CrossMark

ORIGINAL RESEARCH ARTICLE

Phase I Clinical Study of ZYAN1, A Novel Prolyl-Hydroxylase (PHD) Inhibitor to Evaluate the Safety, Tolerability, and Pharmacokinetics Following Oral Administration in Healthy Volunteers

Kevinkumar A. Kansagra[1] · Deven Parmar[1] · Rajendra H. Jani[1] ·
Nuggehally R. Srinivas[1] · Jason Lickliter[2] · Harilal V. Patel[1] ·
Devang P. Parikh[1] · Heather Heading[3] · Hardik B. Patel[1] · Rahul J. Gupta[1] ·
Chintan Y. Shah[1] · Maulik R. Patel[1] · Vyom N. Dholakia[4] · Raghav Sukhadiya[4] ·
Mukul R. Jain[1] · Krupi V. Parmar[1] · Kinjal Barot[1]

Abstract

Objective This phase I study of ZYAN1 was conducted to evaluate the safety, tolerability, and pharmacokinetics following oral administration in healthy volunteers.

Methods The study was a randomized, double-blind, placebo-controlled phase I study carried out in two parts in addition to a third part involving an open-label study to evaluate the food/sex effect. A total of 100 subjects were enrolled into the study as follows: part I—single-dose study with ZYAN1 10, 25, 50, 100, 150, 200, and 300 mg ($n = 56$); part II—multiple-dose study with every other day dosing of ZYAN1 100, 150, 200, and 300 mg ($n = 32$); and part III—sex and food effect study with ZYAN1 150 mg ($n = 12$; open-label).

Results ZYAN1 was well-tolerated after single and multiple oral ascending doses. No drug-related serious adverse events were reported. Following a single ascending dose of ZYAN1, the maximum concentration (C_{max}) ranged from 566.47 ± 163.03 to $17,858.33 \pm 2899.19$ ng/mL and the median time to C_{max} (t_{max}) was approximately 2.5 h for the studied 30-fold oral doses of ZYAN1. Regardless of single or multiple doses, mean C_{max} and area under the concentration–time curve from time zero to time t (AUC_t) values generally showed a dose-proportional increase. The mean elimination half-life ($t_{½}$) of ZYAN1 ranged from 6.9 to 13 h with negligible accumulation. Following a single dose of ZYAN1, the mean serum erythropoietin (EPO) C_{max} values showed dose response (i.e., 6.6 and 79.9 mIU/L for 10 and 300 mg ZYAN1 doses, respectively), while the time to mean maximal serum EPO concentrations ranged from 10 to 72 h.

Conclusion Oral single (10–300 mg) and multiple dosing (100–300 mg) of ZYAN1 in healthy subjects was found to be safe and well-tolerated. With increasing ZYAN1 dose, there was almost a proportional increase in mean C_{max} and AUC_t. The mean serum EPO concentrations showed a trend of dose response. Based on the $t_{½}$, pharmacodynamic activity, and lack of drug accumulation, a once every 2 days dosing regimen of ZYAN1 was appropriate for phase II study.

Trial Registration: Australian New Zealand Clinical Trials Registry trial ID ACTRN12614001240639.

✉ Deven Parmar
   deven.parmar@zyduscadila.com

1   Zydus Research Center, Survey No. 396/403, Sarkhej-Bavla N.H. No. 8A, Moraiya, Ahmedabad, Gujarat 382213, India

2   Division of Nucleus Network, Centre for Clinical Studies (CCS), Melbourne, Australia

3   CPR Pharma Services Pty Ltd, Thebarton, SA 5031, Australia

4   Cliantha Research Limited, Ahmedabad, India

△ Adis

Key Points

A comprehensive phase I study evaluation of ZYAN1, a novel oral prolyl-hydroxylase (PHD) inhibitor for the management of anemia in chronic kidney diseases, was carried out in healthy volunteers.

ZYAN1 was safe and well-tolerated in healthy volunteers following single escalating oral doses (10–300 mg) and multiple (given once every 2 days) escalating oral doses (100–300 mg).

