Safety and Pharmacokinetics of the Orally Available Antiprionic Compound PRI-002: A Single and Multiple Ascending Dose Phase I Study

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Abbreviations: adverse event (AE), Alzheimer´s disease (AD), area under the plasma concentration (AUC), total plasma clearance (CL/f), maximal concentration (C_{max}), steady-state condition (C_{ss}), data

and safety monitoring board (DSMB), electrocardiogram (ECG), first pass effect (FPE), good clinical practice (GCP), elimination rate constants (k_e), first-in-human (FIH), lower limit of quantification (LLOQ), multiple ascending dose (MAD), non-compartment analysis (NCA), pharmacokinetic (PK), single ascending dose (SAD), terminal half-live ($t_{1/2}$), time to reach C_{max} (T_{max})

Abstract

INTRODUCTION: PRI-002 is an orally available anti-Aβ-prionic compound developed for direct disassembly of toxic Aβ oligomers relevant in Alzheimer's disease.

METHODS: Two placebo-controlled clinical phase I trials with oral dosing of PRI-002 were conducted in healthy young subjects: A single ascending dose trial (4, 12, 36, 108 or 320 mg PRI-002 or placebo) in 40 participants followed by a multiple ascending dose study with daily 160 mg PRI-002 for 14 days or 320 mg for 28 days in 24 participants. Main objectives were safety, tolerability, and evaluation of pharmacokinetic (PK) parameters.

RESULTS: PRI-002 was safe and well tolerated after single and multiple oral administration up to the highest doses. PRI-002 was rapidly absorbed and drug exposure increased proportional to dose. During repeated daily administration, the drug accumulated by a factor of about three. Steady-state conditions were reached after 1 to 2 weeks.

CONCLUSIONS: The safety and PK results encourage further clinical development of PRI-002.

1. Introduction

PRI-002 (alias "RD2" in earlier publications, and alias "Contraloid acetate" in regulatory documents) is an all-D-enantiomeric peptide, which was developed to directly destroy toxic and replicating Aβ oligomer prions, by disassembling aggregates into non-toxic Aβ monomers [1 2]. The investigational drug is specifically designed for the curative and/or disease-modifying treatment of cognitive, memory deficits in Alzheimer´s disease (AD) patients. PRI-002 is reaching its target organ, the brain [3] and has

demonstrated target engagement in vitro and in vivo [2 4]. Preclinical proof-of-concept studies in three different transgenic mouse models (APP_{SL}, APP_{swe}/PS1ΔE9, and TBA2.1) of AD in three different laboratories successfully proved therapeutic efficacy of PRI-002 by improving cognition and decelerating neurodegeneration. Therapeutic efficacy could be shown even under truly non-preventive treatment conditions and when applied orally [2 4-6] normalizing cognitive abilities and behavior of transgenic mice up to levels of healthy wild type littermates [4 5].

2. Materials and methods

2.1 Study design

Two consecutive phase-I, randomized, double blind, placebo-controlled clinical studies with PRI-002 were conducted in healthy young subjects. First, a single ascending dose study (SAD; "A Randomized, Double-blind and Placebo-controlled Single Ascending-dose Phase I Study (First-in-human) to Investigate the Safety, Tolerability and Pharmacokinetics of Contraloid Acetate (in Healthy Subjects)."; EUDRA-CT: 2017-000396-93) and second, a multiple ascending dose study (MAD; "Single-center, Randomized, Prospective, Double-blind, Placebo Controlled Phase Ib Study With an Adaptive Multiple Ascending Dose (MAD) Design to Investigate the Safety, Tolerability, Pharmacokinetics of Contraloid Acetate (Healthy Subjects)"; EUDRA-CT: 2018-002500-14) were conducted. Both studies were approved by the Ethics Committee of the Medical University Vienna, Austria. All subjects signed an informed consent form prior to any study-related activity. Both studies were conducted at the Medical University of Vienna, Department of Clinical Pharmacology, Vienna, Austria, under the EU regulations (Directive 2001/20/EC, 2003/94/EC, and 2017/1572/EC), defining Good Clinical Practice (GCP) and Good Manufacturing Practice (GMP) of human drugs as well as whose implementation in national Austrian law.

