min/mL)	2342	4229	4045	5202	9259	9311	13647	17377	11414
Cl (mL/min/kg)	427	236	247	384	216	215	293	230	350
Vss (L/kg)	1.51	0.84	0.88	1.57	0.74	0.72	1.09	1.4	1.75
t _{1/2} (min)	8	13	13	12	12	12	12	31	13

(Table 9, pg. 1358 (in Appendix P, pg. 26) excerpted from Applicant's package)

Dosing Formulation Analysis

Test article concentration was confirmed and was stable (99.3-102.2%) at RT and after freezing throughout the dosing period.

Study title: A 9-Month Intravenous Toxicity Study of Carfilzomib in Cynomolgus Monkeys with an 8-week Recovery Period

Study no.:	TR-0073-171
Study report location:	eCTD: 4.2.3.2.1.TR-0073-171
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 16, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PR-171, 050307 (cycles 1-4), 93.33% and PR-171, PC F001(cycles 5-9), 93.46%

Key Study Findings

- Treatment related mortality was observed in one male and one female at 2 mg/kg, preceded by clinical signs and weight loss. Cause of death was attributed to multiple organ failure.
- Dose-dependent increase in severity of slight to severe glomerulonephropathy was observed at all doses in all animals ≥ 1 mg/kg with increased organ weight, BUN and creatinine and gross findings of pale enlarged kidneys with red foci.
- A number of hematological parameters related were significantly altered consistent with the pharmacodynamic effects.

Methods

Doses:	0.5, 1, 2 mg/kg
Frequency of dosing:	Nine dosing cycles of 28 day: Days 1-2, 8-9, and 15-16 of the 28 day cycle
Route of administration:	IV bolus
Dose volume:	1 mL/kg
Formulation/Vehicle:	10% HPBCD (hydroxypropyl- β -cyclodextrin)/ 50 mM sodium citrate, pH 3.5
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	6 ctrl or HD/sex/group, 4 LD or MD/sex/group, 2 Ctrl and HD animals were permitted to recovery 8 weeks before necropsy
Age:	Not provided/young adult
Weight:	♂:2.3-3.9 kg, ♀:2.0-3.1 kg
Satellite groups:	none
Unique study design:	Each animal was evaluated under restraint in a primate chair and in the home cage. Animals were evaluated at pretest, on Days 73, 76 and 160/161 and 1-2 days prior to necropsy on Days 239 (end of treatment period) and 307 (end of recovery period). Liver samples were frozen and subjected to CYP analysis.
Deviation from study protocol:	Fifteen minor deviations that that did not affect study conduct and interpretation.

Observations and Results

Mortality

Treatment-related mortality of two animals was observed at 2 mg/kg. One male (#4195) was found dead on Day 157 and one female was humanely euthanized as moribund on Day 132. The identified cause of death in the male was multiple organ failure. The Applicant states, *"This animal was found dead on Day 157 (one day after the last dose of Cycle 6). The cause of death was multi-organ toxicities. Moderate non-regenerative anemia (HCT: 31.4%), and renal dysfunction (BUN: 66 mg/dL; creatinine: 1.4 mg/dL) were noted in hematology and clinical chemistry samples on Day 156, although these values were equivalent to group cohorts. Macroscopic examination revealed pale*

kidneys and liver and red mottling of the lung, which corresponded microscopically with altered tubule size and inflammation in the kidney, leukocytosis in the liver, and edema with hemorrhage and inflammation in the lung, respectively. Inflammation in the heart with myocyte hypertrophy and degeneration, inflammation and edema in the lung, and renal dysfunction were considered to have contributed to the demise of this animal.” This animal also collapsed following dosing on Day 44 and was pale, recumbent, and had decreased activity.

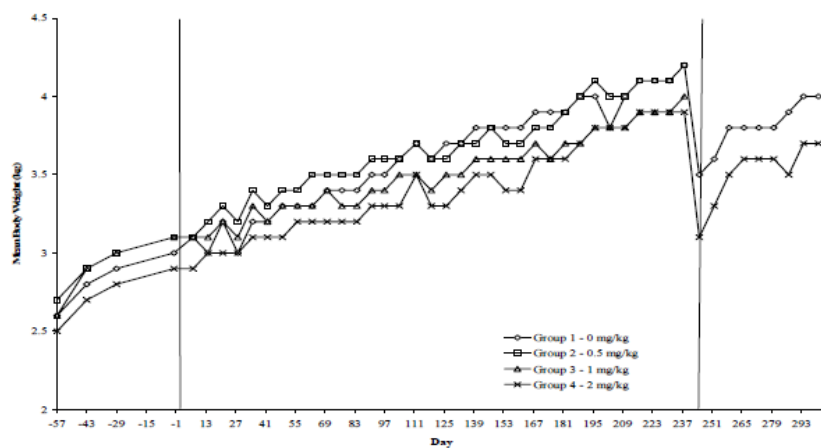
The female progressively declined in health over the course of the study. *“The high-dose female (Animal No. 4695) had a progressive decline in body weight (-0.3 kg compared to the day prior to initiation of dosing) and food consumption. The animal frequently consumed 0 to less than 50% of its daily rations and was given extra treats and supplements (Ensure or meal replacement bars) on a regular basis. On Day 131, a significant amount of subcutaneous fluid was found under the chin during physical examination. Blood samples for hematology, coagulation, and serum biochemistry evaluations were collected on Day 131. The main clinical pathology findings were severe non-regenerative anemia (HCT: 24.4%), severe hypoalbuminemia (ALB: 1.1 g/dL) and moderate increases in liver enzymes, particularly ALT and ALKP. The hypoalbuminemia may have resulted in the subcutaneous swelling found on Day 131. By histopathology, there was generalized atrophy of most organs, correlating with decreased food intake and hypoalbuminemia; decreased bone marrow cellularity, correlating with severe anemia; and marked diffuse hepatocellular glycogen accumulation in the liver.”*

Clinical Signs

Increased incidence of emesis at ≥ 1 mg/kg was observed beginning in males on Day 8 and females on Days 43-58. Hunched appearance was observed at 2 mg/kg in males beginning on Day 93 and in females on Day 37. Decreased activity was observed in females at 1 mg/kg on Day 93 and males on Day 37 and females on Day 30 at 2 mg/kg. Edema and swollen limbs/tail were observed in one male each at 1 and 2 mg/kg on Days 198-122 and 118-122, respectively. The unscheduled euthanatized female had a swollen chin at sacrifice.

Body Weights

Males at ≥ 1 mg/kg had non-significant decreased mean body weights. For females, no trends for mean body weight and body weight gain were noted. There were no body weight differences following recovery.

Figure 42: Body Weights in Male Monkeys Given 6 Months of Intravenous Doses of PR-171

Best Available Copy

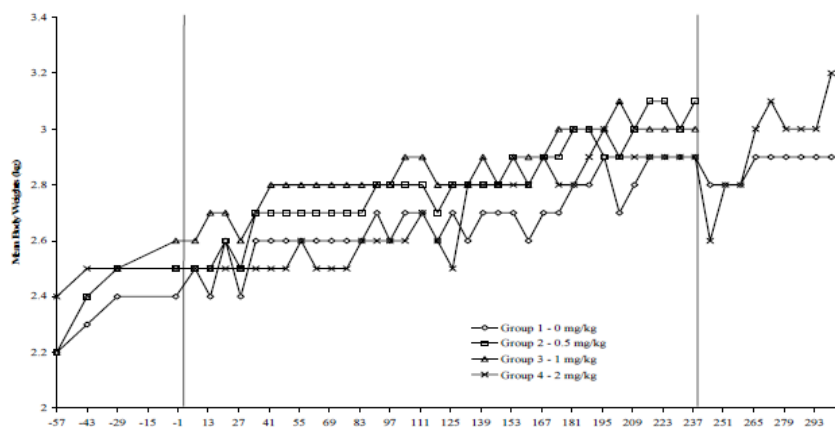
*(Figure 1, pg. 70 excerpted from Applicant's package)***Figure 43: Body Weights in Female Monkeys Given 6 Months of Intravenous Doses of PR-171***(Figure 2, pg. 71 excerpted from Applicant's package)*

Table 71: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) in Monkeys Administered Intravenous PR-171 for 6 Months (n=4-6)

Males	Pre-Dose BWt (g)	Day 238 BWt (g)	% Gain	% Δ
0 mg/kg	2.6	4.2	+62	--
0.5 mg/kg	2.7	4.2	+56	--
1 mg/kg	2.6	4.0	+54	-5
2 mg/kg	2.5	3.9	+56	-7
Females				
0 mg/kg	2.2	2.9	+32	--
0.5 mg/kg	2.2	3.1	+41	+7
1 mg/kg	2.2	3.0	+36	+3
2 mg/kg	2.4	2.9	+21	--

Table 72: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) Following 8 Weeks of Recovery (n=2)

Males	Week 0 BWt (g)	Week 8 BWt (g)	% Gain	% Δ
0 mg/kg	4.2	4.0	-5	--
2 mg/kg	3.9	3.7	-5	-8
Females				
0 mg/kg	2.9	2.9	--	--
2 mg/kg	2.9	3.2	+9	+10

Feed Consumption

Not remarkable.

Hematology

A number of hematological parameters were significantly altered throughout the entire dosing period beginning on Day 2 compared to concurrent controls; this is due to the expected effect of the drug product. There was not a general increase in severity over time.

Termination: Except for an increase in leukocytes noted at ≥ 1 mg/kg in males and not in females, there was little difference between males and females noted. At ≥ 1 mg/kg for both sexes, there was a significant increase in neutrophils, monocytes, leukocytes, and WBCs; fibrin was also significantly increased. There was a numerical increase in reticulocytes at all doses, but it was not significant at any dose in males and at 0.5 mg/kg in females. RBCs were significantly decreased at all doses in males and at ≥ 1 mg/kg in females. Hematocrit at 2 mg/kg in both sexes and hemoglobin were significantly reduced in males at 2 mg/kg and ≥ 1 mg/kg in females, consistent with RBC effects.

Recovery: In males, values showed little recovery at 2 mg/kg and were variable. Some parameters (lymphocytes to 2.6X, WBC to 85%, and reticulocytes to 63%) increased compared to what was observed during the treatment period. For females, most values showed a full recovery compared to controls except for partial recovery of monocytes

(60%), neutrophils (33%) and reticulocytes (50%). The variability is partially explained by only having 2 animals in each group available for evaluation.

Table 73: Change in Selected Mean Hematology Parameters Compared to Control in Monkeys Given 6 Months of Intravenous Doses of PR-171 on Day 241 (24 hours after last dose): % Difference or Fold Difference (n=4-6)

Parameter	Males			Females		
	Dose (mg/kg)					
	0.5	1	2	0.5	1	2
Neutrophils ($10^3/\mu\text{L}$)		+132%*	+151%*	$\uparrow 2.0X$	$\uparrow 4.1X^{**}$	$\uparrow 4.2X^{**}$
Monocytes ($10^3/\mu\text{L}$)		$\uparrow 2.7X^{**}$	+49%	$\uparrow 2.0X$	$\uparrow 3.3X^{**}$	+58%
Leukocytes ($10^3/\mu\text{L}$)		$\uparrow 3.3X^{**}$	$\uparrow 3.2X^{**}$			
Reticulocytes ($10^9/\text{L}$)	+27%	+70%	+46%	+39%	+92%**	+62%**
WBCs ($10^3/\mu\text{L}$)		+79%*	+66%*		+92%	+75%**
RBCs ($10^6/\mu\text{L}$)	-12%*	-11%*	-24%**		-11%*	-29%**
Ht (%)			-23%**			-23%*
Hb (g/dL)		-9%	-25%**		-9%*	-23%*
Fibrin (mg/dL)		+65%**	+73%**		+44%*	+106%*
Prothrombin Time (sec)						-6%*

*, $P < 0.05$; **, $P < 0.01$; *italics*, not significant.

Clinical Chemistry

A number of parameters were significantly altered throughout the entire dosing period beginning on Day 2 compared to concurrent controls. In general, parameters associated with kidney toxicity were more consistently altered and increased in severity over time. In general, the sex had similar findings. However, males showed a more consistent effect on BUN, whereas females had more significant effects on ALT, total protein, and albumin.

Termination: There was a significant dose-dependent increase in BUN in both sexes at ≥ 1 mg/kg reaching a maximum of 2.3- to 2.8-fold. There was a 2.4- to 2.7-fold increase in creatinine at 2 mg/kg in both sexes. Males had a ~17% increase in potassium at ≥ 1 mg/kg, without a comparable change in females (potassium changes were observed at times during the dosing period). ALT was significantly decreased in males at ≥ 1 mg/kg and females at 2 mg/kg. Total protein was significantly decreased in males at 2 mg/kg and females at ≥ 1 mg/kg. Phosphorus was significantly decreased at 2 mg/kg in males, with females at this dose demonstrating a comparable but non-significant decrease. A slight but significant decrease in A/G ratio was observed in males; but not in females; with a concomitant decrease in globulin at 2 mg/kg also noted. Calcium was significantly decreased in females at 2 mg/kg with males only seeing a decrease during the first dosing cycle. Cholesterol was significantly decreased in females at ≥ 1 mg/kg; males never showed a significant change.

In both sexes there were sporadic significant changes noted in triglycerides, Na⁺ and AST at interim timepoints, but no significant differences from control were observed at termination.

Recovery: All parameters recovered with the caveat of n=2/group.

Table 74: Change in Selected Mean Clinical Chemistry Parameters Compared to Control in Monkeys Given 6 Months of Intravenous Doses of PR-171 on Day 241 (24 hours after last dose): % Difference or Fold Difference (n=4-6)

Parameter	Males			Females		
	Dose (mg/kg)					
	0.5	1	2	0.5	1	2
ALT (U/L)		-31%*	-42%**			-96%*
BUN (mg/dL)		↑2.1X**	↑2.8X**		+53%**	↑2.3X**
CREAT (mg/dL)			↑2.7X**			↑2.4X*
Total PROT (g/dL)			-14%*		-11%*	-22%**
ALB (g/dL)		-20%**	-20%**		-13%*	-28%**
GLOB (g/dL)						-18%*
A/G ratio			-7%*			
Phos (mmol/L)			-24%*			-16%
Ca (mmol/L)						-8%*
K (mmol/L)		+16%*	+18%**			
CHOL (mg/dL)					-33%*	-12%*

*, P<0.05; **, P<0.01; *italics*, not significant.

Urinalysis

Not remarkable.

Ophthalmoscopy

Evaluation performed by (b) (4) VMD during pre-test and prior to necropsy.
Not remarkable.

ECG

Evaluation performed by (b) (4) D.V.M., M.S., Ph.D. One male at 2 mg/kg (#4191) had increased R wave amplitude on Day 73 following dosing with the amplitude increasing on Days 161 and 239. There were histopathological correlates of left ventricular enlargement and myocardial hypertrophy with increased heart weight (64.084 g compared to control range of 13.059-18.896g). All other ECGs were considered normal.

Organ Weights

There were increased both absolute and ratio to BW organ weights for spleen and kidney in both sexes at ≥1 mg/kg. The liver, heart and adrenal all showed increased at 2 mg/kg in both sexes. The Table below lists the absolute changes in organ weight.

Table 75: Test Article-Related Absolute Organ Weight Changes on Day 241 (24 hours after last dose): % Difference from Concurrent Vehicle Controls

carfilzomib (mg/kg)	Males			Females		
	0.5	1	2	0.5	1	2
Kidney	-	+82	+139	-	+59	+117
Liver	-	-	+39	-	-	+56
Spleen	-	+47	+68	-	-	+84
Heart	-	-	+120	-	+35	+52
Adrenal	-	-	+11	-	-	+49

- = no test article-related findings.

(Table 3.12-1, pg. 60 excerpted from Applicant's package)

Recovery: In males, there was partial recovery in kidney (↑70% as %BW and 61% absolute) and heart (↑30% as %BW and 21% absolute); with full recovery of liver weights. Females had full recovery, except increased kidney weights (↑57% as %BW and 68% absolute).

Gross Pathology

The kidney had significant findings with microscopic correlates in both sexes at ≥1 mg/kg. Enlarged kidneys were noted in one male and one female at 1 mg/kg and one male and two females at 2 mg/kg. This corresponded to increased organ weight and glomerulonephropathy, tubular alteration and cortex interstitial inflammation/fibrosis. Red foci were noted in four males and four females at 1 mg/kg and one male and two females at 2 mg/kg, corresponding to intratubular red cell casts. Pale kidneys were observed in four males at 1 mg/kg and three males and two females at 2 mg/kg. Enlargement did not recover at 2 mg/kg, being observed in 1 of 2 animals/sex, and pale kidneys were observed in 1 of 2 males.

Table 76: Incidence of Macroscopic Findings in the Kidney 24 hours after the Last Dose of Carfilzomib (Day 241)

carfilzomib (mg/kg)	Males				Females			
	0	0.5	1	2	0	0.5	1	2
Number of animals examined:	4	4	4	3	4	4	4	3
Enlarged	0	0	1	1	0	0	1	2
Pale	0	0	4	3	0	0	0	2
Red foci	0	0	4	1	0	0	4	2

(Table 3.14.1-1, pg. 62 excerpted from Applicant's package)

Histopathology

Adequate Battery- Yes, plus sciatic, sural and peroneal nerves were also examined in all animals and timepoints at 0 and 2 mg/kg.

Peer Review- Peripheral nerves were evaluated by (b) (4) VMD, DACVP

Histological Findings-

The target organs were similar between sexes and included the femoral bone marrow, heart, kidney, and mediastinal LN, with kidney having the most severe toxicities observed. There were injection site findings, but no dose relationship was observed. There were only 3 males (one control and one 2 mg/kg) of apparent sexual maturity based on reproductive tissue findings. The large intestine, lung, pancreas, sciatic nerve, and spleen had some minor and/or nondose related findings that warranted documentation based on other findings or observations from other studies.

The findings in unscheduled deaths are described under mortality. Consistent with scheduled sacrifice, causes of test article-related unscheduled deaths based on histopathology were heart, kidney and/or liver failure. Similar to rats, this is markedly different compared to the milder findings in scheduled necropsies.

Kidney- A dose-dependent increase in severity of slight to severe glomerulonephropathy was observed at all doses in both sexes with all animals ≥ 1 mg/kg being affected. Minimal to slight RBC casts were observed in all treated groups, but not control animals. All males at ≥ 1 mg/kg had slight to marked altered tubular size/cellularity; all treated females had this finding, but the severity increased with dose from minimal at 0.5 mg/kg to slight to marked at 2 mg/kg. There was an increase in severity of chronic interstitial inflammation/fibrosis observed in all treated animals, except for 2 males at 0.5 mg/kg; two control males had minimal findings. Males at ≥ 1 mg/kg (2/3 group) had slight increased subintimal cellularity of arteries.

Lymph Node (Mesdiastinal)- There was slight to moderate medullary granulocytes/granulocytopoiesis in 2/3 animals of both sexes at 2 mg/kg.

Bone Marrow- There was an increase in severity, but not incidence, of hypercellularity noted in both sexes in all groups. Control animals tended to have minimal to slight findings, while treated animals increased to moderate or marked at the high dose. This is an expected response to the increased blood cells noted in the hematology.

Heart- Dose-dependent increases in incidence and severity in both sexes (slight to moderate in all animals at 2 mg/kg) was noted for chronic inflammatory cell infiltrate. This finding was also observed in control animals, albeit with minimal severity. Minimal myocyte degeneration was observed in one of each sex at 1 mg/kg and two males at 2 mg/kg. Minimal to moderate myocyte hypertrophy was observed in all males and 1 female at 2 mg/kg. Edema and atrial endocardial hypertrophy were observed in one male at 2 mg/kg in addition to the other findings.