Without regard to single or multiple oral dose administration, both the mean maximum concentration (C_{max}) and area under the concentration–time curve from time zero to time t (AUC_t) of ZYAN1 generally increased in a ratio comparable to the dose increment ratio. Because the mean elimination half-life of ZYAN1 ranged from 6.9 to 13 h, ZYAN1 was found to have negligible accumulation.

The measurement of serum erythropoietin (EPO) levels in healthy volunteers confirmed the pharmacodynamic effect as EPO increased with increasing ZYAN1 doses in relation to placebo.

On the basis of phase I data, ZYAN1 is recommended for phase II evaluation in a dose range of 100–200 mg every 2 days.

1 Introduction

The causes of anemia may include impaired red blood cell (RBC) production, increased RBC destruction, blood loss, and fluid overload [1, 2]. Anemia can also be caused by a range of serious medical conditions such as chemotherapy-induced anemia (CIA), chronic kidney disease (CKD), and anemia of chronic disease (ACD) [3–5]. Currently available treatments of anemia typically rely on nutritional supplements, treatment of any underlying disorder, and administration of either RBCs or erythropoiesis-stimulating agents (ESAs) [6, 7].

In healthy non-anemic subjects, there is a dynamic, multi-faceted continuous process of erythropoiesis in place to meet the oxygen demands for the normal functioning of the body. Hypoxia-inducible factor (HIF) coordinates this process by mediating genes involved in erythropoiesis [8]. Erythropoietin (EPO) and iron are crucial to proper formation of RBCs. HIF promotes EPO secretion from the kidney and other non-renal sources (e.g., liver) and also up-regulates the EPO receptor in the bone marrow. HIF also activates factors that improve iron absorption from the gut (e.g., transferrin, transferrin receptor, ferroprotein, ceruloplasmin, and divalent metal transporter), mobilization from storage sites, and transport to the bone marrow [9].

It has been observed that erythropoiesis is often suppressed in chronic inflammatory diseases [10]. Moreover, the failing kidneys in CKDs produce inadequate amounts of EPO, resulting in renal anemia [11]. This may be due to expression of hepcidin, an iron regulatory hormone that limits the availability of iron for erythropoiesis. Such functional iron deficiency is a common cause of suboptimal hemoglobin response to EPO analog therapy, and supplemental iron is commonly provided as a necessary adjuvant treatment to EPO analogs. HIF increases renal EPO production, thereby indirectly suppressing hepcidin. Hepcidin suppression in the liver permits increased ferroportin surface expression, which in turn increases iron availability for erythropoiesis [12].

Currently available therapies for anemia have certain safety issues, e.g., risk of iron overload with iron supplements, risk of hypertension, and adverse cardiovascular outcomes with ESAs, etc. [13]. Novel therapeutic strategies are required to replace these therapies and it is believed that modulation of the HIF pathway holds promise for this [14]. Encouraging results by targeting this pathway have already been observed from clinical trials supporting the possibility of pursuing an orally bioavailable therapy that is significantly less expensive than current treatments and thus generally more accessible for certain patient populations [15].

HIF-1 and HIF-2 likely regulate different sets of genes, although their downstream genes largely overlap. *HIF-1α* is expressed under normoxic baseline conditions while *HIF-2α* appears to be a key element in the hypoxic response. It is also observed that in certain situations, *HIF-1α* controls the early response to hypoxia. *HIF-1α* plays an important role in the cell-cycle regulation of hematopoietic stem cells. This effect is independent of EPO [16–18]. HIF-1 is a heterodimer, consisting of an α (the regulatory subunit) and a β subunit [19]. While the presence of the β subunit is generally constant and in excess, the activity of the α subunit within the cell shows an oxygen-sensitive pattern (i.e., inversely related to the oxygen concentration) [20]. This orchestrating mode has been found to be mainly regulated by a family of prolyl-hydroxylase (PHD) enzymes, namely PHD1, PHD2, and PHD3 [21].

The PHD enzymes are critical in regulation of the HIF pathway as they hydroxylate HIF, leading to its ubiquitination and subsequent hydrolysis and destruction. Therefore, the inhibitors of HIF-PHD have the potential to mimic hypoxia, which can be useful for inducing erythropoiesis for the treatment of a variety of diseases such as anemia

and hypoxia-related disorders (e.g., ulcerative colitis, myocardial ischemia, stroke, and metabolic disorders) [22, 23].