The primary end points were safety and tolerability. Secondary objective was the assessment of pharmacokinetic (PK) characteristics of PRI-002.

For the single ascending dose (SAD) study, capsules were filled with PRI-002 acetate without any excipients. The study medication was prepared at the required dose for each treatment prior to administration at the hospital pharmacy. The placebo treatment matched the appearance and shape of PRI-002 capsules. Each subject within the cohort received a single dose of PRI-002 capsules or placebo capsules, under fasting conditions. Capsules were administered with 250 ml of tap water. Fluid intake was restricted during one hour before and one hour after drug administration. Food intake was prohibited for at least 8 hours pre-dose (monitored by glucose testing) and 5 hours post-dose followed by standardized meals.

In the multiple ascending dose (MAD) study, PRI-002 was administered as capsules filled with PRI-002 acetate without any excipients or placebo. Aptuit, Verona, Italy, manufactured the study medication (with 40 mg or 100 mg PRI-002). Capsules were administered with 250 ml of tap water. On days of PK sampling, fluid intake was restricted for one hour before and one hour after drug administration. At the first dosing, food intake was restricted for at least 8 hours pre-dose (glucose testing) and 2 hours post-dose, followed by a standardized meal.

In the SAD first-in-human study, 40 healthy male subjects (19-45 years of age), randomly assigned to the treatment, received one oral dose of study drug in the morning after an overnight fast. Eight healthy subjects were assigned to each of the five sequential dose cohorts (4 mg, 12 mg, 36 mg, 108 mg or 320 mg PRI-002). For each cohort, the first two subjects (sentinels) were randomized in a 1:1 ratio to receive PRI-002 or placebo, and the remaining six subjects in a ratio of 5:1 to receive PRI-002 or placebo. All participants were admitted to the clinical facility on day 1. Sentinels remained at the clinical site through day 4; all other subjects were kept for one day after administration and returned thereafter to the facility once daily for visits. The last follow up visit was one week after study drug administration.

In the MAD study, 24 healthy male subjects (21-43 years of age) were randomly assigned to the treatment and received the fixed dose daily for 14 or 28 days (cohort 1 and cohort 2, respectively). The

study was open for the participation of female subjects, but probably due to the strict inclusion/exclusion criteria, no female subject was recruited.

The sequential cohorts were exposed to an increased dose of PRI-002 in order to identify the maximal administered multiple dose. The study was planned with an adaptive design in order to be able to reduce the dose in case of severe side effects. The first cohort received 160 mg PRI-002, 50 % of the maximal administered single safe dose in the SAD study. In case of severe side effects, the dose for the second cohort would have been reduced to 80 mg. Since no severe side effects occurred in the first cohort, the second cohort was dosed with 320 mg.

For both cohorts, the first four subjects (sentinels) were randomized in a ratio of 2:2 to receive PRI-002 or placebo, and the remaining eight subjects in a ratio of 6:2 to receive PRI-002 or placebo. All participants were admitted to the clinical facility on day 1. Sentinels remained at the clinical site through day 8 and subsequent returned daily for dosing and study procedures to the clinical site until day 14/28 (cohort 1 /cohort 2). All other subjects were allowed to leave the facility on day 2 after administration and returned for dosing and study procedures to the clinical site at a fixed morning time for 11/25 days (cohort 1 /cohort 2). On day 14/28 (cohort 1/cohort 2) all participants were admitted to the clinical site for another 24 hours for PK-sampling. A follow-up was performed on days 16/30, 17/31 and at the end of study visit on day 21/35.

2.2 *Participants*

Healthy subjects (selected by medical history, physical examination, vital signs and laboratory data) aged between 19-45/21-43 years (SAD/MAD) were enrolled. Any medical condition requiring chronic medication, any evidence of active infection requiring antibiotic therapy within 14 days prior to screening, any medical history of vasculitis, autoimmune disease, treatment for cancer within the past 2 years or of acute/chronic hepatitis B or C was exclusionary. All prescription, over-the-counter, and herbal medications were prohibited within 10 days of study dosing (with exception of calcium/vitamin

D supplements, nasal steroids, ocular medications, and paracetamol ≤1000 mg/day at the discretion of the Investigator).