Table 77: Histopathological Findings of Note with Incidence and Severity at Termination in Monkeys Given 6 Months of Intravenous Doses of PR-171

Organ	Finding	Sex	Dose (n)			
			0 mg/kg (4♂, 4♀)	0.5mg/kg (4♂, 4♀)	1 mg/kg (4♂, 4♀)	2 mg/kg (3♂, 3♀)
Bone Marrow (femoral)	Cellularity	M	1-minimal 2-slight 1-moderate	1-minimal 2-slight 1-moderate	2-slight 2-moderate	1-slight 1-moderate
		F	1-minimal 1-slight 2-moderate	1- slight 1- moderate 2-marked	1-minimal 1- moderate 2-marked	3-marked
Cecum	Edema	F			2-slight	
Colon	Edema	F			2-slight	
Rectum	Edema	F			1-minimal 1-slight	
Heart	Chronic inflammatory cell infiltrate	M	1-minimal	1-minimal 3-slight	1-minimal 2-slight 1-moderate	2-slight 1-moderate
		F	3-minimal	2-minimal 2-slight	2-minimal 2-slight	3-slight
	Myocyte degeneration	M			1-minimal	2-minimal
		F			1-minimal	
	Myocyte hypertrophy	M				2-minimal 1-moderate
		F				1-slight
	Edema	M				1-slight
	Atrial endocardial hypertrophy	M				1-slight
Kidney	Glomerulonephropathy	M		2-slight	3-moderate 1-marked	1-slight 1-moderate 1-severe
		F		1-slight	2-slight 2-moderate	2-marked 1-severe
	Altered tubular size/cellularity	M			1-slight 2-moderate 1-marked	1-slight 1-moderate 1-marked
		F		4-minimal	1-minimal 3-slight	1-slight 1-moderate 1-marked
	Chronic interstitial inflammation/fibrosis	M	2-minimal	3-minimal	2-slight 2-moderate	2-slight 1-moderate
		F		4-minimal	1-minimal 3-slight	1-slight 2-moderate
	Increased subintimal cellularity of arteries	M			2-slight	2-slight
	RBC casts	M		1-minimal	1-minimal 3-slight	1-minimal 1-slight
		F		1-minimal	1-minimal 3-slight	3-minimal
Lung	Interstitial inflammation	M			3-minimal	2-minimal 1-slight
		F	1-slight	1-minimal	1-minimal	3-minimal
Mediastinal Lymph Node	Medullary granulocytes/granulocytopoiesis	M				1-slight 1-marked
		F				1-slight 1-marked
Pancreas	Greatly reduced secretory product	M		1-present		
		F			2-present	1-present
Sciatic nerve	Soft tissue f brosis	F		1-slight		
	Soft tissue hemorrhage	F			1-slight	
Skin	Hypotrichosis	F		1-moderate	1-moderate	
	Hyperplasia/hyperkeratosis	M			1-slight	
		F		1-minimal		
Spleen	Decreased marginal zone width and/or cellularity	M				1-slight

Recovery: Partial recovery was observed in males, with a number of minimal and slight findings in the treated animals not observed in controls. Most significantly, there was slight and severe glomerulonephropathy observed in the two animals, respectively. There were two control and one treated apparent sexually immature males based on reproductive tissue findings. The only significant finding at recovery for females was both treated animals had slight glomerulonephropathy.

Table 78: Histopathological Findings of Note with Incidence and Severity in Monkeys Given 6 Months of Intravenous Doses of PR-171 after 8 Weeks Recovery (n=2/sex/group)

Organ	Finding	Sex	Dose (mg/kg)	
			0	2
Heart	Chronic Inflammatory Cell Infiltrate	M	1-minimal	2-slight
	Myocyte degeneration	M		1-minimal
	Myocyte hypertrophy	M		1-minimal
Kidney	Glomerulonephropathy	M		1-slight 1-severe
		F		2-slight
	Chronic interstitial inflammation/fibrosis	M		1-minimal 1-slight
		F	2-minimal	1-minimal
	Increased subintimal cellularity of arteries	M		1-slight
Mediastinal Lymph Node	Intrasinusoidal erythrocytes	M		1-present
Mesenteric Lymph Node	Increased #/size of germinal centers	M		1-slight
	Intrasinusoidal erythrocytes	M		1-present
Spleen	Increased #/size of germinal centers	M		1-moderate

Special Evaluation- Neurobehavioral Assessment

Not remarkable.

Special Evaluation- Pharmacodynamics

Evaluated in blood cell pellets from samples taken at pre-dose and 1 hr post dose timepoints on Days 1, 57, 141, and 225. At all dose levels and times, PR-171 induced >80% inhibition of proteasome activity. There was minimal recovery following the washout periods at all doses and times.

Special Evaluation- CYP Evaluation (ex vivo)

The analysis was conducted by (b) (4) on portions of frozen liver obtained at necropsy. Samples were collected 24 hr after the last dose of the 6- month cycle and recovery (n=2/sex/group). Liver microsomes were prepared for enzymatic evaluation and used with appropriate positive controls for each CYP, and expression was determined by qRT-PCR on another sample.

CYP1A, CYP2C and CYP3A activity decreased in females with RNA correlates, and CYP3A activity increased in males without a correlate in expression. Decreased total

hepatic CYP protein content was observed in both sexes at all doses, but as greater in magnitude in females (maximum: -30% compared to -17% for males at 2 mg/kg)

Toxicokinetics

TK samples were collected at pre-doses, 5, 15, 30 and 60 min post-dose on Days 1, 57, 141, and 225, which were the first days of Cycles 1, 3, 6, and 9, respectively.

As expected for an intravenous drug and comparable to rats, T_{max} was 5-6 minutes at all times and doses; PR-171 was rapidly eliminated and the half-life was 11-15 minutes. Exposure measured by C_{max} and AUC was comparable between the sexes, with males having slightly higher exposure levels. The exposure increased less than dose-linear but proportional for both sexes. There was no clearly observable accumulation of test article.

Table 79: TK Parameters of PR-171 in Monkeys

Gender	Dose (mg/kg)	Day	$t_{1/2}$ (min)	t_{max} (min)	C_{max} (ng/mL)	C_0 (ng/mL)	AUC_{last} (min*ng/mL)	AUC_{inf} (min*ng/mL)
Male	0.5	1	11 ± 1	5 ± 0	41.2 ± 5.0	70.1 ± 9.2	785 ± 90	800 ± 93
		57	11 ± 1	5 ± 0	41.4 ± 5.9	70.3 ± 14.5	779 ± 89	792 ± 87
		141	12 ± 0	5 ± 1	41.1 ± 7.3	68.4 ± 15.2	830 ± 153	848 ± 158
		225	11 ± 0	5 ± 0	43.1 ± 4.6	74.5 ± 5.7	804 ± 99	820 ± 101
	1	1	12 ± 0	5 ± 0	62.3 ± 8.4	111 ± 18	1163 ± 134	1186 ± 134
		57	12 ± 0	5 ± 0	58.9 ± 9.6	102 ± 17	1122 ± 183	1146 ± 185
		141	13 ± 1	6 ± 1	61.4 ± 8.7	112 ± 14	1241 ± 129	1277 ± 128
		225	14 ± 1	5 ± 0	66.8 ± 12.2	133 ± 36	1237 ± 199	1273 ± 203
	2	1	12 ± 1	5 ± 1	103 ± 37	202 ± 74	1934 ± 555	1974 ± 565
		57	13 ± 1	5 ± 0	87.1 ± 15.4	165 ± 41	1581 ± 245	1653 ± 273
		141	15 ± 1	6 ± 1	76.7 ± 22.7	143 ± 35	1572 ± 323	1644 ± 349
		225	15 ± 1	5 ± 0	85.0 ± 7.1	158 ± 16	1624 ± 180	1694 ± 186
Female	0.5	1	12 ± 2	5 ± 1	45.7 ± 5.3	80.3 ± 5.8	890 ± 74	912 ± 75
		57	11 ± 1	5 ± 0	48.5 ± 4.2	82.8 ± 10.0	918 ± 69	937 ± 75
		141	13 ± 2	5 ± 0	46.8 ± 7.2	74.5 ± 15.6	942 ± 136	975 ± 153
		225	11 ± 1	5 ± 0	47.5 ± 9.4	83.4 ± 24.4	886 ± 148	903 ± 149
	1	1	13 ± 1	6 ± 1	51.6 ± 5.6	92.0 ± 5.9	1047 ± 41	1074 ± 41
		57	12 ± 1	5 ± 0	52.3 ± 7.8	87.4 ± 15.7	1009 ± 123	1033 ± 122
		141	14 ± 2	6 ± 2	60.9 ± 14.2	106 ± 22	1236 ± 132	1281 ± 124
		225	12 ± 1	5 ± 0	69.1 ± 8.6	110 ± 23	1449 ± 453	1485 ± 470
	2	1	14 ± 2	5 ± 0	86.9 ± 24.1	151 ± 57	1692 ± 380	1755 ± 380
		57	15 ± 3	5 ± 0	107 ± 34	221 ± 126	2015 ± 515	2081 ± 526
		141	15 ± 2	6 ± 2	81.6 ± 29.3	142 ± 53	1741 ± 376	1827 ± 392
		225	14 ± 2	5 ± 0	114 ± 26	209 ± 58	2129 ± 414	2202 ± 419

Best
Available
Copy

(Table 9, pg. 1280 (in Appendix P, pg. 31) excerpted from Applicant's package)

Dosing Formulation Analysis

Test article concentration was confirmed and was stable at RT and after freezing after cycles 1, 3, 6, and 9 (96.2-105.7%).

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Carfilzomib: Bacterial Reverse Mutation Test

Study no.:	TR-0069-171
Study report location:	EDR, 4.2.3.3.1-TR-0069-171
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 7, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PR-171, 0705-029, ≥95%

Key Study Findings

- PR-171 was not genotoxic in bacteria up to 5000 µg/plate ± metabolic activation under the conditions tested.

Methods

Strains:	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537 <i>Escherichia coli</i> WP2uvrA(pKM101)
Concentrations in definitive study:	+S9: 5, 15, 50, 150, 500, 1500, 5000 µg/plate -S9: 15, 50, 150, 500, 1500, 5000 µg/plate
Basis of concentration selection:	DRF up to 5000 µg/plate
Negative control:	DMSO
Positive control:	+S9 All <i>Salmonella</i> strains: Benzo[a]pyrene, 5 µg/plate WP2uvrA: 2-aminoanthracene, 10 µg/plate -S9 TA98: 2-nitrofluorene, 1.0 µg/plate TA100 & TA1535: sodium azide, 2 µg/plate TA1537: 9-aminoacridine, 50 µg/plate WP2uvrA: 4-nitroquinolone-1-oxide, 2 µg/plate
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Agar overlay method with pre-incubation of 30 min ± S9 metabolic activation using 3 plates/dose, incubation was for ~72 hr.

Study Validity - Valid

1. Sufficient strains were evaluated to cover types of genotoxic mechanisms.
2. Doses were tested to limit of 5000 µg/plate.
3. No cytotoxicity to any tester strain ± S9 metabolic activation observed with viability of 99%.
4. Precipitate was observed at ≥500 µg/plate -S9 and at ≥1500 µg/plate + S9 but did not interfere with evaluation.
5. No bacterial contamination in sterility tests of S9 mixes and test article sham dilutions.
6. No test article-dependent increase in revertant colonies noted ±S9 activation system.
7. Revertant rates were within positive and negative controls historical data range.
8. Positive controls were 3.3 - 53.6x greater than negative DMSO control values.

Results

The sponsor conducted two tests with the first test as a DRF study. Carfilzomib up to 5000 µg/plate were not genotoxic in any bacterial strain tested. No concentration-dependent increase in revertants ± S9 metabolic activation was observed. No single plate count exceeded a positive control mean value. The sponsor's Tables 4 and 5 for Test #2 are included below.

Table 80: Bacterial Reverse Mutation Test Results for PR-171 in the Absence of Metabolic Activation (Test 2)

Without metabolic activation						
Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts
TA98	DMSO		37.0	3.5		39, 33, 39
	Carfilzomib	5 µg	40.0	2.0	1.1	42, 40, 38
		15 µg	47.7	3.5	1.3	51, 44, 48
		50 µg	32.3	2.3	0.9	31, 31, 35
		150 µg	43.0	4.6	1.2	48, 42, 39
		500 µg	34.0	3.0	0.9	37 P, 31 P, 34 P
		1500 µg	31.3	4.9	0.8	37 P, 29 P, 28 P
		5000 µg	25.3	1.2	0.7	24 P, 26 P, 26 P
TA100	DMSO		168.7	12.1		156, 170, 180
	Carfilzomib	5 µg	180.3	35.2	1.1	185, 213, 143
		15 µg	187.3	28.0	1.1	177, 219, 166
		50 µg	176.3	18.0	1.0	194, 158, 177
		150 µg	184.0	14.7	1.1	200, 171, 181
		500 µg	186.7	18.6	1.1	167 P, 189 P, 204 P
		1500 µg	164.7	15.6	1.0	167 P, 179 P, 148 P
		5000 µg	149.0	6.6	0.9	156 P, 143 P, 148 P
TA1535	DMSO		23.7	2.1		23, 26, 22
	Carfilzomib	5 µg	17.3	4.0	0.7	21, 13, 18
		15 µg	21.0	9.5	0.9	16, 15, 32
		50 µg	27.7	4.7	1.2	24, 33, 26
		150 µg	26.7	3.2	1.1	23, 28, 29
		500 µg	22.3	3.5	0.9	22 P, 26 P, 19 P
		1500 µg	23.7	1.5	1.0	25 P, 24 P, 22 P
		5000 µg	17.3	1.5	0.7	17 P, 16 P, 19 P
Key to Plate Postfix Codes						
P Precipitate						

Best Available Copy

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts
TA1537	DMSO		11.0	0.0		11, 11, 11
	Carfilzomib	5 µg	14.0	2.6	1.3	16, 11, 15
		15 µg	13.0	4.6	1.2	18, 12, 9
		50 µg	10.0	0.0	0.9	10, 10, 10
		150 µg	12.3	3.2	1.1	16, 10, 11
		500 µg	10.7	2.9	1.0	14 P, 9 P, 9 P
		1500 µg	11.0	2.6	1.0	8 P, 13 P, 12 P
		5000 µg	7.3	1.5	0.7	7 P, 6 P, 9 P
WP2 uvrA (pKM101)	DMSO		136.0	6.9		132, 144, 132
	Carfilzomib	5 µg	116.3	6.5	0.9	110, 116, 123
		15 µg	130.3	15.9	1.0	126, 148, 117
		50 µg	133.7	6.7	1.0	138, 126, 137
		150 µg	148.3	8.6	1.1	150, 139, 156
		500 µg	132.7	8.5	1.0	133 P, 124 P, 141 P
		1500 µg	132.0	3.6	1.0	129 P, 136 P, 131 P
		5000 µg	107.3	9.6	0.8	116 P, 97 P, 109 P
TA98	2NF	2 µg	319.3	32.5	8.6	352, 319, 287
TA100	NaN3	2 µg	748.0	49.2	4.4	803, 708, 733
TA1535	NaN3	2 µg	973.3	75.0	41.1	887, 1023, 1010
TA1537	AAC	50 µg	424.7	132.3	38.6	577, 358, 339
WP2 uvrA (pKM101)	NQO	2 µg	1934.3	336.0	14.2	1557, 2045, 2201
Key to Positive Controls			Key to Plate Postfix Codes			
2NF	2-Nitrofluorene		P	Precipitate		
NaN3	Sodium azide					
AAC	9-Aminoacridine					
NQO	4-Nitroquinoline-1-oxide					

Best Available Copy

(Table 4, pgs. 25-26, excerpted from Applicant's package)

Table 81: Bacterial Reverse Mutation Test Results for PR-171 in the Presence of Metabolic Activation (Test 2)

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts
TA98	DMSO		46.0	3.6		45, 43, 50
	Carfilzomib	5 µg	46.7	7.1	1.0	53, 39, 48
		15 µg	51.3	3.5	1.1	51, 55, 48
		50 µg	56.7	8.0	1.2	65, 49, 56
		150 µg	51.3	1.5	1.1	53, 51, 50
		500 µg	49.0	4.6	1.1	53, 44, 50
		1500 µg	47.7	4.0	1.0	47 P, 52 P, 44 P
		5000 µg	42.7	3.5	0.9	43 P, 46 P, 39 P
TA100	DMSO		185.3	11.6		172, 193, 191
	Carfilzomib	5 µg	216.7	26.3	1.2	247, 203, 200
		15 µg	181.3	21.0	1.0	203, 161, 180
		50 µg	207.7	22.0	1.1	230, 186, 207
		150 µg	183.3	14.6	1.0	170, 199, 181
		500 µg	201.3	11.4	1.1	198, 192, 214
		1500 µg	178.3	7.5	1.0	178 P, 186 P, 171 P
		5000 µg	174.0	9.5	0.9	179 P, 163 P, 180 P
TA1535	DMSO		19.7	5.7		15, 26, 18
	Carfilzomib	5 µg	18.7	2.1	0.9	18, 17, 21
		15 µg	14.3	3.8	0.7	17, 10, 16
		50 µg	21.0	6.1	1.1	18, 17, 28
		150 µg	22.3	7.0	1.1	15, 23, 29
		500 µg	14.7	1.5	0.7	13, 16, 15
		1500 µg	18.7	4.2	0.9	22 P, 20 P, 14 P
		5000 µg	16.0	2.6	0.8	13 P, 17 P, 18 P
Key to Plate Postfix Codes						
				P	Precipitate	
Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts
TA1537	DMSO		31.3	7.0		38, 24, 32
	Carfilzomib	5 µg	29.3	2.9	0.9	31, 31, 26
		15 µg	30.3	6.4	1.0	34, 34, 23
		50 µg	30.7	1.5	1.0	32, 29, 31
		150 µg	24.0	3.6	0.8	21, 23, 28
		500 µg	29.7	2.1	0.9	32, 29, 28
		1500 µg	24.0	2.0	0.8	24 P, 22 P, 26 P
		5000 µg	23.0	3.5	0.7	19 P, 25 P, 25 P
WP2 <i>uvrA</i> (pKM101)	DMSO		146.3	22.1		123, 167, 149
	Carfilzomib	5 µg	173.0	22.6	1.2	197, 152, 170
		15 µg	144.0	18.7	1.0	127, 164, 141
		50 µg	152.7	7.5	1.0	153, 160, 145
		150 µg	143.7	17.9	1.0	154, 154, 123
		500 µg	167.7	15.0	1.1	185, 159, 159
		1500 µg	162.3	7.5	1.1	155 P, 162 P, 170 P
		5000 µg	136.0	8.2	0.9	145 P, 129 P, 134 P
TA98	B[a]P	5 µg	306.7	35.8	6.7	287, 285, 348
TA100	AAN	5 µg	3158.3	1117.7	17.0	4184, 3324, 1967
TA1535	AAN	5 µg	188.7	22.1	9.6	213, 170, 183
TA1537	B[a]P	5 µg	216.0	65.3	6.9	247, 141, 260
WP2 <i>uvrA</i> (pKM101)	AAN	10 µg	766.3	92.4	5.2	846, 665, 788
Key to Positive Controls						
					Key to Plate Postfix Codes	
B[a]P	Benzo[a]pyrene			P	Precipitate	
AAN	2-Aminoanthracene					

Best Available Copy

(Table 5, pgs 27 and 28, excerpted from Applicant's package)

Study title: Carfilzomib: Bacterial Reverse Mutation Test

Study no.: TR-0425-171
Study report location: EDR, 4.2.3.3.1-TR-0425-171
Conducting laboratory and location: (b) (4)
Date of study initiation: March 18, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: PR-171, 197-36, 94.8%
PR-519: 1.1%
PR-59428: 1.1%
PR-187: 1.3%
PR-59462: 1.2%

Key Study Findings

- Carfilzomib spiked with 4 impurities was not genotoxic in bacteria up to 1500 and 5000 µg/plate with and without metabolic activation, respectively, under the conditions tested.