Considering the therapeutic potential of PHD inhibitors to address the unmet need for anemia, ZYAN1, a novel orally bioavailable drug, is being developed at the Zydus Research Center (Ahmedabad, Gujarat, India). During in vitro analysis in HepG2 cells, ZYAN1 showed HIF stabilization by inhibiting the PHD enzyme (concentration of drug producing 50% inhibition [IC_{50}]: 11.2 μmol/L) [24]. In an acute pharmacodynamic animal model, ZYAN1 showed a dose-related increase in serum EPO levels. In a repeated dose model, ZYAN1 showed a robust increase in erythropoiesis, resulting in normalization of hemoglobin levels in nephrectomized rats and mice. The effective dose for inducing hematopoiesis was observed to be 15 mg/kg, with an alternate dosing pattern for a week. The preclinical pharmacokinetic data indicated that ZYAN1 is well-absorbed and highly bioavailable after oral dosing across the species tested. ZYAN1 is well-distributed, and it is partially excreted as unchanged drug in the urine and bile and showed minimal potential for cytochrome P450 (CYP)-mediated drug–drug interactions [24]. It was observed that in single-dose studies, ZYAN1 was tolerated up to 300 mg/kg in mice, up to 800 mg/kg in rats, and up to 75 mg/kg in beagle dogs by the oral route [24]. Based on 1-month toxicity studies, 60 mg/kg in rats and 15 mg/kg in dogs was considered the maximum tolerated dose (MTD) and 5 mg/kg was considered the no observed adverse effect level (NOAEL) in the 28-day toxicity studies in dogs.

2 Materials and Methods

This was a randomized, double-blind, placebo-controlled, phase I clinical study to evaluate the safety, tolerability, and pharmacokinetics of ZYAN1 following oral administration in healthy volunteers. The study was conducted at the Centre for Clinical Studies (CCS), Melbourne, Victoria, Australia after getting approval by the ethics committee and was duly registered on Australian New Zealand Clinical Trials Registry with trial ID ACTRN12614001240639. The study was conducted as per the International Conference on Harmonisation–Good Clinical Practice (ICH-GCP) guidelines. All study participants provided written informed consent for their willful participation in the study.

The primary objective of the study was to determine safety and tolerability of ZYAN1, which included assessments of adverse events (AEs), clinical and laboratory parameters, and monitoring of electrocardiogram and vital signs. The secondary endpoints were to determine the pharmacokinetics and pharmacodynamics of ZYAN1 after single and multiple oral dose administration in healthy male and female volunteers. Also, exploratory evaluation of sex and food effects was carried out on ZYAN1 for pharmacokinetic parameters.

2.1 Participants

Some of the key eligibility criteria for the study participants are as follows.

Inclusion criteria:

- healthy males or females between 18 and 65 years of age;
- hemoglobin value 12.8–17.5 g/dL for males and 11.3–15.9 g/dL for females;
- body weight >55 kg and body mass index (BMI) within the range of 18–30 kg/m^2; and
- normal health as determined by personal medical history, clinical examination, and laboratory examinations.

Exclusion criteria:

- history or presence of any systemic disorders or diseases;
- abnormality in clinical examination, electrocardiogram (ECG), or laboratory examination; and
- for female volunteers, history of pregnancy or lactation in the past 3 months, fertile females not protected against pregnancy by adequate long-term anti-fertility measures, and those taking hormonal contraceptives.

Healthy male and female volunteers who satisfied the inclusion and exclusion criteria were enrolled into the study.