2.3 *Safety assessments*

Participants were closely monitored for adverse events (AEs) throughout the studies. Safety assessments including physical examination, vital sign measurements, electrocardiogram (ECG) recordings including QTc measurement and clinical laboratory tests were conducted at specified intervals throughout the studies.

The independent Data and Safety Monitoring Board (DSMB) assessed the safety and PK data of the sentinels and the entire cohort carefully and approved the continuation of the study.

2.4 *Pharmacokinetic assessments*

During the SAD study, blood samples for plasma PK analysis were collected pre-dose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 18, 24, 36, 48, and 72 hours post-dose. During the MAD, study blood samples for plasma PK analysis were collected pre-dose and at 0.5, 1, 1.5, 2, 2.5, 4 6, 8, 12, 24 hours post-dose on day 1 for both cohorts and on day 14 (cohort 1) or day 28 (cohort 2). On the remaining study days one blood sample was collected pre-dose. After the last administration, additional blood samples were collected at 36, 48, and 72 hours post-dose and on day 21/35 (cohort 1/ cohort 2). PRI-002 plasma concentrations were quantified using a validated liquid chromatography method with tandem mass spectrometric detection. The method had been qualified in terms of specificity, reproducibility, precision and robustness. The lower limit of quantification (LLOQ) had been set to 0.5 ng/ml. PRI-002 levels of < 0.5 ng/ml were set to zero for PK calculations. In the SAD study, plasma concentrations for each dose level following single oral doses of PRI-002 were used to calculate the following pharmacokinetic parameters: Area under the plasma concentration versus time curve between 0 and t, and from 0 to infinity as the sum of $AUC_{0\text{-}tlast}$ + $AUC_{tlast\text{-}\infty}$ ($AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$), , maximum concentration (C_{max}) and time to reach it (T_{max}), terminal half-life ($t_{1/2}$), and total plasma clearance (CL/f

=Dose/AUC). A non-compartment analysis (NCA) was used to calculate visible disposition phases from semi-log plots, their half-lives ($t_{1/2\ alpha}$, $t_{1/2\ beta}$), and respective elimination rate constants (k_e).

In the MAD study, PRI-002 levels found on day 1 of both cohorts were exclusively evaluated for C_{max}, T_{max}, and $AUC_{0\text{-}24h}$, but not for half-lives because the dosing interval was too short for a reliable estimation of the half-lives of the terminal disposition phase. Trough levels were inspected visually for the time of constant levels and mean values were calculated from those levels representing steady-state conditions (C_{ss}). For cohort 1 this interval was from day 8 to 12 and from day 20 to 28 in cohort 2. Individual mean values were assigned to C_{ss}. PK evaluation at the last study days included C_{max}, T_{max}, $AUC_{0\text{-}24h}$, C_{last}, $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}inf}$, $t_{½\ \alpha}$, and $t_{½\ ß}$.

AUC was calculated by the trapezoidal rule, C_{max}, T_{max}, C_{last} were taken from actual data. For half-lives determination the semi-log plot of individual PRI-002 plasma level profiles were inspected visually and then the time intervals were selected, for which the half-lives (α, ß) were calculated by regression analysis. The calculation of the extrapolated AUC (to infinity) followed the equation $AUC_{tlast\text{-}inf} = C_{last}/k_e$. Extrapolated AUC should account for less than 30 % of total $AUC_{0\text{-}inf}$ because extrapolation is highly dependent on the accuracy of k_e. Total oral clearance is defined by $CL/f = Dose/AUC_{0\text{-}inf.}$ Because the fraction (f) entering the systemic circulation unchanged is not known for PRI002, f was set to 1 for a rough estimate. Accordingly, CL data were not taken up in the result section. The extent of accumulation (accumulation factor, AF) was estimated by the quotient of $AUC_{0\text{-}inf}/AUC_{0\text{-}24h}$ at steady state.

In the MAD study in cohort 2 only one sentinel was available and passed the sentinel study plan including study day 7. In addition, one subject dropped out the study after day 9. No samples were obtained on day 10 or later. Thus, a complete set of data was evaluated for six subjects receiving 320 mg/day of PRI-002.