Methods

Strains: *Salmonella typhimurium* TA98, TA100, TA1535, TA1537
Escherichia coli WP2uvrA(pKM101)
Concentrations in definitive study: +S9: 5, 15, 50, 150, 500, 1500, 5000 µg/plate
-S9: 15, 50, 150, 500, 1500, 5000 µg/plate
Basis of concentration selection: DRF up to 1500 (-S9) & 5000 µg/plate (+S9)
Negative control: DMSO
Positive control: **+S9**
All *Salmonella* strains:
Benzo[a]pyrene, 5 µg/plate
WP2uvrA: 2-aminoanthracene, 10 µg/plate
-S9
TA98: 2-nitrofluorene, 1.0 µg/plate
TA100 & TA1535: sodium azide, 2 µg/plate
TA1537: 9-aminoacridine, 50 µg/plate
WP2uvrA: 4-nitroquinolone-1-oxide, 2 µg/plate
Formulation/Vehicle: DMSO
Incubation & sampling time: Agar overlay method with pre-incubation of 30 min ± S9 metabolic activation using 3 plates/dose, incubation was for ~72 hr.

Study Validity- Valid

- Sufficient strains were evaluated to cover types of genotoxic mechanisms.
- Doses were tested to limit of 5000 µg/plate.

3. No cytotoxicity to any tester strain \pm S9 metabolic activation observed with viability of 99%.
4. Precipitate was observed at ≥ 500 $\mu\text{g}/\text{plate}$ -S9 and at ≥ 1500 $\mu\text{g}/\text{plate}$ + S9 but did not interfere with evaluation.
5. No bacterial contamination in sterility tests of S9 mixes and test article sham dilutions.
6. No test article-dependent increase in revertant colonies noted \pm S9 activation system.
7. Revertant rates were within positive and negative controls historical data range.
8. Appropriate positive controls were 4.2 - 46.6x greater than negative DMSO control values.

Results

This Ames study to evaluated carfilzomib spiked with 4 known process impurities to ~1% of the total drug substance; these amounts were in excess of the levels reported in characterization of the drug substance. The sponsor conducted two tests with the first test as a DRF study. Carfilzomib up to 1500 $\mu\text{g}/\text{plate}$ -S9 activation and 5000 $\mu\text{g}/\text{plate}$ +S9 activation was not genotoxic in any bacterial strain tested. No concentration-dependent increase in revertants \pm S9 metabolic activation was observed. No single plate count exceeded a positive control mean value. The sponsor's Tables 4 and 5 for Test #2 are included below:

Table 82: Bacterial Reverse Mutation Test Results for PR-171 in the Absence of Metabolic Activation (Test 2)

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts
TA98	DMSO		31.7	7.1		24, 38, 33
	Carfilzomib	1.5 µg	28.7	2.5	0.9	31, 26, 29
		5 µg	28.3	5.5	0.9	31, 22, 32
		15 µg	30.0	4.6	0.9	26, 29, 35
		50 µg	32.0	1.7	1.0	31, 31, 34
		150 µg	30.0	2.6	0.9	32, 31, 27
		500 µg	30.0	2.6	0.9	33 P, 28 P, 29 P
		1500 µg	26.7	3.2	0.8	23 P, 28 P, 29 P
TA100	DMSO		150.0	15.4		154, 133, 163
	Carfilzomib	1.5 µg	154.7	31.1	1.0	187, 125, 152
		5 µg	146.3	8.3	1.0	149, 137, 153
		15 µg	146.7	15.6	1.0	163, 132, 145
		50 µg	161.0	20.0	1.1	183, 144, 156
		150 µg	160.3	24.4	1.1	155, 187, 139
		500 µg	156.0	3.5	1.0	160 P, 154 P, 154 P
		1500 µg	140.3	7.1	0.9	134 P, 139 P, 148 P
TA1535	DMSO		24.7	2.9		23, 23, 28
	Carfilzomib	1.5 µg	23.0	3.0	0.9	23, 20, 26
		5 µg	22.7	3.8	0.9	21, 20, 27
		15 µg	26.7	5.1	1.1	28, 31, 21
		50 µg	24.0	3.0	1.0	21, 24, 27
		150 µg	25.3	2.3	1.0	24, 28, 24
		500 µg	25.3	2.3	1.0	28 P, 24 P, 24 P
		1500 µg	22.7	3.1	0.9	22 P, 26 P, 20 P
Key to Plate Postfix Codes						
P Precipitate						
Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts
TA1537	DMSO		11.0	1.7		13, 10, 10
	Carfilzomib	1.5 µg	11.3	4.0	1.0	12, 15, 7
		5 µg	12.3	4.2	1.1	17, 11, 9
		15 µg	12.0	1.0	1.1	13, 12, 11
		50 µg	10.3	1.2	0.9	9, 11, 11
		150 µg	10.7	2.1	1.0	13, 9, 10
		500 µg	8.7	1.5	0.8	10 P, 9 P, 7 P
		1500 µg	8.7	2.1	0.8	11 P, 8 P, 7 P
WP2 uvrA (pKM101)	DMSO		117.0	11.5		104, 121, 126
	Carfilzomib	1.5 µg	122.7	8.5	1.0	114, 123, 131
		5 µg	113.7	10.8	1.0	109, 126, 106
		15 µg	117.7	12.9	1.0	103, 123, 127
		50 µg	133.3	18.5	1.1	133, 115, 152
		150 µg	125.0	11.4	1.1	130, 112, 133
		500 µg	130.3	8.0	1.1	138 P, 131 P, 122 P
		1500 µg	118.7	14.2	1.0	109 P, 112 P, 135 P
TA98	2NF	2 µg	281.7	5.0	8.9	281, 277, 287
TA100	NaN3	2 µg	648.0	71.6	4.3	730, 598, 616
TA1535	NaN3	2 µg	931.7	45.9	37.8	963, 879, 953
TA1537	AAC	50 µg	591.3	13.1	53.8	606, 581, 587
WP2 uvrA (pKM101)	NQO	2 µg	2883.7	190.1	24.6	2912, 2681, 3058
Key to Positive Controls				Key to Plate Postfix Codes		
2NF	2-Nitrofluorene	P Precipitate				
NaN3	Sodium azide					
AAC	9-Aminoacridine					
NQO	4-Nitroquinoline-1-oxide					

(Table 4, pgs. 24 and 25, excerpted from Applicant's package)

Best Available Copy

Table 83: Bacterial Reverse Mutation Test Results for PR-171 in the Presence of Metabolic Activation (Test 2)

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts
TA98	DMSO		46.0	6.6		53, 45, 40
	Carfilzomib	5 µg	48.3	9.0	1.1	38, 53, 54
		15 µg	41.7	6.4	0.9	38, 38, 49
		50 µg	40.7	4.0	0.9	45, 37, 40
		150 µg	37.7	0.6	0.8	38, 37, 38
		500 µg	42.0	6.1	0.9	39, 38, 49
		1500 µg	40.7	7.6	0.9	49 P, 34 P, 39 P
TA100	DMSO		173.0	15.6		191, 163, 165
	Carfilzomib	5 µg	172.3	28.9	1.0	198, 141, 178
		15 µg	182.3	26.7	1.1	152, 202, 193
		50 µg	176.7	30.4	1.0	202, 143, 185
		150 µg	154.7	10.1	0.9	160, 143, 161
		500 µg	161.0	9.8	0.9	172, 158, 153
		1500 µg	163.0	12.8	0.9	174 P, 149 P, 166 P
TA1535	DMSO		27.3	4.0		23, 31, 28
	Carfilzomib	5 µg	22.7	3.1	0.8	20, 26, 22
		15 µg	24.0	2.0	0.9	26, 24, 22
		50 µg	23.3	2.5	0.9	26, 23, 21
		150 µg	25.7	1.5	0.9	27, 24, 26
		500 µg	19.3	2.3	0.7	18, 22, 18
		1500 µg	19.3	1.2	0.7	18 P, 20 P, 20 P
		5000 µg	18.3	3.1	0.7	15 P, 19 P, 21 P

Key to Plate Postfix Codes

P Precipitate

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts
TA1537	DMSO		25.3	3.1		26, 28, 22
	Carfilzomib	5 µg	23.7	7.6	0.9	32, 22, 17
		15 µg	25.0	7.0	1.0	33, 22, 20
		50 µg	25.3	4.0	1.0	26, 29, 21
		150 µg	26.3	2.9	1.0	23, 28, 28
		500 µg	26.0	4.4	1.0	23, 31, 24
		1500 µg	24.7	2.3	1.0	22 P, 26 P, 26 P
5000 µg	23.7	3.8	0.9	22 P, 28 P, 21 P		
WP2 uvrA (pKM101)	DMSO		146.7	14.3		159, 131, 150
	Carfilzomib	5 µg	165.3	16.3	1.1	178, 147, 171
		15 µg	142.0	5.0	1.0	137, 142, 147
		50 µg	150.0	15.6	1.0	159, 132, 159
		150 µg	159.7	20.3	1.1	176, 166, 137
		500 µg	158.3	4.0	1.1	156, 163, 156
		1500 µg	140.7	6.4	1.0	137 P, 148 P, 137 P
5000 µg	136.7	7.5	0.9	129 P, 137 P, 144 P		
TA98	B[a]P	5 µg	212.7	16.2	4.6	222, 194, 222
TA100	AAN	5 µg	3503.0	248.2	20.2	3221, 3688, 3600
TA1535	AAN	5 µg	512.7	13.3	18.8	528, 504, 506
TA1537	B[a]P	5 µg	133.3	22.0	5.3	147, 145, 108
WP2 uvrA (pKM101)	AAN	10 µg	510.0	45.6	3.5	543, 458, 529

Key to Positive Controls

Key to Plate Postfix Codes

B[a]P

Benzo[a]pyrene

P

Precipitate

AAN

2-Aminoanthracene

(Table 5, pgs. 26 and 27, excerpted from Applicant's package)

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Carfilzomib: In Vitro Mammalian Chromosome Aberration Test In Human Lymphocytes

Study no.: TR-0070-171
 Study report location: EDR 4.2.3.3.1-TR-0070-171
 Conducting laboratory and location: (b) (4)
 Date of study initiation: August 7, 2007
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: PR-171, 0705-029, ≥95%

Key Study Findings

- PR-171 causes an increase in chromosome structural aberrations in human peripheral blood lymphocytes, both in the absence and presence of metabolic activation.
- The effect is more potent in the absence of metabolic activation.

Methods

Cell line: Human lymphocytes in whole blood culture
 Concentrations in definitive study: Test 1:
+S9: 0.01, 0.02, 0.039, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10, 15 µg/mL
 Test 2 :
+S9: 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 µg/mL
-S9: 0.001, 0.002, 0.0039, 0.0078, 0.0156, 0.0313, 0.0625, 0.125, 0.25 µg/mL
 Basis of concentration selection: Test 1 DRF: Precipitate was noted at ≥ 312.5 µg/mL and cytotoxicity was noted at ≥0.625 µg/mL -S9 and ≥5 µg/mL +S9.
 Test 2 DRF: Doses based on Test 1; Mitotic indices reductions (~50%) were not sufficient +S9, thus doses were increased compared to -S9.
 Negative control: DMSO
 Positive control: +S9: cyclophosphamide (5 µg/mL)
 -S9: Mitomycin C (Test 1, 0.2 µg/mL; Test 2, 0.1 µg/mL)
 Formulation/Vehicle: DMSO
 Incubation & sampling time: 2 plates/treatment
 Test 1- 3 hr treatment, 18 hr recovery

Test 2- +S9: 3 hr treatment, 18 hr recovery
-S9: 21 hr treatment

Study Validity- Valid

1. Negative controls fell within historical range of proportion of cells with chromosomal aberrations.
2. Appropriate positive controls induced appropriate increase in rate of chromosomal aberrations over threshold value \pm S9 metabolic activation and within historical range.
3. Cytotoxicity (~10-20% viability) was observed in least one dose used in the initial study.
4. At least 3 concentrations in the two studies did not show excessive cytotoxicity (>50%) and were available for metaphase analysis.
5. A sufficient number of cells were evaluated (100/plate, total of 200 cells).

Results

The sponsor performed two, duplicate plate tests with a separate DRF for each. Dosing was limited by cytotoxicity. The first test used the same incubation times of 3 hr, while the second test had the 3 hr incubation +S9, but 21 hr -S9.

Test 1- Significant cytotoxicity with a steep concentration response was observed in the first test, with no mitotic cells present at any concentration. In a repeat test, there was a mitotic index value of 55% at 0.313 μ g/mL -S9 compared to control. This concentration was considered to be the high dose in the absence of metabolic activation and used for the metaphase analysis (see Methods above with a single underline). In the presence of metabolic activation, PR-171 caused a reduction of the mitotic index to 55% at 2.5 μ g/mL compared to control and was selected as the high dose (see Methods above with a double underline).

Following 3 hr of exposure to PR-171 in the absence of metabolic activation, there was significant increase in the proportion of metaphase figures containing observable chromosomal aberrations all three doses. At 0.078 and 0.156 μ g/mL, there was a comparable increase of 6.0% and 6.5% mean cells with aberrations (excluding gaps) and 6.5 and 7.5% (including gaps), respectively. These exceeded the historical range of 0-3.0% for % cell aberrations with gaps excluded, and equaled or exceeded historical range of 0-6.5% for % cells with gaps included. At 0.313 μ g/mL of PR-171, the values were 17% and 18.5% for % cell aberrations with gaps excluded and included, respectively. There was also a concentration-dependent decrease in mitotic index observed.

In the presence of metabolic activation, PR-171 at 2.5 μ g/mL caused a significant increase in the proportion of metaphase figures containing observable chromosomal aberrations of 12% and 12.5% for % cell aberrations with gaps excluded and included, respectively. The mitotic index compared to control was reduced at ≥ 1.25 μ g/mL.

Test 2- For continuous treatment of 21 hr, there was a mitotic index value of 49% at 0.0625 μ g/mL -S9 compared to control. This concentration was considered to be the high dose in the absence of metabolic activation (see Methods above in **bold and**

single underline). In the presence of metabolic activation, PR-171 for 3 hr caused a reduction of the mitotic index to 52% at 5.5 µg/mL compared to control and was selected as the high dose (see Methods above in **bold and double underline**).

Table 84: Summary of Metaphase Analyses ± S9 Metabolic Activation

Test 1

Exposure period (hr)	S9 mix (v/v)	Nominal concentration of carfilzomib (µg/mL)	Cells with aberrations excluding gaps			Cells with aberrations including gaps			Relative mitotic index (%)	Polyploidy frequency
			Individual values (%)	Mean (%)		Individual values (%)	Mean (%)			
3	-	0 (DMSO)	0.0	1.0	0.5	0.0	3.0	1.5	100	0
		0.078	8.0	4.0	6.0***	8.0	5.0	6.5**	92	4
		0.156	5.0	8.0	6.5***	6.0	9.0	7.5**	74	1
		0.313	16.0	18.0	17.0***	18.0	19.0	18.5***	55	1
		0.2 (Mitomycin C)	16.0	32.0	24.0***	17.0	33.0	25.0***	-	1
3	+ (2%)	0 (DMSO)	3.0	1.0	2.0	4.0	1.0	2.5	100	1
		0.625	2.0	4.0	3.0	2.0	6.0	4.0	106	0
		1.25	1.0	3.0	2.0	4.0	3.0	3.5	89	2
		2.5	8.0	16.0	12.0***	8.0	17.0	12.5***	55	2
		5 (Cyclophosphamide)	16.0	17.0	16.5***	18.0	19.0	18.5***	-	0

Test 2

Exposure period (hr)	S9 mix (v/v)	Nominal concentration of carfilzomib (µg/mL)	Cells with aberrations excluding gaps			Cells with aberrations including gaps			Relative mitotic index (%)	Polyploidy frequency
			Individual values (%)	Mean (%)		Individual values (%)	Mean (%)			
21	-	0 (DMSO)	1.0	0.0	0.5	4.0	4.0	4.0	100	0
		0.0156	1.0	2.0	1.5	4.0	2.0	3.0	82	1
		0.0313	2.0	2.0	2.0	5.0	6.0	5.5	62	3
		0.0625	11.0	7.0	9.0***	15.0	10.0	12.5***	49	7**
		0.1 (Mitomycin C)	12.0	12.0	12.0***	17.0	23.0	20.0***	0	0
3	+ (5%)	0 (DMSO)	1.0	0.0	0.5	1.0	1.0	1.0	100	3
		2.5	3.0	5.0	4.0	4.0	5.0	4.5	97	1
		4.5	5.0	6.0	5.5**	6.0	10.0	8.0***	72	4
		5.5	11.0	17.0	14.0***	11.0	18.0	14.5***	52	2
		5 (Cyclophosphamide)	24.0	14.0	19.0***	28.0	14.0	21.0***	-	0

One-tailed Fisher's exact test

*** $p < 0.001$

** $p < 0.01$

Otherwise $p \geq 0.01$

(Table 1, pg. 23, excerpted from Applicant's package)

PR-171 in the absence of metabolic activation at 0.0625 µg/mL caused a significant increase in the proportion of metaphase figures containing observable chromosomal aberrations of 9 and 12.5% for % cell aberrations with gaps excluded and included, respectively. There also was a concentration-dependent increase in polyploidy frequency significant at 0.0625 µg/mL with a value of 7% (control: 0%). There was also a concentration-dependent decrease in mitotic index observed.

In the presence of metabolic activation, PR-171 caused a dose-dependent increase in the proportion of metaphase figures containing observable chromosomal aberrations that was significant at ≥ 4.5 $\mu\text{g/mL}$. At 5 $\mu\text{g/mL}$, there were maximal values of 14 and 14.5% for % cell aberrations with gaps excluded and included, respectively. The mitotic index was reduced at ≥ 4.5 $\mu\text{g/mL}$ compared to control.

Study title: Carfilzomib: In Vitro Mammalian Chromosome Aberration Test In Human Lymphocytes

Study no.:	TR-0426-171
Study report location:	EDR 4.2.3.3.1-TR-0426-171
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 19, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PR-171, 197-36, 94.8%
	PR-519: 1.1%
	PR-59428: 1.1%
	PR-187: 1.3%
	PR-59462: 1.2%

Key Study Findings

- The results for PR-171 spiked with impurities are similar to a PR-171 drug substance with >95% purity.
- PR-171 spiked with impurities causes an increase in chromosome structural aberrations in human peripheral blood lymphocytes at comparable concentrations.

Methods

Cell line:	Human lymphocytes in whole blood culture
Concentrations in definitive study:	+S9: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 $\mu\text{g/mL}$ -S9: 0.04, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, 0.25, 0.30, 0.35 $\mu\text{g/mL}$
Basis of concentration selection:	DRF: significant cytotoxicity was noted at ≥ 0.14 $\mu\text{g/mL}$ -S9 and ≥ 5 $\mu\text{g/mL}$ +S9.
Negative control:	DMSO
Positive control:	+S9: cyclophosphamide (5 $\mu\text{g/mL}$) -S9: Mitomycin C (0.2 $\mu\text{g/mL}$)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	2 plates/treatment 3 hr treatment, 18 hr recovery

Study Validity- Valid

1. Negative controls fell within historical range of proportion of cells with chromosomal aberrations.
2. Appropriate positive controls induced appropriate increase in rate of chromosomal aberrations over threshold value and within historical range+S9 metabolic activation, but above historical range-S9- 69.0% vs. 62.5%.
3. Cytotoxicity (~10-20% viability) was observed in least one dose used in initial study.
4. At least 3 concentrations did not show excessive cytotoxicity (>50%) and were available for metaphase analysis.
5. A sufficient number of cells were evaluated in the presence of metabolic activation (100/plate, total of 200 cells). In the absence of metabolic activation, significantly fewer than 100/plate were evaluated (see Table below). However, since there were clear concentration-dependent signs of clastogenicity and cytotoxicity, the evaluation was adequate.

Table 85: Metaphase Analysis Data - Number of Cell Examined

Nominal concentration of Carfilzomib (µg/mL)	No. cells examined
0 (DMSO)	100 100
0.04	68 76
0.08	30 36
0.12	31 27
0.2 (Mitomycin C)	16 13

(Table 3, pg. 27, excerpted from Applicant's package)

Results

Significant cytotoxicity with a steep concentration response was observed in the first test, with no mitotic cells present at 1-10 µg/mL -S9 and 5-10 µg/mL+S9. In a repeat test, there was a mitotic index value of 53% at 0.22 µg/mL -S9 compared to control with no toxicity evident at 0.01 - 0.05 µg/mL. A second repeat was performed; was a mitotic index value of 49% at 0.12 µg/mL -S9 compared to control. This concentration was considered to be the high dose in the absence of metabolic activation and used for the metaphase analysis (see Methods above with a single underline). In the presence of metabolic activation, PR-171 caused a reduction of the mitotic index to 49% at 3 µg/mL compared to control and was selected as the high dose (see Methods above with a double underline).