2.2 Methodology

The study schematic comprising parts I, II, and III is given in Fig. 1. Briefly, the study involving the following:

1. Part I: single ascending-dose trial. Involved seven independent panels, each of which comprised eight subjects of whom six were given successive single escalating doses of ZYAN1 (10, 25, 50, 100, 150, 200, and 300 mg) and two were given placebo. The oral administration of various ZYAN1 doses and matching placebo was carried out under fasting conditions (i.e., overnight fast).
2. Part II: multiple ascending-dose trial. Involved four independent panels, each of which consisted of eight subjects of whom six were given multiple escalating doses of ZYAN1 (100, 150, 200, and 300 mg) and two were given placebo on an every 2 days regimen (i.e., alternate-day dosing on Days 1, 3, and 5). Oral
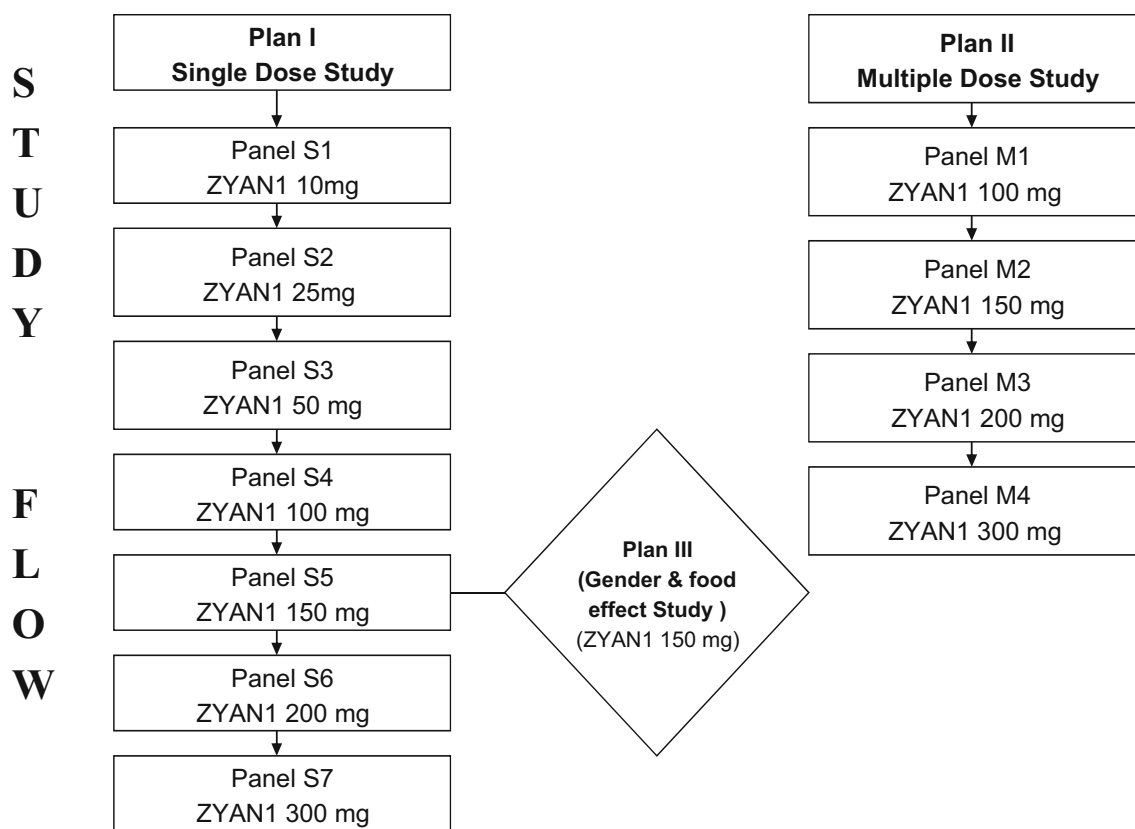
STUDY FLOW

```
Plan I
Single Dose Study

Panel S1
ZYAN1 10mg

Panel S2
ZYAN1 25mg

Panel S3
ZYAN1 50 mg

Panel S4
ZYAN1 100 mg

Panel S5
ZYAN1 150 mg

Panel S6
ZYAN1 200 mg

Panel S7
ZYAN1 300 mg
```

```
Plan III
(Gender & food
effect Study )
(ZYAN1 150 mg)
```

```
Plan II
Multiple Dose Study

Panel M1
ZYAN1 100 mg

Panel M2
ZYAN1 150 mg

Panel M3
ZYAN1 200 mg

Panel M4
ZYAN1 300 mg
```

Fig. 1 Study flow

administration of various ZYAN1 doses and matching placebos was carried out under fasting conditions.