2.5 *Statistical analysis*

Both phase I studies were conducted with a sufficient number of volunteers to fulfill the study objectives of safety and PK. Descriptive statistics was used to evaluate the data. The PK and statistical analysis of the SAD and MAD studies was conducted by the clinical research team from the Clinical Unit of Hospital de La Princesa, Madrid, Spain, using the program WinNonlin 7 (Pharsight Corporation, Cary, NC, USA). Further data evaluation and interpretation were conducted by KAIROS*metics* UG, Berlin, Germany.

3. RESULTS

3.1 Safety and tolerability results

3.1.1 Safety summary for the SAD study

There were no deaths, serious AEs, severe AEs, or discontinuations because of a treatment-emergent AE reported in the SAD study. A total of six (20 %) participants given PRI-002 reported non-serious AEs considered probably or possibly related to study treatment compared with three (30%) participants who received placebo. A summary of these AEs is presented in Table 1. The severity of eight of these treatment-emergent AEs was deemed mild and included electroencephalography (EEG) abnormalities only, while the severity of one AE (headache) was graded as moderate. No dose-related trend in the incidence of AEs was observed with increasing doses of PRI-002.

Table 1 Summary of adverse events of the SAD study considered probably or possibly related to study treatment

	PRI-002						
System organ class, preferred term, n (%)	Cohort 1 (4 mg) n=6	Cohort 2 (12 mg) n=6	Cohort 3 (36 mg) n=6	Cohort 4 (108 mg) n=6	Cohort 5 (320 mg) n=6	Placebo n=10	total n=40
Nervous system disorders							
EEG abnormal (grade 1)	1 (16.7 %)	1 (16.7 %)	3 (50.0 %)	0	0	3 (30 %)	8 (20 %)

Headache (grade 2)	0	0	1 (16.7 %)	0	0	0	1 (2.5 %)
Total Number of Events	1 (16.7 %)	1 (16.7 %)	4 (66.7 %)	0	0	3 (30 %)	9 (22.5 %)

Overall, there were no major treatment emergent changes in blood biochemistry and hematological variables. There were no clinically significant alterations in the physical exploration, vital signs, ECG or EEG during the course of the study. No apparent treatment- or dose-related trends in the 12-lead ECG parameters were noted, following any dose of PRI-002 or placebo.

3.1.2 Safety summary for the MAD study

There were no deaths, serious AEs, severe AEs, or discontinuations because of a treatment-emergent AE reported in the MAD study. One subject was withdrawn of the study due to an unrelated serious adverse event (broken lower jaw due to bicycle accident). A total of one (6.7 %) participant given PRI-002 reported non-serious AEs compared with one (12.5 %) participant who received placebo. A summary of these AEs is presented in Table 2.

Table 2: Summary of adverse events of the MAD study considered probably or possibly related to study treatment

	PRI-002			
System organ class, preferred term, n (%)	Cohort 1 (160 mg)	Cohort 2 (320 mg)	Placebo	total
	n=8	n=7	n=8	n=23
Nervous system disorders				
Disoriented for 3 times	1 (12.5 %)	0	0	1 (4.3 %)
Headache (grade 2)	0	0	1 (12.5 %)	1 (4.3 %)

Total Number of Events	1 (12.5 %)	0	1 (12.5 %)	2 (8.7 %)

There were two non-serious AEs assessed by the principal investigator to be possibly related to the study treatment [Table 2]. One in the low dose and one in placebo, none in the high dose group. The severity of both AEs was deemed mild. The occurrence of these adverse events does not appear to be dose-related.

Overall, there were no major changes in the course of the blood chemistry and hematology attributable to study treatment. Clinically significant alterations in the physical exploration, vital signs, oral body temperature, or ECG were not found during the study. Although minor and transient changes in blood pressure and pulse rate were noted at isolated time points for some subjects, none of these findings were clinically relevant.