In the absence of metabolic activation, there was significant increase in the proportion of metaphase figures containing observable chromosomal aberrations all three doses. At 0.04 µg/mL of PR-171, the mean values were 13.9% and 14.6% for %

cell aberrations with gaps excluded and included, respectively. At 0.08 and 0.12 µg/mL, there was a comparable increase of 30.3% and 34.5% mean cells with aberrations (including and excluding gaps were identical values) respectively. These exceeded the historical range of 0-3.0% for % cell aberrations. There was also a concentration-dependent decrease in mitotic index observed.

In the presence of metabolic activation, PR-171 at 3 µg/mL caused a significant increase in the proportion of metaphase figures containing observable chromosomal aberrations of 15.2 % for % cell aberrations both with gaps excluded and included. There was also a concentration-dependent decrease in mitotic index observed.

Table 86: Summary of Metaphase Analyses ± S9 Metabolic Activation

Exposure period (hours)	S9 mix (v/v)	Nominal concentration of Carfilzomib (µg/mL)	Cells with aberrations excluding gaps			Cells with aberrations including gaps			Relative mitotic index (%)	Polyploidy mean incidence (%)
			Individual values (%)	Mean (%)		Individual values (%)	Mean (%)			
3	-	0 (DMSO)	0.0	1.0	0.5	0.0	1.0	0.5	100	0.5
		0.04	14.7	13.2	13.9***	14.7	14.5	14.6***	97	2.1
		0.08	33.3	27.8	30.3***	33.3	27.8	30.3***	87	0.0
		0.12	32.3	37.0	34.5***	32.3	37.0	34.5***	49	0.0
		0.2 (Mitomycin C)	62.5	76.9	69.0***	62.5	76.9	69.0***	-	0.0
3	+ (2%)	0 (DMSO)	0.0	4.0	2.0	4.0	4.0	4.0	100	0.0
		1	0.0	6.0	3.0	1.0	9.0	5.0	93	0.0
		2	6.0	4.0	5.0	6.0	7.0	6.5	81	0.5
		3	26.3	11.0	15.2***	26.3	11.0	15.2***	49	0.0
		5 (Cyclophosphamide)	25.6	32.3	28.6***	30.8	35.5	32.9***	-	0.0

One-tailed Fisher's exact test

*** $p < 0.001$

Otherwise $p \geq 0.01$

(Table 1, pg. 22, excerpted from Applicant's package)

7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Carfilzomib Mouse Micronucleus Test

Study no: TR-0071-171
 Study report location: EDR, 4.2.3.3.2-TR-0071-171
 Conducting laboratory and location: (b) (4)
 Date of study initiation: August 28, 2007
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: PR-171, 06RD30, 95.3%

Key Study Findings

- PR-171 did not cause a significant increase in the number of micronucleated polychromatic erythrocytes in either sex under the conditions tested.
- Dose limiting toxicities such as weight loss and mortality with clinical signs that included flattened posture, piloerection, inactivity, irregular breathing, ungroomed coat and cyanosis was observed in both sexes at ≥ 2.5 mg/kg. These clinical signs were also observed in surviving animals and all treated animals lost weight.

Methods

Doses in definitive study: ♂: 1.25, 2.5, 5 mg/kg; ♀: 0.31, 0.63, 1.25 mg/kg
 Frequency of dosing: Twice, 24 hr apart
 Route of administration: IV
 Dose volume: 10 mL/kg
 Formulation/Vehicle: 10% HPBCD (hydroxypropyl- β -cyclodextrin)/ 50 mM sodium citrate, pH 3.5
 Species/Strain: Mouse/CD-1 (b) (4)
 Age: ~6 weeks
 Weight: ♂ 28.0 - 32.0 g; ♀ 22.0 - 25.4 g
 Number/Sex/Group: 5/sex/group
 Satellite groups: none
 Basis of dose selection: MTD in DRF studies- female MTD set at 1.25 mg/kg based on mortality at 2.5 and 5 mg/kg; male MTD set at 5 mg/kg based on mortality at 7.5 mg/kg.
 Negative control: vehicle
 Positive control: Mitomycin C (12 mg/kg) at 20 mL/kg

Bone marrow sampling occurred at 24 hr after last dose. Bone marrow from both femurs was flushed and smears were taken on slides. 2000 polychromatic erythrocytes (PCE) per animal were evaluated for incidence of micronuclei. The proportion of PCEs out of 1000 normochromatic erythrocytes (NCE) was performed in parallel with number of mature micronucleated erythrocytes recorded. The sponsor's Acceptance Criteria are listed below:

1. Each treated and control group should include at least 5 analysable animals.
2. Vehicle control values for micronucleated polychromatic erythrocytes must be consistent with the laboratory historical negative control data.
3. Positive controls must show clear unequivocal positive responses.
4. The proportion of polychromatic erythrocytes among total erythrocytes in treated groups is not less than 20% of the control value.

Study Validity- Valid

The study was adequate for the following criteria:

- 1) Dosing appeared to be adequate based on the dose-ranging study results.
- 2) Preparation and administration of the test substance was acceptable.

- 3) Negative and positive controls were within historical range for (PCE/NCE) ratio and % Mononucleated Polychromatic Erythrocytes (% MN-PCE)
- 4) Positive controls exhibited appropriate responses within historical range
- 5) Proportion of immature erythrocytes (PCE) among total erythrocytes was not less than 20% of the control value
- 6) Positive control must induce a statistically significant ($P < 0.05$) response of rate of MN-PCEs relative to the vehicle control
- 7) Based on toxicity differences between the sexes, both sexes were evaluated in the definitive study.

The Applicant reported that the study did not meet its own Criteria #1 with only 4/6 males at 5 mg/kg surviving to termination; “*As insufficient animals survived in this treatment group (i.e. less than 5), this group was excluded and no slide analysis was conducted.*” The Applicant did not measure exposure of PR-171 in mice; no comparison to human clinical exposure of drug is possible. However, since the Applicant made a reasonable effort to dose to the MTD, the overall study is considered valid by this reviewer.

Results

Mortality

Preliminary study: Treatment-related mortality was observed in females at ≥ 2.5 mg/kg and males at 7.5 mg/kg. One female at 5 mg/kg was found dead on Day 3 (prior to termination) with clinical signs that included flattened posture, piloerection, inactivity, irregular breathing, ungroomed coat and cyanosis. One female at 2.5 mg/kg was found dead on Day 3 (prior to termination) with clinical signs that included flattened posture, inactivity, irregular breathing, ungroomed coat, and abnormal gait. One male at 7.5 mg/kg was sacrificed *in extremis* on Day 2, with severe clinical signs that included flattened posture, piloerection, reduced activity, irregular breathing, partially closed eyelids and cyanosis.

Definitive study: One male at 2.5 mg/kg (#226M) and two males at 5 mg/kg (#233M and #501M) were found dead with clinical signs. Oddly, the sponsor did not provide individual animal data that reported the specific findings for each animal, only a group summary (see below in Clinical Signs).

Clinical Signs

Preliminary study: On Day 3, clinical signs included flattened posture, piloerection, reduced activity, irregular breathing, ungroomed coat and cyanosis at ≥ 2.5 mg/kg in both sexes. No significant signs were observed in females at 1.25 mg/kg.

Definitive study: On Day 3, similar clinical signs as the preliminary study were observed in males at all doses and females at 1.25 mg/kg. Singular instances were observed in males at ≥ 2.5 mg/kg on Day 2. No significant signs were observed in females at ≤ 0.63 mg/kg.

Body Weights

Definitive study: All animals treated with PR-171 weighed less at the study termination compared to pre-dose weights. The maximal mean weight loss was ~6-7% in males at 1.25 and 2.5 mg/kg and females at 1.25 mg/kg. The weight loss in males at 5 mg/kg was 9%, but this group was not included in micronucleus analysis. Animals treated with Mitomycin C gained weight.

Micronucleus Evaluation

PR-171 did not cause a significant increase in the number of micronucleated polychromatic erythrocytes in either sex at any dose. The %PCE was also unaffected by treatment.

Table 87: Summary of Mouse Micronucleus Test Results for Intravenous PR-171

MALE DATA					
Sampling time after second dose	Treatment	Dose (mg/kg/day)	Proportion % PCE #	MPCE # (Group Mean)	MNCE # (Group Mean)
24 Hours	Vehicle	0	40.7	0.3	0.0
	carfilzomib	1.25	38.5	0.8	0.3
	carfilzomib	2.5	42.1	0.6	0.4
	Mitomycin C ^a	12	41.9	54.4**	0.4

FEMALE DATA					
Sampling time after second dose	Treatment	Dose (mg/kg/day)	Proportion % PCE #	MPCE # (Group Mean)	MNCE # (Group Mean)
24 Hours	Vehicle	0	42.1	0.3	0.0
	carfilzomib	0.31	43.3	0.3	0.2
	carfilzomib	0.63	39.9	0.2	0.3
	carfilzomib	1.25	44.7	0.3	0.0
	Mitomycin C ^a	12	45.7	66.0**	0.2

Vehicle 10% (w/v) sulfobutylether beta-cyclodextrin and 10 mM citrate buffer (pH 3.5)

PCE Polychromatic erythrocytes

MPCE Number of micronucleated cells observed per 2000 polychromatic erythrocytes examined

MNCE Number of micronucleated normochromatic erythrocytes observed

^a Positive control, dosed on one occasion only 24 hours prior to termination

Occasional apparent errors of $\pm 1\%$ may occur due to rounding of values for presentation in the table

Results of statistical analysis using the appropriate nonparametric method of analysis based on permutation (one-sided probabilities):

** $P < 0.01$ (significant)
 otherwise $P > 0.01$ (not significant)

(Table 1, pg. 22, excerpted from Applicant's package)

Study title: Carfilzomib Mouse In Vivo Micronucleus Test

Study no:	TR-0422-171
Study report location:	EDR, 4.2.3.3.2-TR-0422-171
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 12, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PR-171, 186146TS, 92.3% PR-59428 (1.2%) PR-187 (0.77%) PR-519 (1.6%)

Key Study Findings

- PR-171 spiked with 3 process impurities did not cause a significant increase in the number of micronucleated polychromatic erythrocytes in either sex under the conditions tested.
- Clinical signs and weight loss were comparable to Study #TR-0070-171.

Methods

Doses in definitive study:	0.625, 1.25, 2.5 mg/kg
Frequency of dosing:	Twice, 24 hr apart
Route of administration:	IV
Dose volume:	10 mL/kg
Formulation/Vehicle:	10% HPBCD (hydroxypropyl- β -cyclodextrin)/ 50 mM sodium citrate, pH 3.5
Species/Strain:	Mouse/CD-1 (b) (4)
Age:	~6 weeks
Weight:	♂ 28.2 - 32.8 g; ♀ 21.4 - 25.0 g
Number/Sex/Group:	6/sex/group
Satellite groups:	none
Basis of dose selection:	MTD in DRF studies- female MTD set at 1.25 mg/kg based on mortality at 2.5 and 5 mg/kg; male MTD set at 5 mg/kg based on mortality at 7.5 mg/kg. Clinical signs were observed
Negative control:	vehicle
Positive control:	Mitomycin C (12 mg/kg) at 20 mL/kg

As for Study #TR-0070-171, above, except the sponsor's Acceptance Criteria:

1. Each treated and control group should include analyzable smears from at least 5 individual animals.
2. Vehicle control values for micronucleated polychromatic erythrocytes must be consistent with the laboratory historical negative control data.
3. Positive controls must show clear unequivocal positive responses.

Study Validity- Valid

The study was adequate for the following criteria:

- 1) Dosing appeared to be adequate based on the dose-ranging study results.
- 2) Preparation and administration of the test substance was acceptable.
- 3) Negative and positive controls were within historical range for (PCE/NCE) ratio and % Mononucleated Polychromatic Erythrocytes (% MN-PCE)
- 4) Positive controls exhibited appropriate responses within historical range
- 5) Proportion of immature erythrocytes (PCE) among total erythrocytes was not less than 20% of the control value
- 6) Positive control must induce a statistically significant ($P < 0.05$) response of rate of MN-PCEs relative to the vehicle control
- 7) Based on toxicity differences between the sexes in Study# TR-0070-171, both sexes were evaluated.

The sponsor reported that the study did not meet its own Criteria #1 with only 4/5 males at 2.5 mg/kg surviving to termination; *“As insufficient animals survived in this treatment group (i.e. less than 5), this group was excluded and no slide analysis was conducted.”* The sponsor did not measure exposure of PR-171 in mice; no comparison to human clinical exposure of drug is possible. However, since the sponsor made a reasonable effort to dose to the MTD, the overall study is considered valid by this reviewer.

Results

Mortality

Preliminary study: Treatment-related mortality was observed at 5 mg/kg. On Day 2, one female and one male at 5 mg/kg were sacrificed *in extremis* with severe clinical signs that included hunched posture, piloerection, closed left eyelid, unsteady gait, reduced activity, partially closed eyelids, slow breathing, muscle tremors and paleness.

Definitive study: One male at 2.5 mg/kg (#231M) was found dead following clinical signs.

Clinical Signs

Preliminary study: On Day 3, clinical signs included piloerection, partially closed eyelids, reduced activity, paleness, hunched posture, deep breathing and unsteady gait at ≥ 2.5 mg/kg in both sexes. No significant signs were observed in females at 1.25 mg/kg.

Definitive study: On Day 3, similar clinical signs as the preliminary study were observed in males at all doses and females at 2.5 mg/kg. No significant signs were observed at ≤ 1.25 mg/kg.

Body Weights

Definitive study: In both sexes, four of five animals at 1.25 mg/kg and all animals at 2.5 mg/kg weighed less at the study termination compared to pre-dose weights. The maximal mean weight loss was 5% in males at 1.25 and 6% in females at 1.25 mg/kg. The weight loss in males at 5 mg/kg was 5%, but this group was not included in micronucleus analysis. Animals treated with Mitomycin C gained weight.

Micronucleus Evaluation

PR-171 spiked with 3 process impurities did not cause a significant increase in the number of micronucleated polychromatic erythrocytes in either sex at any dose. The %PCE was also unaffected by treatment.

Table 88: Summary of Mouse Micronucleus Test Results for Intravenous PR-171 Spiked with Three Process Impurities

Male Data				
Sampling time after 2 nd dose	Treatment	Dose (mg/kg/day)	Proportion of PCE (%) #	Incidence MPCE (mean) #
24 Hours	Vehicle	-	51.5	1.4
	carfilzomib	0.625	50.4	0.8
	carfilzomib	1.25	54.3	1.0
	Mitomycin C ^a	12	37.5**	79.2**
Female Data				
Sampling time after 2 nd dose	Treatment	Dose (mg/kg/day)	Proportion of PCE (%) #	Incidence MPCE (mean) #
24 Hours	Vehicle	-	53.7	1.2
	carfilzomib	0.625	54.3	0.6
	carfilzomib	1.25	51.7	1.4
	carfilzomib	2.5	50.8	0.6
	Mitomycin C ^a	12	38.4**	65.2**

Vehicle 10% (w/v) sulfobutylether beta-cyclodextrin and 10 mM citrate buffer (pH 3.5)

PCE Polychromatic erythrocytes

MPCE Number of micronucleated polychromatic erythrocytes observed per 2000 polychromatic erythrocytes examined

^a Positive control dosed once only 24 hours prior to termination

Occasional apparent errors of $\pm 1\%$ may occur due to rounding of values for presentation in the table.

Results of statistical analysis using the appropriate nonparametric method of analysis based on permutation (one-sided probabilities):

** $p < 0.01$ (significant)

(Table 1, pg. 25, excerpted from Applicant's package)

9 Reproductive and Developmental Toxicology

9.2 Embryonic Fetal Development

Study title: Preliminary Intravenous Embryo-Fetal Toxicity Study in Rats

Study no.:	TR-0074-171
Study report location:	eCTD: 4.2.3.5.2.TR-0074-171
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Sept. 19, 2007
GLP compliance:	no
QA statement:	no
Drug, lot #, and % purity:	PR-171, 050307, 93.33%

Key Study Findings

- Doses investigated induced a 5% decrease in body weight gain during dosing (not statistically significant).
- Test article-related increases in % post-implantation loss at all doses due to early resorptions (not statistically significant).

Methods

Doses:	0.5, 1, and 2 mg/kg
Frequency of dosing:	daily
Dose volume:	IV bolus
Route of administration:	1 mL/kg
Formulation/Vehicle:	10% HPBCD (hydroxypropyl- β -cyclodextrin)/ 50 mM sodium citrate, pH 3.5
Species/Strain:	Rat/ Sprague-Dawley [CrI:CD® (SD)IGS BR]
Age:	10-12 weeks
Weight:	238-291g
Number/Sex/Group:	8/group
Satellite groups:	none
Study design:	Dosing from Gestation Days (GD) 6 to 17, with Cesarean section on GD 20
Deviation from study protocol:	2 minor deviations

Observations and Results

Mortality

None

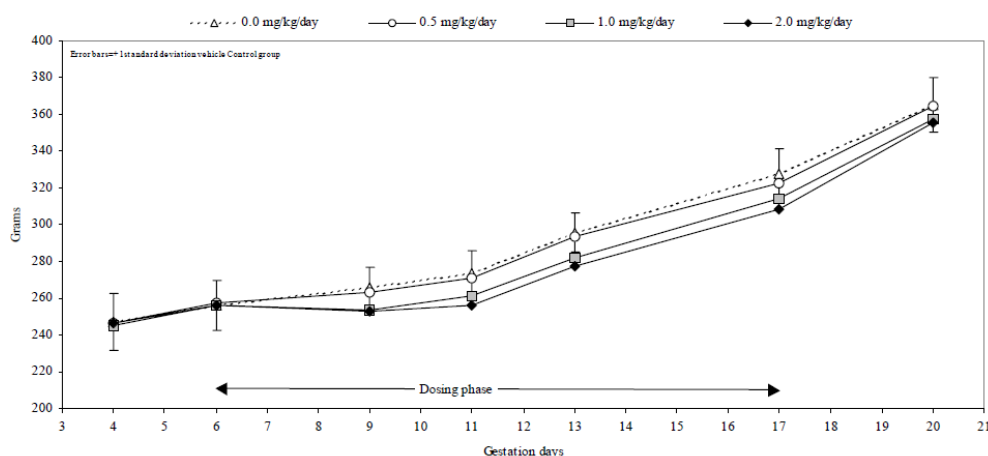
Clinical Signs

Not remarkable.

Body Weight

There was a non-significant dose-dependent decrease in body weight and body weight gain that was maximally -3% and -5%, respectively, compared to control for GDs 6-20. There was larger body weight gain differences observed early during the dosing period on GD2 6-9, but the weight gain recovered by the end of the in-life period.

Figure 44: Body Weight in Pregnant Rats Given Intravenous Administration of PR-171 from GD6-17



Best Available Copy

(Figure excerpted from Applicant's package)

Feed Consumption

Food consumption correlated with the body weight values.

Necropsy

Not remarkable.

Cesarean Section Data

All treated animals were pregnant and with viable fetuses. No findings were different from control with statistical significance. There was an increase in % post-implantation loss in all treated groups. Values for post-implantation loss were 9.3%, 5.1%, and 5.6% at 0.5, 1, and 2 mg/kg, respectively, compared to control value of 1.6%. This was due to increased mean early resorptions with values of 1.4, 0.8, and 0.8, respectively, compared to 0.1 for control. There were no late resorptions noted for any group. There was a decrease in gravid uterine weight in treated groups that did not highly correlate with the above noted increase in implantation losses.

Offspring

Not remarkable.