3.2 Pharmacokinetic results

3.2.1 Pharmacokinetic evaluation of the SAD study

Plasma levels of PRI-002 at the two lower doses were below 0.5 ng/ml (LLOQ). At the dose level of 36 mg, levels were close to the determination limit and reliable PK parameters could not be calculated for all subjects. Therefore, no stable means were presentable. Mean PK parameters of PRI-002 are presented in Table 3 and Figure 1.

Table 3: Mean pharmacokinetic parameters of PRI-002 in subjects (SAD-study)

PRI-002 dose (mg)	Study day	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-24} (ng*h/ml)	$AUC_{0-tlast}$ (ng*h/ml)	AUC_{0-inf} (ng*h/ml)	$t_{1/2}$ (h)
Cohort 4 (108 mg) (n=6)	Day 1	2.92	3.08	26.4	26.8	39.2	14.9
Cohort 5 (320 mg) (n=6)	Day 1	19.6	1.17	104	131	157	26.5

Following oral administration of the two higher doses (108 and 320 mg), PRI-002 plasma levels were detectable after 0.25 to 0.5 hours and mean C_{max} levels were 2.92 ng/ml or 19.6 ng/ml reached at mean T_{max} of 3.08 or 1.17 hours in cohorts 4 and 5, respectively. $AUC_{0\text{-}inf}$ accounted to 39.2 ng*h/ml (dose 108 mg) or 157 ng*h/ml (dose 320 mg). Whereas the ratio of C_{max} was 1:6.8 between both dose groups, the $AUC_{0\text{-}inf}$ ratio of 1:4 was close to the dose ratio (1:3). According to the rather limited time interval of measurable plasma levels (about 18 hours after administration at the 108 mg dose), the calculation of the half-lives of the terminal disposition phase was not sufficiently accurate. In the case of the 320 mg cohort, plasma levels were above the LLOQ for up to 48 hours. The estimated plasma half-life of PRI-002 of 26.5 hours in subjects receiving the 320 mg dose can be regarded as fairly reliable, because the documented time interval of this phase was roughly twice the half-life at least. The limited data for the SAD study do not allow a statement about dose linearity of PRI-002 PK over the investigated dose range.

3.2.2 Pharmacokinetic evaluation of the MAD study

Table 4 Mean pharmacokinetic parameters of PRI-002 in subjects (MAD-study)

PRI-002 dose (mg)	Study day	C_{max} (ng/ml) (CV %)	T_{max} (h) (CV %)	AUC_{0-24} (ng*h/m) (CV %)	$AUC_{0-tlast}$ (ng*h/m) (CV %)	AUC_{0-inf} (ng*h/ml) (CV %)	$t_{1/2\alpha}$ (h) (CV %)	$t_{1/2\beta}$ (h) (CV %)	C_{last}	$C_{ss\ trough}$
Cohort 1 (160 mg) (n=8)	day 1	5.6 (58 %)	1.3 (50 %)	35.6 (38 %)						
	days 8 - 12									1.28 (99 %)
	day 14	7.2 (53 %)	1.3 (37 %)	62.7 (64%)	147 (92 %)	181 (64 %)	4.6 (20 %)	38.5 (34 %)	0.6 (34 %)	
Cohort 2 (320 mg) (n=6)	day 1	13.8 (76 %)	1.3 (54 %)	76.2 (75 %)						
	days 20 - 28									2,76 (57 %)
	day 28	33.5 (45 %)	0.9 (64 %)	193 (28 %)	513 (40 %)	656 (51 %)	3.7 (25 %)	74.2 (39 %)	1.4 (47 %)	

CV = coefficient of variation

Mean plasma levels

Table 4 shows the mean plasma PK parameters of cohort 1 and cohort 2. Figure 1 shows the mean PRI-002 plasma concentrations at certain time points (full PK profile at day 1 and day14/28) [A-D] and over the total treatment period [E-F].

Mean plasma levels of the cohort 1 show a sharp peak on both full study days, a steady state of pre-treatment levels after about 8 days and a slow decrease of plasma levels at the end of treatment [Figure 1E]. The CVs of C_{max}, T_{max} and AUC_{0-24h} were 58 %, 50 %, and 38 %, respectively. Mean PK parameters of day 14 showed a similar variation as on day 1 with CVs of 53 %, 37 %, 64 % and 34 % for C_{max}, T_{max}, AUC_{0-24h} and C_{last}, respectively. The mean value of $t_{1/2\ \alpha}$ was 4.6 hours showing a low CV of 20 %. The mean $t_{1/2\ ß}$ was 38.5 hours (CV = 34 %), which is in agreement with the observation that steady-state conditions were reached after 8 days (corresponding to 4-5 half-lives). The extrapolated part of the total AUC was 20 % on average and thereby met the rule of not being ≥ 30 %. The accumulation

factor at steady state was estimated by the quotient of $AUC_{0\text{-}inf}$ /$AUC_{0\text{-}24h}$ at day 14. The mean accumulation factor was 2.9 for the daily treatment interval.

Mean plasma levels of the cohort 2 again show a sharp peak on both full study days, a steady state of pre-treatment levels after about 15-20 days and a slow decrease of plasma levels at the end of treatment [Figure 1 F]. The CVs of C_{max}, T_{max} and $AUC_{0\text{-}24h}$ were 76 %, 54 %, and 75 %, respectively. Mean PK parameters of day 28 showed a somewhat lower variation compared to day 1 with CVs of 45 %, 64 %, 28 % and 47 % for C_{max}, T_{max}, $AUC_{0\text{-}24h}$ and C_{last}, respectively. The mean value of $t_{1/2\ \alpha}$ was 3.7 hours showing a low variance of CV = 25 %. The mean $t_{1/2\ ß}$ was 74.2 hours (CV = 39 %) which again is in agreement with the observation that steady-state conditions were reached after 15 days (corresponding to 4-5 half-lives). The extrapolated part of the total AUC was 21 % on average. The accumulation at steady state was 3.0 (using the quotient of Css (2.76ng/ml) and $C_{24h,day1}$ (0.92ng/ml)) or 3.4 using the quotient of day 28 $AUC_{0\text{-}inf}$ (656ng*h/ml)/$AUC_{0\text{-}24h}$(193ng*h/ml) Thus, as in cohort 1, the mean accumulation factor of PRI-002 found in cohort 2 is about 3 for a daily treatment interval.

Comparing the dose-dependent parameters in both treatment groups, there was a dose-proportional increase from 160 mg to 320 mg for day 1 and C_{max}, $AUC_{0\text{-}24h}$ and C_{ss} with Cohort2/Cohort1-quotients of 2.5, 2.1, and 2.2. Comparing days 14 and 28, there was, however, a dose over-proportional increase in C_{max}, $AUC_{0\text{-}24h}$, $AUC_{0\text{-}tlast}$ and total $AUC_{0\text{-}inf}$ with quotients of 4.6, 3.1, 3.5, and 3.6. Differences are generated by the slower elimination of PRI-002 in subjects of cohort 2. The mean terminal half-life calculated for cohort 2 was 2-fold higher than for cohort 1. Because of small group sizes, half-life differences are regarded as chance results.

Oral clearance rates in humans had been estimated to 2500 to 3500 l/hour and in the present study mean oral clearance of cohort 5 (SAD-study) was calculated to be 566L/hour.