Study title: Carfilzomib: An Intravenous Embryo-Fetal Toxicity Study in Rats (GLP)

Study no.: TR-0075-171
Study report location: eCTD: 4.2.3.5.2.TR-0075-171
Conducting laboratory and location: (b) (4)
Date of study initiation: January 7, 2008
GLP compliance: yes
QA statement: yes
Drug, lot #, and % purity: PR-171, PCF001, 93.46%

Key Study Findings

- PR-171 was not tolerated at 2 mg/kg with clinical signs of piloerection, hunched posture, decreased activity, and feed consumption, a significant decrease in absolute body weight and in body weight gain and two animals found dead.
- Increased pre-implantation loss and early resorptions at ≥ 1 mg/kg
- No remarkable fetal malformations, variations or retardations were observed.

Methods

Doses: 0.5, 1, and 2 mg/kg
Frequency of dosing: daily
Dose volume: IV bolus
Route of administration: 1 mL/kg
Formulation/Vehicle: 10% HPBCD (hydroxypropyl- β -cyclodextrin)/ 50 mM sodium citrate, pH 3.5
Species/Strain: Rat/ Sprague-Dawley [CrI:CD® (SD)IGS BR]
Age: 10-12 weeks
Weight: 217-286 g
Number/Sex/Group: 22♀/group
Satellite groups: TK: 6♀/group
Study design: Dosing from Gestation Days (GD) 6 to 17, with Cesarean section on GD 20
Deviation from study protocol: None reported

Observations and Results**Mortality**

Two dams at 2 mg/kg were found dead on GDs 9 (#4512) and 15 (#4507), respectively. The deaths were considered test article-related. Implantation sites with normal fetuses were found in both animals and the clinical signs observed were similar to those observed in the other dams at 2 mg/kg (hunched appearance, labored breathing, decreased activity, and piloerection). Both dams had thin fluid in thorax at necropsy. Animal #4507 had an enlarged heart, distended colon, discolored jejunal serosa, and small spleen.

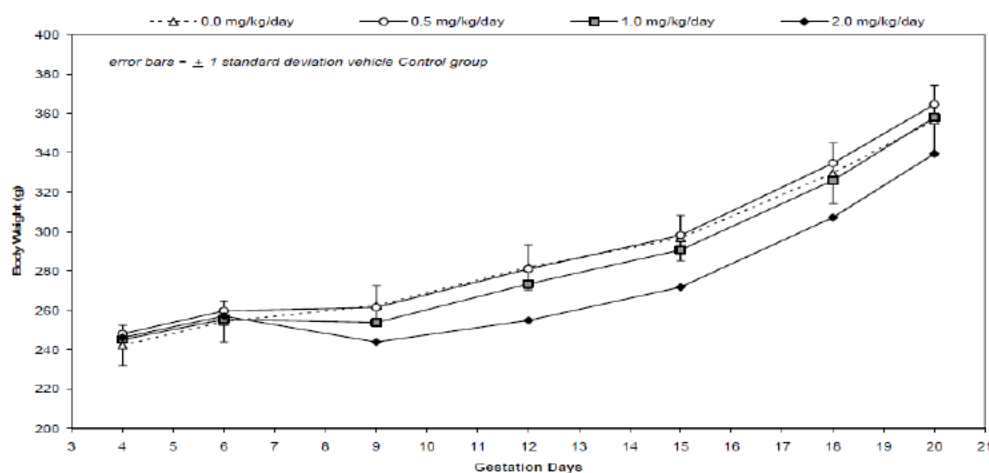
Clinical Signs

In general, there were a number of signs observed at 2 mg/kg beginning on GD 9 (except for piloerection in 5 dams at 1 mg/kg on GD 12). Piloerection, decreased food consumption and hunched appearance was observed in all animals at 2 mg/kg with incidence increasing with duration. Anogenital staining, paleness, labored breathing, and decreased activity were also observed in some dams at 2 mg/kg.

Body Weight

There was a decrease of 5% in absolute body weight at 2 mg/kg compared to control at termination based on pre-treatment weights. The % body weight gain was also reduced compared to control, but all dams that survived did gain weight. All treated groups had significant dose-dependent mean reduced weight changes during the GD 6-9 dosing period, with mean absolute weight losses at ≥ 1 mg/kg reaching -12.5 g at 2 mg/kg, compared to control. Following the dosing period, dams at ≥ 1 mg/kg had significantly increased mean weight changes compared to control.

Figure 45: Gestational Body Weights in Pregnant Rats Treated with Intravenous PR-171 from Gestational Day 6 to 17 (n=20-22)



Best Available Copy

(Figure 1, pg. 45. excerpted from Applicant's package)

Table 89: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) in Pregnant Rats Administered Intravenous PR-171 from Gestational Day 6-17 (n=20-22)

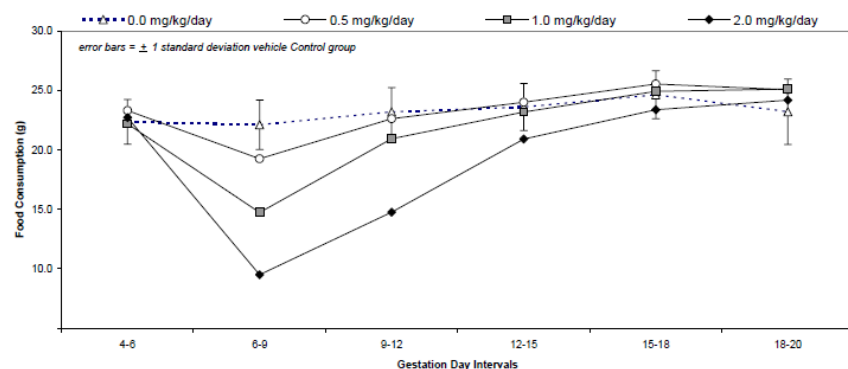
Dose (mg/kg)	GD 4 (pre-dose) BWt (g)	GD 20 BWt (g)	% Gain	% Δ
0	242.3	356.5	+47	--
0.5	248.1	364.6	+47	-5
1	245.2	358.0	+46	+4
2	246.2	339.6*	+38	-5

*, P<0.05

Feed Consumption

Feed consumption was consistent with noted treatment-related body weight changes.

Figure 46: Feed Consumption in Pregnant Rats Treated with Intravenous PR-171 from Gestational Day 6 to 17 (n=20-22)



Best Available Copy

(Figure excerpted from Applicant's package)

Toxicokinetics

TK samples were collected from 3 dams/group/time point on GDs 6 and 17 at pre-dose, 5, 15, 30, and 60 min post-dose.

As expected for an IV drug, T_{max} was 5 min at all doses and days. On GD 6, both C_{max} and AUC were dose-linear from 0.5 to 1 mg/kg and were greater than dose-proportional from 1 to 2 mg/kg. On GD17, PR-171 exposure as measured by C_{max} and AUC were linear and dose-proportional from 0.5 to 1 mg/kg; AUC was less than proportional and C_{max} was greater than proportional from 1 to 2 mg/kg. Exposure of all three measured metabolites increased dose proportionally. The abundance in descending order was PR-389 followed by PR-519 and then PR-413.

Table 90: TK Parameters of PR-171 and its Main Metabolites in a Rat Embryo-Fetal Toxicity Study

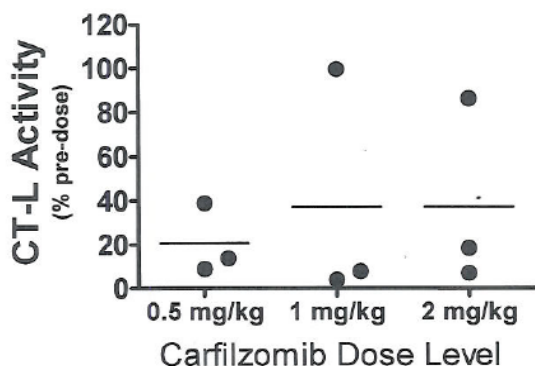
		carfilzomib		PR-389		PR-413		PR-519	
		GD 6	GD 17	GD 6	GD 17	GD 6	GD 17	GD 6	GD 17
0.5 mg/kg/day	C_{max} (ng/mL)	95.1	85.5	95.6	103	28	26.7	59.8	40.9
	T_{max} (min)	5	5	5	5	5	5	5	5
	AUC_{inf} (ng*min/mL)	1799	1675	4875	4911	372	455	723	471
1.0 mg/kg/day	C_{max} (ng/mL)	170	173	183	188	52.4	50.7	107	60
	T_{max} (min)	5	5	5	15	5	5	5	5
	AUC_{inf} (ng*min/mL)	2892	3157	8862	10231	777	949	1430	839
2.0 mg/kg/day	C_{max} (ng/mL)	536	246	347	464	112	109	227	119
	T_{max} (min)	5	5	5	5	5	5	5	5
	AUC_{inf} (ng*min/mL)	13200	4509	14755	18064	1521	1696	2603	1442

(Table, pg. 6, excerpted from Applicant's package)

Special Evaluation- Pharmacodynamics

Evaluated in blood cell pellets from samples taken at pre-dose and 1 hr post dose timepoints from 3 animals/group on GD 6. At all dose levels and times, PR-171 induced >60% inhibition of mean proteasome activity; however in all cases, 2/group had >80%, and the final animals ranged from 0-60%.

Figure 47: Protease Activity in Whole Blood Samples from Pregnant Rats



(Figure 1, pg. 429, Appendix S, excerpted from Applicant's package)

Dosing Formulation Analysis

Test article concentration was confirmed at both RT and -70°C within 10% of the nominal doses (Day 1: 101.2 - 103.5%; Day 19: 91.0 - 99.8%).

Necropsy

Not remarkable, except for the two deaths at 2 mg/kg as noted under mortality.

Cesarean Section Data

All treated animals except one dam at 2 mg/kg were pregnant and with viable fetuses. No differences in findings compared to control were statistically significant. There was an increase in early resorptions that resulted in a decreased % pre-implantation loss at ≥ 1 mg/kg. There was a slight dose-dependent increase in gravid uterine weight in treated groups that did not correlate with any notable increase in viability.

Table 91: Selected Gestation Day 20 Laparohysterectomy Data Obtained from a Rat Embryo-Fetal Reproductive Toxicity Study of Daily IV PR-171 Administration (N=20-22/group)

Parameter	Dose (mg/kg)			
	0	0.5	1	2
No. Initial Females in Study	22	22	22	22
Pregnant Females	22	22	22	22
Pregnant Females at Termination	22	22	22	19
No. Females w/ Total Implantation Loss	0	0	0	1
Mean Gravid Uterine Weight (g)	77.05	77.92	80.07	82.82
Mean Early Resorptions/dam	0.8	0.7	1.0	1.4
Pre-implantation loss (%)	5.0	3.1	2.8	2.1
Post-implantation loss (%)	6.7	5.1	7.5	5.4
Mean Viable Fetuses/dam	12.5	12.7	12.6	13.1
Mean Fetal Weight (g)	4.0	4.0	4.1	4.1

*, P<0.05 v. control

Offspring

Not remarkable.

Study title: Preliminary intravenous Embryo-Fetal Toxicity Study in Rabbits (non-GLP)

Study no.: TR-0123-171
Study report location: eCTD: 4.2.3.5.2.TR-0123-171
Conducting laboratory and location: (b) (4)
Date of study initiation: March 4, 2008
GLP compliance: no
QA statement: no
Drug, lot #, and % purity: PR-171, 050307, 93.33%

Key Study Findings

- One doe at 0.8 mg/kg was found dead on GD 8 with test article-related pathological findings of cardiac and pulmonary edema.
- Statistically significant maternal body weight and feed consumption decreases were noted at 0.8 mg/kg compared to control.
- There was a statistically significant increase in % pre-implantation loss at ≥ 0.4 mg/kg, and an increase in early resorptions at 0.8 mg/kg (not statistically significant).
- There was increased post-implantation loss and decreased mean viable fetuses and fetal weight at 0.8 mg/kg (not statistically significant) that was driven primarily by one litter from a dam that exhibited maternal toxicity.

Methods

Doses: 0.2, 0.4, and 0.8 mg/kg

Frequency of dosing: daily
Dose volume: IV bolus
Route of administration: 1 mL/kg
Formulation/Vehicle: 10% HPBCD (hydroxypropyl- β -cyclodextrin)/ 50 mM sodium citrate, pH 3.5
Species/Strain: Rabbit/New Zealand White
Age: ~5 months
Weight: 3.0 - 3.8 kg
Number/Sex/Group: 8/group
Satellite groups: none
Study design: Dosing from Gestation Days (GD) 6 to 19, with Cesarean section on GD29
Deviation from study protocol: none reported

Observations and Results

Mortality

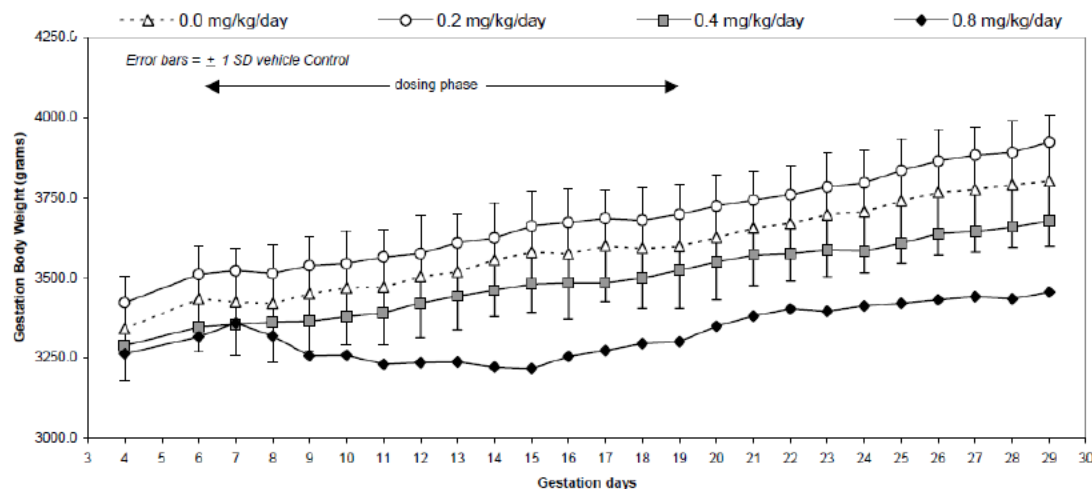
One female (#4503) at 0.8 mg/kg was found dead on GD8 preceded by reduced food intake. At necropsy, the findings were severe accumulation of thin red fluid in the thoracic and pericardial cavities, a severely edematous pericardium, a moderately enlarged mediastinal lymph node, and a moderately edematous thymus.

Clinical Signs

No overt dose-dependent signs of test article-related toxicities were observed. Reduced fecal output at 0.8 mg/kg was correlative to decreased food consumption. One female at 0.8 mg/kg had green fluid in the vagina and decreased food intake from GDs 8 to 16 (see below under C-section).

Body Weight

There was a dose-dependent trend in body weight gain reduction with a statistically significant decrease of 8% in mean body weight at 0.8 mg/kg compared to control on GDs 19 and 29. Starting on GD 11, the body weights at 0.8 mg/kg were significantly different.

Figure 48: Body Weight in Pregnant Rabbits Given Intravenous Administration of PR-171 from GD6-19Best Available
Copy

(Figure 1, pg. 32 excerpted from Applicant's package)

Table 92: Body Weight Gain (% Gain) and Body Weight Gain Compared to Control (% Δ) in Pregnant Rabbits Administered Intravenous PR-171 from GD 6-19 (n=7-8)

Dose (mg/kg)	GD 4 BWt (g)	GD 20 BWt (g)	% Gain	% Δ	GD 29 BWt (g)
0	3341	3599	+8	--	3803
0.2	3422	3699	+8	+3	3924
0.4	3289	3524	+7	-3	3677
0.8	3264	3301**	+1	-8	3456**

**, P<0.01

Feed Consumption

Feed consumption correlated with body weight reductions with significant decreases at 0.8 mg/kg noted during the whole dosing period, but recovered by GD 24.

Necropsy

Not remarkable.

Cesarean Section Data

There was one female each at 0, 0.2, and 0.8 mg/kg that was not pregnant at necropsy. (The female at 0.8 mg/kg found dead on GD 8 had 7 implantations). One female at 0.8 mg/kg had green fluid in the vagina (#4506). There were 4 live fetuses, but green-stained amniotic fluid. This is a sign of meconium-containing amniotic fluid and fetal toxicity. This female had decreased food intake from GDs 8 to 16, which may have precipitated abortions. Significantly increased % pre-implantation loss was observed at ≥0.4 mg/kg. There were also increased early resorptions and post-implantation loss at 0.8 mg/kg. The post-implantation loss was driven by the female noted above with clinical and gross pathological observations; without this female, the value was 7.4% post-implantation loss. Overall, these parameters likely contributed to the reduced-

albeit not significant- mean viable fetuses/doe. Fetal weight was decreased by 9% compared to control; while not a statistically significant difference, it potentially reflects fetal-maternal toxicities. It should be noted that this decrease is largely driven by the reduced weights of the 4 fetuses from Doe #4506 which had reduced food consumption, macroscopic findings of green fluids in the vagina.

Table 93: Selected Gestation Day 29 Laparohysterectomy Data Obtained from a Rabbit Embryo-Fetal Reproductive Toxicity Study of Daily IV PR-171 Administration (N=6-8/group)

Dose (mg/kg)	0	0.2	0.4	0.8	Historical Control Mean (range)
No. Initial Females in Study	8	8	8	8	
Pregnant Females	7	7	8	7	
Pregnant Females at Termination	7	7	8	6	
Mean Early Resorptions/doe	0.3	0.6	0.4	1.5	
Pre-implantation loss (%)	1.8	3.0	14.7*	8.1*	8.4 - 18.2
Post-implantation loss (%)	5.8	8.0	4.9	16.1 (7.4 ^a)	1.6 - 8.8
Mean Viable Fetuses/doe	8.9	9.0	7.1	6.8	
Mean Fetal Weight (g)	41.7	42.0	44.3	37.9	NR

*, p<0.05 v. control

^a, post-implantation loss at 0.8 mg/kg without data from female # 4506 described above.
NR, Not reported

Offspring

Omphalocele was noted in 2/97 fetuses at 0.8 mg/kg. While within historical control values, this finding was not observed in the other groups.

10 Special Toxicology Studies

Study TR-0424-171: A three-part, non-GLP study investigating the effect of proteasome inhibition on endotoxin challenge in mice.

Study #LPS01- Male BALB/c mice were administered intravenous bolus of 3 or 5 mg/kg PR-171 ± 50 µg of intraperitoneal lipopolysaccharide (LPS). This dose of LPS is pyrexia, but sublethal. PR-171 was given 1 hr prior to (-1 hr), and +1, +24, or +48 hr following LPS challenge (n=5/group). Mortality was 100% at -1 hr and +1 hr in the co-treatment groups, with no deaths noted in any single treatment of LPS or test article, or in controls.

Study #LPS02- Male BALB/c mice were administered intravenous bolus of 3 or 5 mg/kg PR-171 ± 50 µg of intraperitoneal lipopolysaccharide (LPS). The related agent bortezomib was also examined. PR-171 and bortezomib were given 1 hr after LPS was given. Blood samples were obtained 12 hrs following LPS challenge (n=5/group).

Clinical chemistry signs of end organ failure were noted in animals dosed with PR-171 plus LPS or bortezomib plus LPS with increases in CK, AST, ALT, BUN and phosphorus and a decrease in albumin.

Study #SNMouseCytokineAnalysis- Male BALB/c mice were administered intravenous bolus of 5 or 10 mg/kg PR-171 \pm 50 μ g of intraperitoneal lipopolysaccharide (LPS). PR-171 was given 1 hr after LPS was given and blood samples were obtained 1.5, 6, and 12 hrs following LPS challenge (n=5/group). Carfilzomib administered 1 hr prior to LPS resulted in a significant increase in RANTES, MCP-1, IL-6, IL-1 β , IFN- γ , and IL-17 at 6 or 12 hr following LPS challenge.