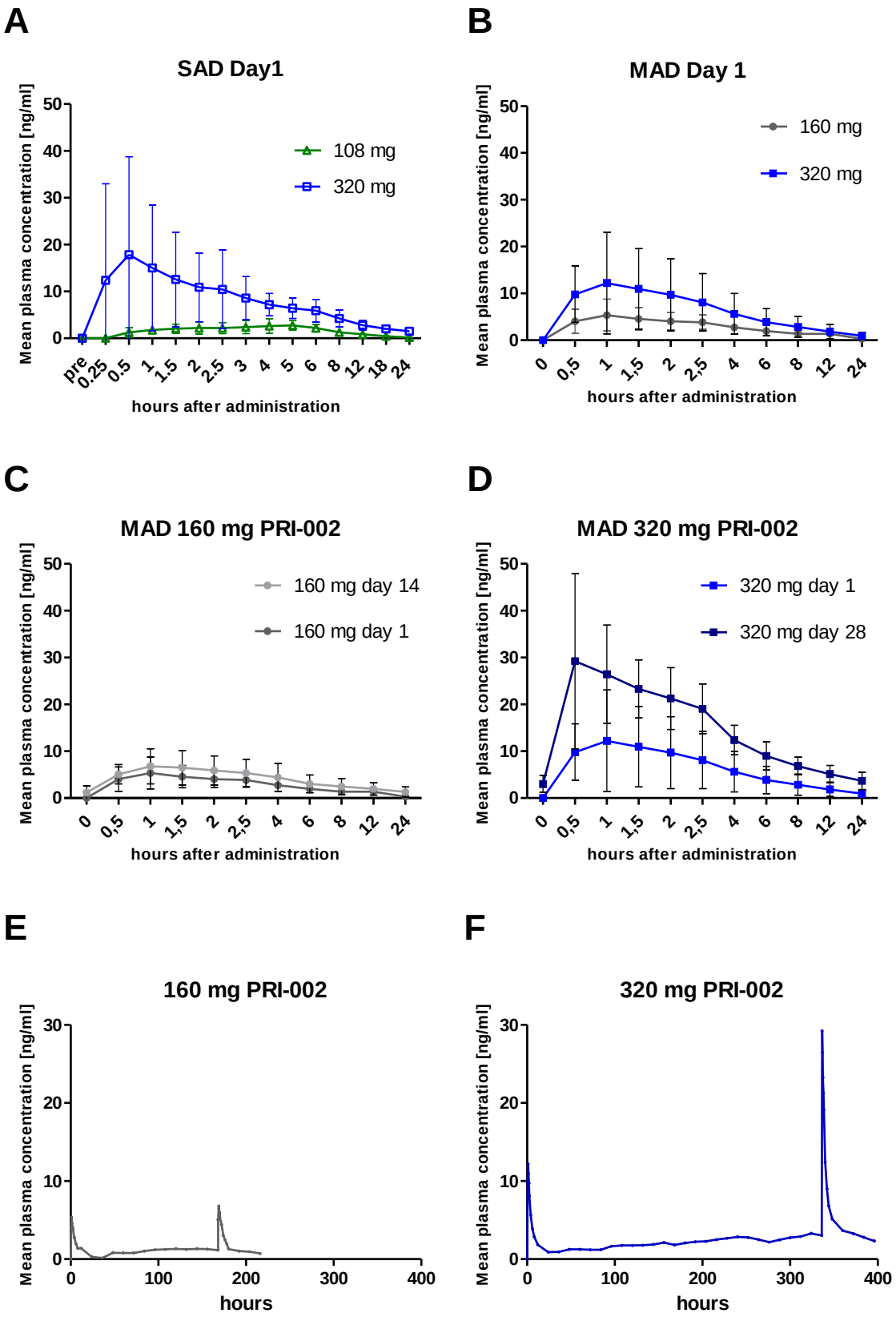

Figure 1 Mean plasma concentrations of PRI-002 over time. (A) Pharmacokinetic profile of PRI-002 after single oral doses. (B) Pharmacokinetic profile of PRI-002 after a single oral dose of 160 mg compared to 320 mg. (C) and (D) Comparison of pharmacokinetic profiles of PRI-002 between a single

dose (day 1) and multiple oral doses (day 14/28) of 160 mg/320 mg. (E) and (F) Mean plasma concentrations of PRI-002 over time for the 160 mg and 320 mg cohorts. Please note that PK sampling was done only during the first 24 hours and after the last dosing for each cohort. On all other days, only one measurement has been done, pre-dose. The lower limit of quantification (LLOQ) had been set to 0.5 ng/ml. PRI-002 values of <0.5 ng/ml were set to zero for PK calculations.