11 Integrated Summary and Safety Evaluation

The applicant has developed carfilzomib (PR-171) for treatment of relapsed/refractory multiple myeloma. Carfilzomib is an irreversible inhibitor of the chymotrypsin-like activity of the 20S proteasome, which is comparable in activity and toxicity to its chemical analogue, bortezomib (Velcade®, a FDA-approved reversible protease inhibitor for treatment of relapsed/refractory multiple myeloma). The Applicant hypothesizes that carfilzomib may have improved efficacy due to its longer sustained action *via* lack of reversible binding and less toxicity since no neuropathy was noted in the nonclinical studies.

Pharmacology/Pharmacokinetics:

Carfilzomib is an irreversible inhibitor targeting the chymotrypic active sites of the 20S proteasome (PR-171 potency = chymotrypsin-like > PGPH-like > trypsin-like). Carfilzomib potency at chymotrypsin-like (IC₅₀ = 4 nM) and trypsin-like (IC₅₀ = 2400 nM) active sites is similar to other proteasome inhibitors; however, carfilzomib is less potent at PGPH-like sites, making it more selective for chymotrypsin-like sites. Carfilzomib is also an NK₂ receptor antagonist, but with 100-fold less potency than for the 20S proteasome. Carfilzomib leads to cell death in human tumor cell lines *in vitro* via activation of the apoptotic pathway.

Cells resistant to bortezomib are not cross-resistant to carfilzomib, even though both target the 20S proteasome. These findings suggest that carfilzomib differs functionally enough to circumvent resistance to bortezomib. It is unclear if these differences are purely due to increased specificity of carfilzomib to the chymotrypic active sites, off-target binding, or compound-specific resistance mechanisms. Carfilzomib is also a substrate for P-gp proteins (\geq 100 nM), suggesting that it may be susceptible to multidrug resistance protein 1 (MDR1)-mediated drug resistance.

In heart tissue, carfilzomib also inhibits caspase-like and trypsin-like activity, but this seems to be specific for the heart as it was not seen in other tissues (blood, adrenal, bone marrow, spleen, liver, lung, and kidney). Carfilzomib significantly inhibits the hERG channel potassium current at doses \geq 1.5 μ M, which is more than 300-fold higher than the concentration at which it inhibits 20S proteasome chymotrypsin-like activity. In a safety pharmacology study in monkeys, a single bolus intravenous injection of 3

mg/kg carfilzomib resulted in increased ventricular premature complex, ST segments and T wave amplitudes in one male, and increased heart rate and troponin-T levels and decreased blood pressure, PR interval, QRS interval and QT interval in the other male tested. Both males administered 3 mg/kg died within 73 hours after dosing. Although unclear, it is possible that heart-specific activities of carfilzomib at one or all of the above described non-specific sites are responsible for the cardiac toxicities observed in animals at high doses.

Carfilzomib has high plasma protein binding (93-97%), but with a high degree of degradation in plasma (39-66%) due to peptidase cleavage, which also occurs in liver, lung and kidney. Carfilzomib has short half-life (<20 min); however, recovery is dependent on new proteasome protein production due to the irreversible binding, which occurs within 24 hours in some tissues (adrenal gland, liver, and PBMC), but longer in others (erythrocytes, bone marrow, heart). Due to the slow turnover rate of erythrocytes, only minimal recovery occurs in whole blood after 9 days in monkeys. Therefore, recovery is prolonged in tissues unable or slow to synthesize new proteasomes.

Although carfilzomib was rapidly and widely distributed to most tissues, it did not cross the blood-brain barrier. Significant (80% to complete) proteasome inhibition was evident in whole tissues after single or a minimal number of IV bolus infusions in monkeys (≥ 0.5 mg/kg or ≥ 6 mg/m²) and rats (≥ 2 mg/kg or ≥ 12 mg/m²). *In vivo* pharmacodynamics in rats and monkeys were comparable to humans, wherein 76% to 92% proteasome inhibition was evident after single doses at ≥ 15 mg/m² and persisted for ≥ 48 hours (Study #PX-171-005, #PX-171-006, and PX-171-007). In mice, only a twice daily schedule (5 mg/kg/dose or 15 mg/m²/dose X 2 = 10 mg/kg/day or 30 mg/m²/day) exhibited an anti-tumor response towards xenografts (human H-29 colon cancer cells), whereas twice weekly dosing of 10 mg/kg/dose or 30 mg/m²/dose did not inhibit tumor growth. Since the mouse exposures following either administration schedule were similar to rat exposures that are sufficient to completely inhibit proteasome activity in blood for > 24 hours and up to 4 days, it is unclear why only a twice daily schedule is sufficient for anti-tumor drug efficacy in mouse xenografts. Since carfilzomib is rapidly distributed throughout the body, it is likely that myeloma cells may receive similar drug exposures as the xenografts. However, it is also possible that myeloma cells may differ in susceptibility to carfilzomib-mediated activity; therefore, it is uncertain how the anti-tumor activity in these mouse xenograft studies correlate with potential anti-myeloma activity in humans.

Carfilzomib is predominantly metabolized via peptidase cleavage and epoxide hydrolysis, but is not significantly metabolized by cytochrome P450 enzymes *in vivo*. Profiles of major carfilzomib metabolites in monkey and rat plasma and urine are similar to those of human plasma and urine. The carfilzomib diol (M16) is the predominant metabolite, but is also rapidly metabolized and has a short half-life ($T_{\max} = 30$ min). The other 2 predominant metabolites M14 and M15 are due to peptidase cleavage and were identified in all rat blood and tissue homogenates with M14 being more abundant than M15 in all tissues. Metabolites identified in ³H-carfilzomib include tyrosine (M1) and

phenylalanine (M2) amino acids. None of the five carfilzomib metabolites tested (M3, M4, M14, M15 and M16) significantly inhibited proteasome activity *in vitro*. In rats, ³H-carfilzomib pharmacokinetics does not mirror carfilzomib pharmacokinetics, suggesting contamination of radiolabeled peptidic metabolites (i.e. Phe) that cannot be differentiated from endogenous components and are likely incorporated into new proteins, giving the appearance lingering carfilzomib exposure even after 1 week.

Carfilzomib is primarily eliminated via non-hepatic mechanisms in humans, monkeys, and rats. However, in mice, clearance is comparable to liver blood flow, which does not necessarily implicate non-hepatic clearance of carfilzomib in mice. Carfilzomib is largely and rapidly (within 4 hours) cleared extrahepatically via biliary excretion and kidney excretion. In monkeys, the majority of the parent drug clearance occurs in the first minute with a biphasic, multi-exponential profile via an unknown mechanism. Moreover, these studies further suggest that while some elimination mechanisms are similar between species, there are also some species-specific clearance and/or metabolism mechanisms that may ultimately lead to differences in exposures after repeat dosing between species.

Since the half-life of carfilzomib is very short, the observed increases in exposure with repeat dosing are likely due to decreased elimination rather than drug accumulation. Since renal clearance accounts for 30% of carfilzomib elimination, it would be reasonable that drug-related renal toxicities could be associated with decreased renal clearance, leading to increased drug C_{max} and AUC with repeat dosing. It is unclear if renal impairment would increase the risk of drug toxicity in humans.

Carfilzomib weakly to modestly inhibits CYP3A4, CYP1A2, and CYP2C *in vitro* and *in vivo*.

Toxicology:

In single dose studies in rats, signs of hepatotoxicity and nephrotoxicity based on gross pathology, hematology and clinical chemistry were observed. The sponsor conducted 3- and 4-week, and 3/6-month repeat-dose toxicology studies in rats, and 4-week and 9-month repeat-dose toxicology studies in monkeys. Rats were administered 1, 2, or 4 mg/kg (6, 12 or 24 mg/m²) and monkeys were administered 0.5, 1, or 2 mg/kg (6, 12 or 24 mg/m²). All chronic repeat-dose studies used an administration schedule that is the same as the proposed clinical regimen and an intravenous bolus injection of carfilzomib. Bolus intravenous administration was used in *in vivo* genetic toxicity and reproductive and developmental toxicity studies, but for practical considerations did not use the clinical administration schedule. All treated groups evaluated in the repeat-dose studies (chronic and reproductive and developmental toxicity) had levels of protease inhibition in blood consistent with tumor cell inhibition in mouse xenograft studies, but minimal recovery was observed. Significant and consistent dose-dependent mortality and toxicities were observed in all studies conducted with carfilzomib. Interestingly, animals that survived the first dosing cycle in the repeat-dose studies often had reduced evidence of significant toxicity. Many findings were exaggerated pharmacology related to the proteasome inhibition activity of carfilzomib, consisting of effects on hematological

parameters and hematopoietic organs in rats and monkeys. No sex differences were noted except in one of two mouse micronucleus studies. Overall, at the significantly toxic doses there were signs of cardiac failure, prerenal azotemia, acute phase response, and thrombocytopenia. Notable carfilzomib toxicity and target organ effects are detailed below in descending order of severity and/or contribution to mortality.

The following table shows exposure multiples in the rat and monkey chronic toxicity studies and rat Embryo-fetal Toxicity (EFT) study compared to human AUC in patients after one cycle of dosing at 20 mg/m² on Day 1 and Day 2 and at 27 mg/m² on Days 8, 9, 15 and 16. The rat and monkey TK and human PK were highly variable but did not suggest significant accumulation. The mean AUC value obtained during the each study was used since there were minimal sex-dependent differences observed, i.e., male rats and female monkeys.

Table 94: Exposure Multiples Based on AUC and Body Surface Area (BSA) for Carfilzomib Following Repeat Dose Intravenous Administration to Rats, Rabbits and Monkeys Compared to Humans at 27 mg/m²

Species	Sex	Study	Dose (mg/kg)	AUC _{0-inf} (ng*min/mL)	Exposure Multiple	
					AUC	BSA
Rat	M/F	3/6 month	1	4407	0.2	0.2
	M/F	3/6 month	2	9780	0.4	0.4
	M/F	3/6 month	4	11904	0.6	0.9
	F	EFT	0.5	1675	.01	0.1
	F	EFT	1	3157	0.1	0.2
	F	EFT	2	4509	0.2	0.4
Rabbit	F	EFT	0.2	N/D	N/A	0.1
	F	EFT	0.4	N/D	N/A	0.2
	F	EFT	0.8	N/D	N/A	0.4
Monkey	M/F	9 month	0.5	862	0.04	0.2
	M/F	9 month	1	1379	0.1	0.4
	M/F	9 month	2	1948	0.1	0.9
Human	M/F	PX-171-007	20/27 mg/m ²	20,940	--	

M, Male; F, Female; NOAEL, No Adverse Effect Level; NOAEL, Lowest Observed Adverse Effect Level; AUC_{0-inf}, Area Under the Curve for 0-infinity; STD₁₀, Significantly Toxic Dose in 10% of the Animals; EFT, Embryo-fetal Toxicity; N/D, Not Done; N/A, Not Applicable

Mortality and Clinical Signs:

In all pivotal studies conducted in rats, monkeys and mice, significant mortality and morbidity were observed. Death was often preceded by weight loss and clinical signs; however, surviving animals also exhibited body weight gain decreases and similar clinical signs. Rodents tended to die earlier than monkeys following initiation of dosing, which was likely due to higher mean exposures in rodents, although a clear exposure relationship does not appear to be evident.

Rats showed a dose-dependent increase in mortality at ≥ 2 mg/kg in both sexes preceded by a number of clinical signs including signs of stress; deaths occurred earlier

as dose increased. Causes of deaths were attributed to GI tract hemorrhage/necrosis, and cardiac or kidney failure (described below). At shorter durations, mortality was observed at doses ≥ 6 mg/kg. In a 3 week study, one rat died at 6 mg/kg on Day 3 of dosing with notable weight loss. In three single dose studies, there was also mortality at doses ≥ 6 mg/kg with clinical signs including lethargy and piloerection; weight changes were inconsistent.

In monkeys, mortality was observed in one male and one female at 2 mg/kg on Days 157 (found dead) and 132 (moribund), respectively, preceded by clinical signs including emesis, hunched posture, lethargy and weight loss. Cause of death was attributed to multiple organ failure.

In the mouse micronucleus study, there was mortality at ≥ 2.5 mg/kg preceded by signs that included piloerection, partially closed eyelids, lethargy, paleness, hunched posture, deep breathing and unsteady gait; these findings were also observed in animals that survived.

Body Weights:

As mentioned above, mean body weight decreases were observed in rats and mice and often the most profound losses were in animals that died. Feed consumption was correlated to body weight findings, especially preceding deaths.

Male rats had 13-16% and females had 4-8% body weight decreases following 6 months of administration, but weight gain and feed consumption reductions were observed following each of the two days of dosing during each cycle with recovery observed during the 2 weeks between dosing cycles. In the mouse micronucleus study, all treated groups had mean body weight losses at ≥ 0.31 mg/kg after two doses with most individual animals losing weight.

Monkeys showed little mean body weight differences. Males had a maximum mean reduction of 7% at 2 mg/kg compared to controls after 6 months, but females showed no reduction at any dose. However, the two animals that died had body weight losses preceding death.

Heart:

Cardiotoxicity was observed in both species and was considered a cause of death in some animals (see **Mortality**, above). In male rats after 3 weeks twice weekly dosing, there were treatment-related findings of mild to moderate myocardial hypercellularity with myofiber atrophy at ≥ 2 mg/kg, and mild to moderate necrosis at ≥ 4 mg/kg without dose-dependency to incidence and severity. In the chronic study after 26 weeks, the most significant histopathological findings were minimal myofiber degeneration/necrosis in two males at ≥ 2 mg/kg and a single male at 2 mg/kg with moderate myocyte hypertrophy. Minimal fibrosis was observed at 4 mg/kg in one male at 12 weeks and one female at 26 weeks. Minimal congestion/hemorrhage in one male at 1 mg/kg after 26 weeks, and cardiomyopathy in both sexes at ≥ 1 mg/kg after 13 and 26 weeks were also noted. There were increased mean heart weights in males at all doses and females at ≥ 2 mg/kg. Fibrin increases were noted at all doses in males and at ≥ 2 mg/kg in females. The causes of death of one male at 2 mg/kg and two males and one female at 4 mg/kg were determined to be cardiac fibrosis and two females at 4 mg/kg died of cardiac failure. The findings were more severe than in animals that survived to

scheduled sacrifice. In the three-week study, there was mild to moderate myocardial hypercellularity with myofiber atrophy; and mild to moderate necrosis at ≥ 4 mg/kg was noted in most animals.

In the chronic monkey study, heart weights at 2 mg/kg were increased compared to control by 11% and 49% for males and females, respectively, with some histopathological findings. Minimal myocyte degeneration was observed in one male and one female at 1 mg/kg and two males at 2 mg/kg. Minimal to moderate myocyte hypertrophy was observed in all males and one female at 2 mg/kg. Dose-dependent increase in incidence of slight to moderate chronic inflammatory cell infiltrate was observed at all doses; control animals had minimal findings. A male at 2 mg/kg that was found dead late in the study (Day 157) had cardiac findings consistent with multiple organ failure with congestive heart failure including inflammation in the heart with myocyte hypertrophy and degeneration. One male at 2 mg/kg had increased R wave amplitude on Day 73 following dosing with the amplitude increasing on Days 161 and 239 and with histopathological correlates of left ventricular enlargement and edema and atrial endocardial hypertrophy with increased heart weight. The findings at 2 mg/kg did not recover after 8 weeks. In a two dose- seven day study in monkeys, there were elevated levels serum CRP, fibrinogen, and troponin I at 2 mg/kg. One animal had myocardial degeneration correlated with high troponin I and CRP levels that did not recover. The data suggest that carfilzomib caused cardiotoxicity in rats and monkeys at AUCs approximately comparable to AUCs in humans receiving 27 mg/m² and this impairment is likely permanent. Overall, carfilzomib appears to induce congestive heart failure in rats and monkeys.

Kidney:

Essentially all studies submitted had clinical chemistry, gross pathology and/or histopathology findings consistent with renal toxicity. In rats after 3 weeks, tubular vacuolation was noted at all doses with a dose-dependency to incidence and severity. Casts and epithelial necrosis were noted in one animal at 6 mg/kg. In the chronic study, kidney toxicity in both sexes was noted, indicated by increased BUN, creatinine, and phosphorus with enlarged kidneys, and microscopic findings of chronic progressive nephropathy and glomerulonephropathy. There was a dose- and duration-dependent effect with increasing incidences and severity of minimal to moderate chronic progressive nephropathy in both sexes at ≥ 2 mg/kg at 12 weeks and all doses at 26 weeks, and slight (2 mg/kg at 12 weeks) to moderate (4 mg/kg at 26 weeks) glomerulonephropathy noted in males. Minimal to moderate tubular vacuolation was observed at 26 weeks in males at ≥ 2 mg/kg; females at 26 weeks and males at 12 weeks had no dose relationship for this finding. Kidney weights were significantly increased at all doses; and one male at 12 and 26 weeks each had enlarged, pale kidney correlating to nephropathy findings. BUN was significantly increased by 6.1-fold in males and 38% in females at 4 mg/kg and creatinine was increased 2.3-fold in males at 4 mg/kg. Total protein, albumin and globulin were significantly decreased and phosphorus was significantly increased at 4 mg/kg, but at more modest levels. Some causes of deaths were attributed to kidney failure, specifically the deaths of two males at 2 mg/kg were attributed to glomerulonephropathy and one female at 4 mg/kg died of renal tubular necrosis. Renal toxicity was often evident in the supportive single dose

toxicology studies based on BUN, creatinine and phosphorus increases and total protein and albumin decreases.

In monkeys, there was minimal to moderate mononuclear cell infiltration and minimal to mild increased mesangial matrix at 2 mg/kg after two doses of carfilzomib with elevated serum BUN, creatinine, and decreased total protein, albumin and phosphorus. In the chronic study, dose-dependent increases in severity of slight to severe glomerulonephropathy was observed in most animals at ≥ 0.5 mg/kg with increased organ weights and gross findings of pale enlarged kidneys with red foci at ≥ 1 mg/kg, increased serum BUN (1.5-2.8-fold over control) at ≥ 1 mg/kg and creatinine (> 2 -fold over control) at 2 mg/kg, and decreased albumin at ≥ 1 mg/kg and total protein at 2 mg/kg. In the male that died at 2 mg/kg, renal failure was considered contributory.

The renal toxicity appears to be prerenal azotemia based on the observed findings. There was elevated BUN, creatinine, and phosphorus with pale, enlarged kidneys with correlative increased organ weight and glomerulonephropathy in both rats and monkeys. Prerenal azotemia is often a consequence of congestive heart failure, which was observed (see **Heart**, above). Additionally, increased mean adrenal gland weights were observed in high dose animals of both species and transient cortical vacuolation was observed in male rats at 4 mg/kg. This is often a response of the Renin-Angiotensin-Aldosterone-System resulting in increases in aldosterone synthesis (not evaluated in toxicity studies). The Applicant did not measure Glomerular Filtration Rate (GFR) for definitive diagnosis.

Liver:

Rats and monkeys had signs consistent with an Acute Phase Response (APR), but the rats had generally more severe pathological findings. APR was often evident in the supportive single dose toxicology studies. Both species had increased liver weights and decreased ALT in the chronic studies. Additionally, both species had increased CRP, neutrophils, monocytes, and fibrinogen with decreased albumin consistent with APR. These occurred at all doses in rats while the monkeys tended only to have these findings at the high dose of 2 mg/kg. In the chronic rat study, additional findings of increased triglycerides, AST and cholesterol with centrilobular hepatocellular hypertrophy and periportal hepatic fatty vacuolation at all doses were noted at 12 weeks and at 26 weeks in females; ALP was increased at 4 mg/kg. After 26 weeks, males at 4 mg/kg also had increased decreased triglycerides and AST. Decreased total hepatic CYP protein content was observed in both sexes at 4 mg/kg. Males at 4 mg/kg after 13 weeks of administration and after recovery had comparable microscopic findings as females, but these were not found at 26 weeks. In the three week study, there was necrosis of varying severity noted at ≥ 2 mg/kg and neutrophilic infiltration at all doses. After 6 months, monkeys had changes discussed above in both sexes but without any histopathological correlates. However, increased ALP and ALT, decreased albumin, and marked diffuse hepatocellular glycogen accumulation in the liver was noted as contributory to the death of the female at 2 mg/kg that was euthanized on Day 132. Elevated serum CRP, AST and fibrinogen were noted at 2 mg/kg following two days of intravenous administration suggesting an APR. The differences may be related to increased exposure in rats or better adaptation to the carfilzomib by monkeys.