4. Discussion

A disease-modifying therapy against AD is of fundamental interest for aging societies, health care systems as well as for each single current and future AD patient. Since Aβ oligomers are the major neurotoxic agent responsible for disease development and progression [7-11] and growing evidence suggest that Aβ oligomers, or at least sub-fractions of them, are able to replicate in a prion-like fashion [12-14], anti-Aβ-prionic compounds eliminating toxic Aβ-oligomers are a promising new treatment strategy for AD [1]. PRI-002 was specifically designed to eliminate these toxic oligomers and already demonstrated its pre-clinical *in vivo* efficacy, including *in vivo* target engagement [4]. In this first-in-human study we could show that single and multiple oral doses of PRI-002 were safe and well tolerated by healthy subjects when administered at single doses ranging from 4 to 320 mg (SAD study) and in the MAD study at daily oral doses of 320 mg for up to 28 days. In both studies (SAD and MAD), AEs were generally mild and balanced between the treatment groups (PRI-002 vs. placebo), indicating that they were potentially not drug and dose-related. The only serious adverse event, a broken lower jaw due to a bicycle accident, was unrelated to the study medication and lead to withdrawal of the subject from the study.

Pharmacokinetic assessment revealed a rapid liberation and absorption of PRI-002 acetate from the formulation (lyophilized powder in hydroxypropyl methylcellulose (HPMC) capsules without excipients). T_{max} was found as early as 0.5 hours after administration in fasted condition and in the MAD study mean T_{max} values ranged between 0.9 and 1.3 hours.

Values for oral clearance in humans in the SAD study are unphysiologically high (10- to 70-fold the physiological plasma flow through the liver of 48 l/hour [15]). The reason is that within the equation

defining the oral clearance (CL/f = Dose/AUC) the fraction of the dose that is systemically available (f) is not known. In principal, f can become < 1 by two mechanisms. The first is incomplete enteral absorption and the second is pre-systemic elimination by biotransformation before absorption, during absorption and first liver passage (FPE). The metabolic stability of PRI-002 was found to be high in gastric and intestinal simulated fluid as well as in liver microsomes [16]. It is therefore rather unlikely that PRI-002 is rapidly degraded before (gastric or intestinal fluid), during (gut wall) or shortly after absorption (first pass effect, FPE). Therefore, f has most probably been lowered mainly by incomplete absorption. This is not in contrast to the fact that absorption was rapid and occurred shortly after treatment. Of note, never a second peak or a late T_{max} occurred.

The terminal disposition half-life of 34 hours (cohort 1) or 78 hours (cohort 2) might reflect the relatively high protease-resistance of D-amino acid peptides [16 17]. Due to the limited data of the here presented studies, it is not possible to make a statement regarding the excretion of PRI-002. The present data clearly demonstrate that there is a dose-proportional increase of dose-dependent PK parameters like C_{max}, AUC´s, and C_{ss}. It is suggested that the over-proportional increase of AUC´s in cohort 2 compared to cohort 1 is subject to chance and based on the longer $t_{1/2ß}$ of subjects in cohort 2.

When administered daily, the accumulation factor is about 3. Steady-state plasma levels were reached after about 5 $t_{1/2ß}$ half-lives. They increased proportional to dose and reached 2.8 ng/ml (1.8 nM) at an oral dose of 320 mg PRI-002/d. When compared to plasma levels yielded in successful proof-of-concept studies with transgenic AD mice, plasma levels achieved in humans already after single oral dosing, are in the same range of those observed in transgenic mice that received a high therapeutic dose [4]. Therefore, it can be assumed that the necessary plasma levels required for the treatment of AD patients with PRI-002 can be accomplished. The presented data have been obtained in healthy young volunteers and does not allow a sample size calculation of a phase II a proof-of-concept trial in AD patients. Additionally, metabolization and BBB penetration in the AD study population can be different to young volunteers. An allometric scaling should be considered for getting a more precise estimation

on the clearance in the intended study population and calculating the dosing for a proof-of-concept trial. In any case the presented plasma levels of the SAD and MAD study offer an adequate range for the desired treatment of AD in patients. In conclusion, safety and pharmacokinetic data support further clinical development of PRI-002.

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Conflict of interest/ disclosure statement

The authors have no conflict of interest to report.

D.W. is co-inventor of patents covering the composition of matter of RD2. D.W., D.J, K.A. and A.W. are co-founders and shareholders of the company “Priavoid GmbH”, which is further developing PRI-002 for clinical use. J.K., C.F. M.W. M.H. W.R. and M.W. declare no competing interests.

Supplement

None

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