Large Intestine:

The findings in rats as related to mortality warrants increased concern. In the chronic rat study 3 deaths (all male) at 4 mg/kg were attributed to GI tract hemorrhage/necrosis. In a 3 week study, one animal at 6 mg/kg had moderate fibrosis, glandular dilatation, and mucosal epithelial hyperplasia and two animals had mild neutrophilic mononuclear infiltration. Some female monkeys at 1 mg/kg (the mid-dose group) had edema in sections of the large intestine. There were no significant findings at the end of dosing in the chronic studies suggesting that some adaptive response may be occurring.

Neurotoxicity:

The applicant conducted a 4-week neurological evaluation study in rats with twice weekly intravenous administration of 2 mg/kg carfilzomib and 0.2 mg/kg bortezomib as a positive control. This study was inadequate due to lack of positive control effect for the functional assays and histopathology. Neurotoxicity (functional or histopathological findings) was not clearly evident at the doses used for either drug. Since bortezomib is reportedly neurotoxic, the study is not considered useful in assessing neurotoxicity of carfilzomib. However, no signs of neurotoxicity were present in any toxicity study.

Lymph Node:

In the chronic rat study, discolored lymph nodes were observed at 26 weeks with minimal to slight increased mast cells in males at ≥ 2 mg/kg at all time points, (except: minimal findings at 4 mg/kg after recovery); and in females at ≥ 1 mg/kg at 13 and 26 weeks. Intrasinusoidal erythrocytes and minimal findings of brown pigment were present at ≥ 2 mg/kg in both sexes at 26 weeks. Minimal to slight increased lymphocytes were noted in males at ≥ 1 mg/kg at 13 weeks, and moderate increased lymphocytes were noted in females at both 13 weeks and recovery, but not at 26 weeks. In the monkey chronic study, there was slight to moderate medullary granulocytes/ granulocytopenia in of the mediastinal lymph node in 2/3 animals of both sexes at 2 mg/kg. The findings did not recovery fully in either species.

Bone:

In rats and monkeys at ≥ 1 mg/kg, there were increases in neutrophils, monocytes, reticulocytes, WBCs and leukocytes and decreased RBCs noted with microscopic correlates such as bone marrow hypercellularity (increased reticulocytes). There was an increase in severity, but not incidence, of hypercellularity in both sexes of monkeys in all groups. Control animals tended to have minimal to slight findings, while treated animals increased to moderate or marked at the high dose. In shorter duration studies, decreased platelets and hematocrit indicative of thrombocytopenia were observed. In rats, there was a less pronounced treatment-related effect that showed some sex-dependent differences. This is an expected response to the increased blood cells noted in the hematology. These findings were reversible.

Spleen:

Along with the related changes in hematopoietic parameters described above, there was decreased marginal zone width and/or cellularity, with increased spleen weight in

rats after 6 months. The monkey findings at 6 months were milder: one of 4 monkeys at 2 mg/kg had comparable findings. The findings partially recovered.

The following organs had significant findings, but were restricted to shorter duration studies in rats. This suggests that these findings are transient and adaptive.

Lung:

In a three-week toxicity study in rats, there were treatment-related findings in lung with little or no dose-dependency to incidence and severity at 3 weeks. Mild to moderate lung histopathological findings suggestive of pneumonia were noted in all treated animals with neutrophilic infiltration at all doses. There was some minimal inflammatory cell infiltration noted in high dose group only at 3 weeks. Other shorter, non-GLP studies with higher doses showed fluid in the thoracic cavity.

Since pneumonia was not seen in any other study, the relationship to carfilzomib is unclear. It may be that carfilzomib made the animals more sensitive to an opportunistic infection, as is evidenced by data that mice treated with carfilzomib and given an endotoxin challenge had increased mortality with increasing markers of inflammatory responses.

Pancreas:

In a three-week toxicity study in rats, necrosis of varying severity was noted in pancreas at ≥ 2 mg/kg at 3 weeks. Minimal to slight findings were present at 2 mg/kg only after 26 weeks in the chronic toxicity study in rats.

Intestinal Fat:

In a three-week toxicity study in rats, there were findings of intestinal fat with no dose-dependency to incidence and severity, and neutrophilic infiltration in fat at all doses after three weeks.

Genetic Toxicity:

Carfilzomib was not genotoxic in bacteria up to 5000 $\mu\text{g}/\text{plate}$ \pm metabolic activation. When spiked with 4 process impurities, no genotoxicity was observed up to 1500 and 5000 $\mu\text{g}/\text{plate}$ with and without metabolic activation, respectively. Carfilzomib caused an increase in chromosome structural aberrations in human peripheral blood lymphocytes *in vitro*, both in the absence (≥ 0.0625 $\mu\text{g}/\text{mL}$) and presence (2.5 $\mu\text{g}/\text{mL}$) of metabolic activation. The results for carfilzomib spiked with 4 impurities showed a similar increase in chromosome structural aberrations in the absence (≥ 0.04 $\mu\text{g}/\text{mL}$) and presence (3 $\mu\text{g}/\text{mL}$) of metabolic activation. The results are consistent with the mechanism of action. Bortezomib, a related protease inhibitor, has clastogenic activity *in vitro* based on the package insert. Carfilzomib and bortezomib were reported to have cytotoxic activity via apoptosis on lymphoma cell lines *in vitro*.⁷ Although not definitive, this study would be consistent with clastogenicity. Carfilzomib was clastogenic at lower concentrations in the absence of metabolic activation, suggesting that the S9 fraction

⁷ Demo, et al. (2007) *Cancer Research*; **67**: 6383.

inactivates carfilzomib to a degree. This would be consistent with the observation that the clastogenic effects noted on lymphocytes are pharmacodynamic in nature. This is also consistent with the fact that CYPs are not believed to be major components in metabolism of carfilzomib, thus complete inactivation was not observed. Carfilzomib, and carfilzomib spiked with 4 impurities, did not cause a significant increase in the number of micronucleated polychromatic erythrocytes in either sex in the *in vivo* micronucleus assay.

Reproductive Toxicology:

A preliminary EFT study in rats up to 2 mg/kg did not induce significant maternal or fetal toxicity; non-significant carfilzomib-related increases in % post-implantation loss at all doses due to early resorptions were noted; the definitive EFT study was conducted using the same doses as the preliminary study. There were significant maternal toxicities observed at 2 mg/kg (approximately 20% the AUC in patients receiving 27 mg/m² for two weeks), with clinical signs of piloerection, hunched posture, decreased activity, and feed consumption, a significant decrease in absolute body weight and in body weight gain with mortality (two animals found dead at 2 mg/kg). One dam at 2 mg/kg had total implantation loss. Increased pre-implantation loss and early resorptions were observed at ≥ 1 mg/kg/day (approximately 10% the AUC in patients), although no findings were statistically significant compared to control.

In rabbits, only a preliminary EFT study up to 0.8 mg/kg was conducted. Significant maternal toxicity was noted at 0.8 mg/kg (approximately 40% the recommended dose in humans of 27 mg/m² based on body surface area) with significant body weight and feed consumption decreases compared to control. One dam at was found dead on GD 8 with pathological findings of cardiac and pulmonary edema. There was a statistically significant increase in % pre-implantation loss at ≥ 0.4 mg/kg (approximately $\geq 20\%$ the recommended human dose of 27 mg/m²). There was a non-statistically significant increase in post-implantation loss, and decreased mean viable fetuses and fetal weight at 0.8 mg/kg. The increased post-implantation loss was primarily driven by one litter in a female with gross pathological and clinical findings, supporting the possibility that some degree of embryo-fetal toxicity observed may be related to maternal toxicity.

Carfilzomib caused no overt teratogenicity. However, these studies were limited in the ability to adequately assess teratogenicity due to the inability to reach high exposures due to maternal dose-limiting toxicity. Embryo-lethality occurred at or below human exposures based on findings of increased post-implantation loss from early resorptions in rats and rabbits. The maternal findings for carfilzomib in pregnant rats and rabbits were consistent with those previously reported chronic toxicity studies (decreased weight gain, feed consumption, piloerection and hunched posture). Inappetance in rabbits is a known cause for abortions and embryo-lethality. There were no significant toxicities observed in reproductive tissues in the chronic studies in rats and monkeys. The weight of evidence suggests risk to a pregnant woman because of embryo-fetal lethality secondary to maternal toxicities but without overt teratogenicity at clinically relevant exposures.

12 Attachments

SAFETY PHARMACOLOGY:

1. Cardiovascular, respiratory, and central nervous system assessments of PR-171 when administered intravenously to conscious monkeys.

▪ Key study findings:

- PR-171 at 3 mg/kg showed cardiovascular toxicity and mortality.
- Proteasome activity reduced ~80% at all doses tested.

Study no.:

04-6576

Sponsor Study No.:

TXC-005

Volume #, and page #:

Volume 6, page 1

Conducting laboratory and location:

(b) (4)

Date of study initiation:

3 March 2005

GLP compliance:

Yes

QA report:

yes () no (X)

Drug, lot #, and % purity:

PR-171 in 10% sulfobutylether β -cyclodextrin, pH 3.5
Batch # 014167A, B, C, purity assumed ~100%.

Formulation/vehicle:

10% sulfobutylether beta-cyclodextrin, 5 mM citrate
(pH 3.5), batch # 014167A

Methods:

Doses:

Phase No.	Group No.	Dosage Administration ^a				Total Number of Animals	
		Dose No.	Dose Level (mg/kg)	Dose Volume (mL/kg)	Concentration (mg/mL)	Male	Female
I	1	1	0	1	0	2	2
		2	1	1	1		
II	2	1	0	1	0	2	2
		3	2	1	2		
III	3	1	0	1	0	2	2
		4	3 ^b	1	3		

^aDoses represent active ingredient. At the start of each phase, animals assigned to that phase were dosed with the vehicle (10% sulfobutylether beta-cyclodextrin, 10 mM citrate, pH 3.5) then 2 or 3 days later were dosed with the test article at the selected dose level.

^bThe dose level for Phase III, Dose No. 4, was determined based on cardiovascular data obtained from the 2.0 mg/kg dose (Phase II, Dose No. 3).

The first day of dosing in each phase was identified as Day 1.

Species/strain:

Cynomolgus monkeys

(*Macaca fascicularis*) – purpose bred

Number/sex/group or time point (main study):

2

Route, formulation, volume, and infusion rate:

Intravenous, 1 mL/kg, bolus

Satellite groups used for toxicokinetics or recovery:

No

Age:

3.0 to 7.0 years

Weight:

Males 2.8 to 4.3 kg

Females 2.5 to 2.9 kg

Best Available Copy

Observation and Times:

<u>Clinical signs:</u>	Twice daily
<u>Body weights:</u>	Pre- and post-administration
<u>Cardiovascular parameters:</u>	10 Lead electrocardiograms (Systolic, diastolic and mean blood pressures, ECG and heart rate) From 2 h before and 6 h after dosing - every 5 minutes From 6 h to 24 h after dosing – every 15 minutes.
<u>Respiratory parameters:</u>	Respiratory rate, minute volume, and tidal volume – twice pretest and ~6 h post-dose.
<u>Neurobehavioral evaluation:</u>	Mental status, muscle tone, muscle movements, eye movements, pupil size, lacrimation and salivation Pretest and ~24 h post-dose
<u>Blood samples:</u>	Collected pretest and ~24 h after administration for myocardial biomarkers (Troponin T) and pharmacodynamic evaluations
<u>Gross pathology:</u>	Group 3 animals only

Results:

<u>Mortality:</u>	Group 3 – 2 • within 24 h after dosing at 3 mg/kg 2 • within 73 h after dosing at 3 mg/kg Group 2 – 1 • 6 days after dosing
<u>Clinical signs:</u>	Group 3 – Decreased activity, pale, hunched, leaning against the wall of their cages, decreased fecal volume, and/or unformed stool. Group 2 – Decreased fecal volume and/or unformed stool
<u>Food consumption:</u>	Group 3 - Reduced
<u>Cardiovascular parameters:</u>	Group 1 and 2 – No effects on PR interval, QRS interval, QT interval, QTc interval, heart rate or blood pressure. Group 3 – Males Increased ventricular premature complex (animal # 3005), increased ST segments and T wave amplitudes (animal # 3006). Blood pressure↓, heart rate↑, PR interval↓, QRS interval↓, and QT interval ↓ (animal # 3006)
<u>Respiratory parameters:</u>	No effects on respiratory rate, tidal volume or minute volume.
<u>Neurobehavioral evaluation:</u>	No indication of primary neurologic effect
<u>Myocardial biomarker:</u>	Troponin-T increased in one 3 mg/kg animal (• # 3006) after 22 h post-dose.
<u>Pharmacodynamics:</u>	Chymotrypsin-like proteasome activity reduced ~80% in all PR-171 treated animals
<u>Gross pathology:</u>	Peribronchial edema in the soft tissue of the hilus (animal # 3005 & 3006). Hydrothorax/hydropericardium (animal # 3006), discoloration of the left ventricle and interventricular septum (animals # 3006), soft cardiac ventricles (animal # 3505).

Safety pharmacology conclusions:

PR-171 at 3 mg/kg caused cardiovascular toxicity in monkeys.

TOXICOLOGY:**Multiple Doses****1. A 4-week repeat dose intravenous toxicity study of PR-171 in rats.****Key study findings:**

- The IV administration of 1, 2, 4 or 6 mg/kg/day PR-171 was associated with unscheduled death/sacrifices.
- All but one of the unscheduled deaths occurred during the first dosing cycle.
- The cause of early death may be due to cardiac failure or lung hemorrhage/ inflammation.
- The NOAEL and STD₁₀ values for PR-171 were 0.5 and 2 mg/kg/day, respectively.

Study no: 04-2867 **Sponsor Report No.** TR-0014-171

Volume #, and page #: Volume 2, page 1

Conducting laboratory and location:

(b) (4)

Date of study initiation: 10 January 2005

GLP compliance: Yes

QA report: yes () no (X)

Drug, lot #, radiolabel, and % purity: PR-171, lot # 0411-139, purity not provided.

Formulation/vehicle: 10% sulfobutylether beta-cyclodextrin, pH 3.5

Dosing:

Species/strain: Albino rats (outbred) VAF/Plus, CD (Sprague-

Dawley derived) CrI:CD (SD)IGS BR

#/sex/group or time point (main study): 10 or 13

Satellite groups used for toxicokinetics or recovery: yes

Age: 5-6 weeks

Weight: • 211-409 g, • 155-278 g

Doses in administered units:

Group	Daily Doses			Number of Animals													
				Toxicity Groups		Toxicokinetic/ Pharmacodynamic Groups ^b		Clinical Pathology ^d		Necropsy/Terminal Clinical Pathology						Microscopic Pathology	
	Total	Days 0 ^c and 18		Days 0 ^c , 19 and 28		Day 19		Day 28		Day 42							
Dose ^a (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
1	0	5	0	13	13	8	8	10	10	5	5	5	5	3	3	13	13
2	2	5	0.4	13	13	8	8	10	10	5	5	5	5	3	3	13	13
3	4	5	0.8	13	13	8	8	10	10	5	5	5	5	3	3	13	13
4	6	5	1.2	13	13	8	8	10	10	5	5	5	5	3	3	13	13
5	0.5	5	0.1	10	10	8	8	5	5	5	5	5	5	-	-	10	10
6	1	5	0.2	10	10	8	8	5	5	5	5	5	5	-	-	10	10

^aDoses represent active ingredient.

^bToxicokinetic samples were collected from up to 4 animals/sex/group/time point (predose and 15 minutes and 1 and 24 hours post-dose on Days 0 and 18. Pharmacodynamic samples (packed whole blood, PBMC, and adrenal, brain and liver tissue, bone marrow) were collected from up to 4 animals/sex/group on Day 18 at 1 and 24 hours post-dose. Packed whole blood (blood cell pellets) was also obtained at 1 and 24 hours post-dose on Day 0.

^cDay 0 was the day of first dose.

^dAnimals scheduled for necropsy on Days 19 and 28 (up to 5/sex/group).

^eCollection of predose clinical pathology samples using CO₂/O₂ anesthesia was not performed for Groups 5 and 6.

The complete tissue list was examined for all animals in the control and high-dose groups (Groups 1 and 4). Target tissues only were examined for all other groups.

M = Male, F = Female, mg/kg = milligrams of test article per kilogram of body weight

Route, form, volume, and infusion rate:

Intravenous bolus

Observations and times:

Clinical signs: Once daily
 Body weights: Twice weekly
 Food consumption: Weekly
 Functional observational battery (home page evaluation, handling evaluation, open field evaluation, reflex assessment, grip strength, landing foot splay, and air righting ability) at pretest, end of the dosing period and prior to necropsy.
 Ophthalmoscopy: Pretest, at the end of dosing and prior to necropsy.
 Hematology: Days 0, 19, 28 and 42
 Clinical chemistry: Days 0, 19, 28 and 42
 Urinalysis: Prior to necropsy (Days 19, 28, and 42)
 Gross pathology: Days 19, 28, and 42
 Organs weighed: See histopathology inventory for this IND
 Histopathology: See histopathology inventory
 Toxicokinetics: Days 0 and 18 at predose and 0.25, 1 and 24 hours post dose.

Results:

Mortality: Unscheduled deaths

Sub-Group	Dose (mg/kg/day)	No. rats / sex/group	Males			Females		
			No. Deaths	Study Day ^a	No. Doses	No. Deaths	Study Day ^a	No. Doses
Tox	0.5	10	0	-	-	0	-	-
TK		8	0	-	-	0	-	-
Tox	1	10	1	15	6	0	-	-
TK		8	0 ^b	-	-	0	-	-
Tox	2	13	0 ^c	-	-	1	1	2
TK		8	0	-	-	1	3	3
Tox	4	13	2 ^c	0-3	1-3	1 ^c	8	5
TK		8	2	3	3-4	3	3-4	3-4
Tox	6	13	1	1	1	1	3	3
TK		8	3	4-5	4-5	5	2-3	2-3

Tox- Toxicity sub-group

TK-Toxicokinetic/Pharmacodynamic sub-group (No histopathology)

^aTest article was administered on Days 0-4 and 14-18^bExcludes one animal euthanized because of an eye injury^cOne additional animal died shortly after the first dose and was replaced (not included in totals presented above).

Best Available Copy

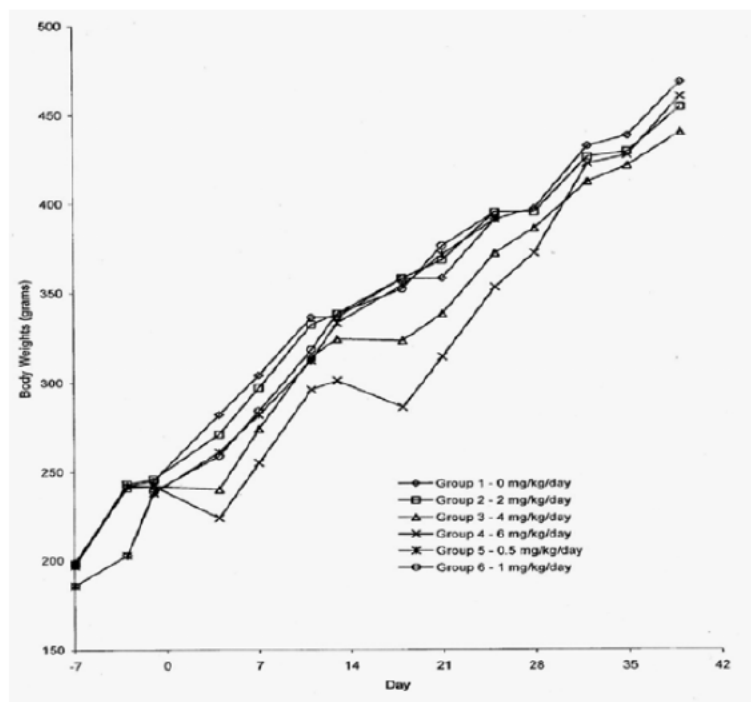
Clinical signs: 4 & 6 mg/kg – Decreased food intake, decreased activity, ano-genital staining, and poor condition of animals.

Body weights:

Dose mg/kg/day	Day 4 (End of 1 st Cycle)		Day 18 (End of 2 nd Cycle)	
	Males	Females	Males	Females
4	↓ 15 %**	↓ 10 %**	↓ 10 %*	↓ 5 %
6	↓ 21 %**	↓ 13 %**	↓ 20 %**	↓ 9 %**

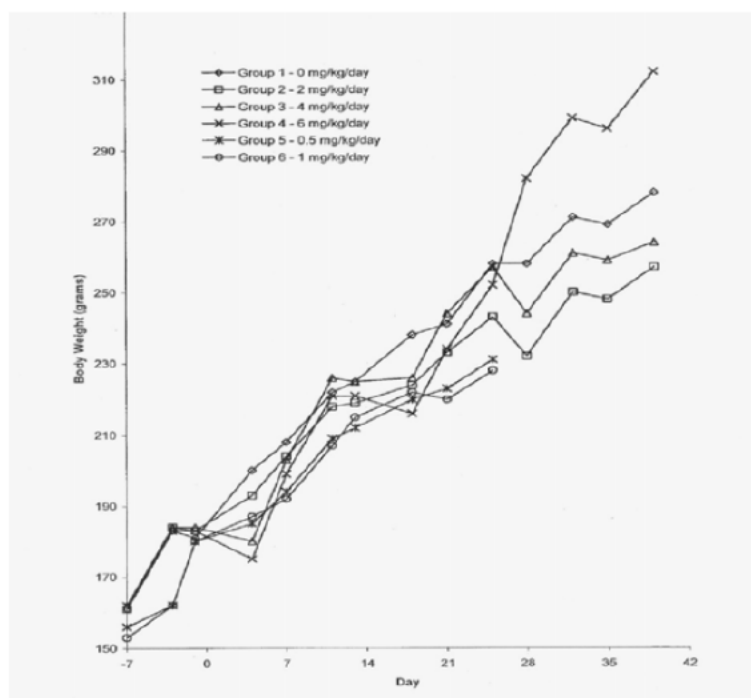
Absolute values significantly different from controls at $p \leq 0.05$ (*) or $p \leq 0.01$ (**)

Males



Best Available Copy

Females



Food consumption: 4 & 6 mg/kg – Decreased (~15%)
Ophthalmoscopy: No ocular abnormalities.
Functional observational battery: No apparent neurological alteration.

Hematology:

Hematology profile relative to control animals

Group	Dose (mg/kg)	Sex	Day	RBC	HGB	HCT	PLT
3	4	M	19	13 % ↓	11% ↓		31% ↓
			28	14% ↓			
4	6		19	27% ↓	25% ↓	22% ↓	58% ↓
			28	14% ↓			
3	4	F	19	14% ↓	10% ↓		
			28	18% ↓			
4	6		19	26% ↓	20% ↓	17% ↓	30% ↓
			28	14% ↓			

Clinical chemistry: 2-4 mg/kg – Cholesterol (~2X of the control values)
6 mg/kg - Cholesterol (~2x), triglycerides (2-5 X)

Organ weights:

Organ	Dose (mg/kg)			
	4		6	
	••	••	••	••
Liver (absolute)		32% ↑	26% ↑	68% ↑
Thymus (absolute)	27% ↓	35% ↓	62% ↓	54% ↓

Gross pathology: Unscheduled deaths: Red discoloration of the lung and heart, fluid in the lung and pericardial and thoracic cavities, and discoloration of the stomach and liver.

End of dosing period (Day 19 necropsy): High dose animals – Red discoloration of the lung, heart, and stomach, thin red fluid in the thoracic cavity and enlarged liver and spleen.

Day 28 and 42 necropsies (recovery animals): Showed recovery except white discoloration in the heart of one 4 mg/kg female and splenic enlargement in one male and one female animal.

Histopathology:

Unscheduled deaths

Organ/finding	Dose group					
	Sex					
	Animal examined					
	3	4	6	2	3	4
	••			••		
	2	1	1	1	1	1
Heart, cardiomyopathy	1				1	1
Myocardial congestion	1					
Epicardial congestion/hemorrhage	1					
Myocardial degeneration/necrosis/ Inflammation	1					
Kidney, tubular vacuolation	1					1
Liver, degeneration/necrosis/hemorrhage			1	1		

Organ/finding	Dose group					
	Sex					
	Animal examined					
	3	4	6	2	3	4
	• •			• •		
	2	1	1	1	1	1
Lungs, interstitial inflammation	1	1	1	1		
Increased alveolar macrophages	1	1		1		
Hemorrhage	1	1				1
Congestion		1	1	1		

Scheduled euthanasia

Day 19 Heart, lung, liver, spleen, thymus, stomach, small intestine, large intestine, pancreas, bone marrow 5/sex/group)

Organ/finding	Dose group						Sex					
	Animal examined						• •					
	5	5	5	5	5	5	5	5	5	5	5	5
Colon, epithelial hyperplasia			1	5					4	5		
Duodenum, epithelial hyperplasia			3	5						5		
Femoral marrow, megakaryocytes,				5						5		
Heart, cardiomyopathy				2					1	3		
Valve, increased cellularity			1	1						1		
Myocardium, perivalvular hypercellularity				2								
Ileum, epithelial hyperplasia				5						4		
Kidneys, tubular vacuolation	1		1	3			1			3		
Tubular dilation				2						2		
Liver, periportal, hepatocellular hypertrophy				5					5	5		
Lungs, interstitial inflammation		2	5	5		1		4	5	5		3
Spleen, marginal zone, decreased width and/or altered cellularity		3	3	3				2	4	5		
Red pulp, congested		3	3	4				4	2	4		
Spleen marrow, megakaryocytes, increased number		5	5	5		4		5	5	5		5
Stomach, glandular stomach, regenerative epithelial Hyperplasia				1						1		
Testes, tubular degeneration				1								
Thymus, lymphocyte depletion			1	5						3		

Terminal sacrifice (day 28): heart (valve, increased cellularity), lungs (increased alveolar macrophages), spleen (marginal zone, decreased width and/or altered cellularity), thymus (lymphocytolysis).

Recovery sacrifice (day 42): spleen (red pulp, congested)

Toxicokinetics:

Plasma levels in rats at 15 minute post-dose

Dose level (mg/kg)	Average (\pm std dev) PR-171 plasma levels (ng/mL)			
	Males		Females	
	Day 1	Day 19	Day 1	Day 19
0.5	2 \pm 0.3	3 \pm 0.5	2 \pm 0.3	2 \pm 0.3
1.0	4 \pm 0.8	6 \pm 0.8	5 \pm 1.1	6 \pm 2.9
2	21 \pm 5.3	10 \pm 2.0	20 \pm 6.2	8 \pm 0.5
4	59 \pm 21.1	17 \pm 0.9	40 \pm 3.5	13 \pm 1.2
6	74 \pm 17.1	19 \pm 2.2	52 \pm 12.1	18 \pm 1.6

Pharmacodynamics: Proteasome inhibition was observed in whole blood, adrenal glands, bone marrow, liver and PMBC. No proteasome inhibition was detected in the brain.

2. A 4-week repeat dose intravenous toxicity study of PR-171 in cynomolgus monkeys.

Key study findings:

- Intravenous administration of PR-171 at 2 mg/kg/day for 5 days caused 60% mortality.
- The apparent causes of unscheduled deaths were pulmonary hemorrhage and cardiac inflammation/hemorrhage.
- HNSTD was 1 mg/kg/day.

Study no:

04-3088

Sponsor report No.:

TR-0017-171

Volume #, and page #:

Volume 4, page 1

Conducting laboratory and location:

(b) (4)

Date of study initiation:

9 February 2005

GLP compliance:

Yes

QA report:

yes () no (X)

Drug, lot #, radiolabel, and % purity:

PR-171, lot # 0411-139, purity not provided

Formulation/vehicle:

10% sulfobutyl ether β -cyclodextrin, lot # 026999A2

Dosing:

Species/strain:

Cynomolgus monkeys
(Macaca fascicularis) –purpose bred

#/sex/group or time point (main study):

See below table

Satellite groups used for toxicokinetics or recovery:

See below

Age:
Weight:

3-6 years
Males 2.4 – 4.4
Females 2.2 – 3.2

Doses in administered units:

The test or control articles were administered by intravenous (bolus) injection to monkeys for 5 consecutive days (Days 0-4) followed by 9 days of rest, and then followed by a second 5-day dosing period (Days 14-18), as detailed below.

Group	Daily Doses ^a			Number of Animals																	
				Total	Toxicokinetic / Pharmacodynamic ^b		Clinical Pathology								Necropsy / Pharmacodynamic ^c				Microscopic Pathology		
							Pretest (2x)		Days 5 and 13		Day 19		Day 28		Day 19		Day 28				
	Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
1	0	1	0	5	5	5	5	5	5	5	5	5	5	2	2	3	3	2	2	5	5
2	0.5	1	0.5	5	5	5	5	5	5	5	5	5	5	2	2	3	3	2	2	5	5
3	1	1	1	5	5	5	5	5	5	5	5	5	5	2	2	3	3	2	2	5	5
4 ^d	2	1	2	5	5	5	5	5	5	2	2	0 ^e	0 ^e	2	2	0	0	2	2	5	5

^aDose levels for Groups 3 and 4 were based on the tolerability of the low-dose level (Group 2 was stagger-started ahead of Groups 3 and 4), as well as on results of a previous range-finding study (TXC-03).

^bToxicokinetic samples were collected from all animals on Days 0 and 14 (predose and at 15 minutes and 1 and 24 hours post-dose). Pharmacodynamic samples were collected as follows: packed whole blood – predose and 1 hour post-dose on Days 1 and 14; PBMC – 1 hour post-dose on Days 0 and 14, pre-dose on Day 1 and on Day 7.

^cPharmacodynamic samples at necropsy consisted of packed whole blood, PBMC, and adrenal, brain, heart and liver tissue on Days 19 and 28.

M = Male, F = Female, mg/kg = milligrams of test article per kilogram of body weight

^d Due to lethality in the first dosing cycle, Group 4 animals did not receive PR-171 administrations on Days 14 – 18.

^e Three males and three females from Group 4 were sacrificed in poor condition after 3 to 5 doses (see Section 3.2).

Route, form, volume, and infusion rate:

Intravenous (bolus)

Observations and times:

Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Daily
Ophthalmoscopy: Pretest, near the end of the treatment, and recovery period.
EKG: Prestudy, end of dosing, and end of recovery.
Neurological evaluation: Pretest, near the end of the treatment, and recovery period
Hematology: Prestudy, on days 5, 13, 19, and 28
Clinical chemistry: Pre-dosing and on days 5, 13, 19, and 28
Urinalysis: Days 19 or 28
Gross pathology: Day 19 and 28
Organs weighed: See histopathology inventory for IND 71,057
Histopathology: See histopathology inventory.
Toxicokinetics: Days 0 and 14 at pre-dose and 0.25, 1, and 24 hours post-dose
Pharmacodynamic: Pre-dose and 1 h after dosing on days 0 and 14 and prior to scheduled necropsy (days 19 and 28).

Results:

Mortality: Group 4 (2 mg/kg/day) – 3 • and 3 • (60%) died or euthanized in moribund condition after 3 to 5 doses
Clinical signs: Unscheduled death – Hunched appearance, decreased activity and respiratory distress.
Body weights: No effect

Food consumption:	Similar for all groups
Ophthalmoscopy:	No ocular abnormalities.
Neurological evaluations:	Normal (mental status, muscle tone, muscle movements, palpebral closure, pupil size, visual tracking, lacrimation, salivation, posture, locomotor activity, motor function, gait, and climbing ability),
Electrocardiography:	Not submitted
Hematology:	1 mg/kg – Platelets (40-50% ↓ of control values) on day 5 2 mg/kg – RBC, Hgb and Hct (7-17% ↓) on day 5 platelets (~90% ↓)
Clinical chemistry:	2 mg/kg – BUN ↑ on day 5
Urinalysis:	No effect
Organ weights:	1 mg/kg - day 19 sacrifice – Lung 55% ↑ than control animals
Gross pathology:	Unscheduled deaths – Cardiac inflammation/hemorrhage and pulmonary hemorrhage and edema.

Histopathology:

Unscheduled deaths

Organ/finding	Dose (mg/kg)	2	2
	Sex	••	••
	Animal examined	3	3
Brain, meninges, hemorrhage		2	
Cecum, parasitic granuloma		1	1
Colon, parasitic granulomas			3
Epididymis, immaturity		3	
Heart, acute inflammation, hemorrhage, necrosis & edema		2	2
Myocardium increased cellularity		2	
Subepicardial inflammatory cell infiltrate/foci		2	
Subepicardial hemorrhage		2	3
Endocardium hemorrhage			2
Injection site, acute inflammatory cell infiltrate		2	2
Hemorrhage		3	1
Kidney, chronic inflammatory cell infiltrates		2	2
Cortical mineral deposits		1	2
Papillary mineral deposits		3	
Liver, degeneration, necrosis, hemorrhage, inflammation		2	
Parasitic granulomas		1	2
Congestion		1	
Lung, interstitial, acute inflammatory cell infiltrate		2	2
Intraalveolar hemorrhage, fibrin, edema		2	3
Alveolar chronic inflammatory cell foci		2	1
Pleural edema			2
Salivary gland, chronic inflammatory cell infiltrates		2	1
Spleen, follicles, decreased size		2	2
Sternal marrow, increased megakaryocytes		3	3
Testes, immaturity		3	
Thymus, lymphocytes decreased		1	3

Scheduled necropsy (day 19)

Organ/finding	Dose (mg/kg) Sex Animal examined	0	0.5	1	0	0.5	1
		Males			Females		
		3	3	3	3	3	3
Brain, choroid plexus/subependyma, hemorrhage						1	1
Cecum, parasitic granuloma						1	2
Heart, myocardium hypertrophy				1			
Injection site, vein acute, inflammatory cell infiltrate				1			
Kidney, papillary mineral deposits			2		1	2	1
Liver, degeneration/necrosis/hemorrhage/inflammation		3	1	1	1	3	2
Lungs, intraalveolar hemorrhage/fibrin/edema				1			2
Pleural edema							2
Pituitary gland, chronic inflammatory cell infiltrate				1		2	
Prostate, chronic inflammatory cell infiltrate				1			
Salivary gland, chronic inflammatory cell infiltrates		1	1	2	1	1	3
Spleen, follicles, decreased size				1	1	2	
Sternal marrow, increased megakaryocytes				3			3
Thymus, lymphocytes, decreased		1	3	3	3	3	3

Recovery animals (day 28):

2 mg/kg/day (animals were not dosed after day 5) – minimal increased myocardial cellularity in 1 male and 1 female

1 and 0.5 mg/kg/day – No differences between control and treated animals.

Toxicokinetics:

Average (\pm STD dev) plasma PR-171 levels (ng/mL) 15 minutes post-dose

Dose level (mg/kg)	Males		Females	
	Day 0	Day 14	Day 0	Day 14
0.5	9 \pm 5.5	10 \pm 1.2	11 \pm 2.8	9 \pm 0.9
1	10 \pm 1.4	11 \pm 3.7	5 \pm 3.8	4 \pm 2.4
2	18 \pm 10.3	N/A	15 \pm 3.0	N/A

Pharmacodynamics:

PR-171 caused 80% proteasome inhibition in whole blood.

High variability of proteasome inhibition was seen in PBMC.

Lack of inhibition was seen in adrenal, liver and brain.

Histopathology Inventory for IND # 71,057

Study	04-2867	04-3088
Species	Rat	Monkey
Study duration		
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	X
Brain	X*	X*
Cecum	X	X
Cervix	X	
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder		X
Gross lesions		
Harderian gland	X	X
Heart	X*	X*
Ileum	X	X
Injection site	X	
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland	X	X
Larynx		
Liver	X*	X*
Lungs	X	X*
Lymph nodes, cervical		
Lymph nodes mandibular		X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		
Optic nerves	X	X
Ovaries	X*	X*
Pancreas	X	X
Parathyroid	X	
Peripheral nerve		
Pharynx		
Pituitary	X*	X*

Study	04-2867	04-3088
Species	Rat	Monkey
Study duration		
Prostate	X*	X*
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles		
Skeletal muscle	X	X
Skin		X
Spinal cord	X	X
Spleen	X*	X*
Sternum	X	
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X*	X*
Tongue		X
Trachea	X	X
Urinary bladder	X	X
Uterus	X*	X*
Vagina	X	X
Zymbal gland		
Standard List		

X, histopathology performed

*, organ weight obtained

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JESSICA J HAWES
06/01/2012

TODD R PALMBY
06/01/2012

JEFFREY D BRAY
06/01/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA

NDA Number: 202714

**Applicant: Onyx
Pharmaceuticals**

Stamp Date: September 27, 2011

Drug Name: carfilzomib

NDA Type: 505(b)1 standard

60-Day Filing Review Date:

November 8, 2011

74-Day Letter Date:

November 26, 2011

Expected Date of Draft Review:

PDUFA Goal date:

July 27, 2012

Jeffery Bray is reviewing the nonclinical toxicology section of this NDA submission; Jessica Hawes is reviewing the pharmacology, safety pharmacology, and ADME sections. The applicant submitted an apparently complete nonclinical toxicology package that included:

4 acute toxicity studies (3 rat, 1 nonhuman primate)

9 repeat dose toxicity studies (6 rat, 3 nonhuman primate)

6 genotoxicity studies (4 in vitro, 2 mouse micronucleus)

3 reproductive toxicity studies (2 rat, 1 rabbit embryofetal toxicity)

No fertility and early embryonic development, peri-postnatal development, or carcinogenicity studies were submitted based on ICH S9.

On **initial** overview of the NDA application for RTF:

	Content Parameter	Yes	No	Comment
1	On its face, is the pharmacology/toxicology section of the NDA organized (in accord with 21 CFR 314 and current guidelines for format and content) in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section of the NDA indexed and paginated in a manner allowing substantive review to begin?	X		
3	On its face, is the pharmacology/toxicology section of the NDA legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted in this NDA (carcinogenicity, mutagenicity*, teratogenicity*, effects on fertility, juvenile studies, acute and repeat dose adult animal studies*, animal ADME studies, safety pharmacology, etc)?	X		No phototoxicity evaluation was conducted.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route	X		Applicant states that the to-be-marketed formulation was used in the pivotal nonclinical studies.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA

	Content Parameter	Yes	No	Comment
	intentionally and by desire of the FDA).			
6	On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the sponsor <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		The applicant stated in Section 2.6.6 , " <i>All pivotal nonclinical studies were conducted consistent with ICH Nonclinical Testing Guidelines and in compliance with the Good Laboratory Practice (GLP) Regulations.</i> "
8	Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the sponsor?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m ² or comparative serum/plasma levels) and in accordance with 201.57?		X	There are some deficiencies since the applicant was not consistent in use of exposure multiples as mg/m ² . Nonclinical sections of the labeling will appear to require some modification.
10	If there are any impurity – etc. issues, have these been addressed? (New toxicity studies may not be needed.)	X		The applicant details the qualification of impurities in Section 3.2.S.3.2.3 . The response appears to be adequate without substantive review.
11	Has the sponsor addressed any abuse potential issues in the submission?		n/a	
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?		n/a	
13	From a pharmacology/toxicology perspective, is the NDA fileable? If ``no`` please state below why it is not.	X		

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

Any Additional Comments: none

Jeffrey Bray, Ph.D.

Reviewing Pharmacologist

November 1, 2011

Date

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA

Jessica Hawes, Ph.D.

October 28, 2011

Reviewing Pharmacologist

Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

TODD R PALMBY

11/04/2011

I am the Acting Pharmacology Supervisor for this communication. By signing this review I am providing my concurrence as a secondary reviewer on the conclusions drawn by the primary reviewers, Dr. Bray and Dr. Hawes, who have previously signed this review.