3. Part III: sex and food effect trial. Single-sequence, open-label, randomized, single-dose crossover study of two treatments (fasted vs. fed) of ZYAN1 with a washout period of at least 14 days between the two treatments. During period I, 12 subjects (six men and six women) received a single 150 mg oral dose of ZYAN1. During period II, ten subjects (five men and five women) received a high-fat and high-calorie (approximately 800–1000 calories) breakfast, which derives approximately 150, 250, and 500–600 calories from protein, carbohydrate, and fat, respectively, 30 min prior to the administration of a single 150 mg oral dose of ZYAN1. In both periods, the participating subjects observed an overnight fast that lasted for at least 10 h prior to the oral dosing of ZYAN1 the next day.

2.3 Safety Assessment

All clinical signs and symptoms were monitored and frequent vital signs measurement, general and systemic clinical examination, 12-lead ECGs, as well as continuous ECG monitoring for 24 h post-dose (in parts I and II) were conducted to assess safety. Blood samples were collected to evaluate the safety of the standard battery of laboratory parameters after dosing. All AEs, including both observed and volunteered problems, complaints, signs, and symptoms were recorded irrespective of their association/causality with the investigational products and reported to the Institutional Review Board/Institutional Ethics Committee (IRB/IEC) of Centre for Clinical Studies (CCS), regulatory bodies, and the sponsor following respective guidelines. All AEs were coded using the current version of MedDRA® (*Medical Dictionary for Regulatory Activities*) (version 18.0) and concomitant medications were coded using the current version of the *WHO Drug Dictionary*

2.4 Blood Sample Collection

For the single ascending-dose study (part I), samples for the pharmacokinetic assessment were collected before dosing, and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 24.0, 36.0, 48.0, and 72.0 h after the administration of the drug. For the multiple-dose study (part II), samples for the pharmacokinetic assessment were collected before dosing and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 24.0, and 36.0 h after administration of Day 1 and Day 5 dosing. In addition, samples were collected before dosing and at 12.0,

24.0 and 36.0 h after administration of Day 3 dosing and 48.0 and 72.0 h after administration of Day 5 dosing. Total blood loss for participation in the study was 217 ± 5, 452 ± 5, and 418 ± 5 mL in parts I, II, and III, respectively.

2.5 Urine Sample Collection

For part I and III, urine samples were collected before dosing and then 0–12, 12–24, 24–36, and 36–48 h following drug administration, while for part II, urine samples were collected before dosing, and then 0–12, 12–24, 24–36, and 36–48 h after each dosing.

3 Pharmacokinetic Blood Sampling

In each study panel, a venous blood sample of 6 mL was collected for pre-dose and post-dose investigational medicinal product (IMP) in K_2EDTA vacutainers through a fresh venepuncture at various timepoints. The blood samples were placed in an ice bath, followed by dilution of the blood sample with equal volume of purified water (1:1) using a calibrated pipette. Samples were mixed well by vortex mixing and stored in freezer at -80 ± 10 °C until analysis.

Urine samples were collected at various timepoints in each study panel. The total volume of urine voided over each collection interval was collected in a single container and stored in a refrigerated condition. The total volume of urine collected was measured and recorded in the case report form. Aliquots of 5 mL of urine sample were prepared in pre-labelled tubes, and stored in a freezer at -80 ± 10 °C until analysis..

3.1 Bioanalytical Procedures

The estimation of ZYAN1 in diluted blood and urine was performed using a sensitive and specific liquid chromatography–tandem mass spectrometry (LC-MS/MS) method. The method was validated in accordance with the US Food and Drug Administration (FDA) *Guidance for Bioanalytical Method Validation* (May 2001)[25]. An analog internal standard (IOX2) was used for quantitative analysis of ZYAN1 in blood as well as in urine sample analysis. Chemically, IOX2 belongs to the quinolone chemotype class and has a glycinamide side chain, which are common between IOX2 and ZYAN1. IOX2 possess similar physicochemical properties to ZYAN1 and is highly suitable to use as an internal standard for mass spectrometry detection of ZYAN1. ZYAN1 and internal standard were separated on a C18 high-performance liquid chromatography (HPLC) column, and monitored by an API4000 MS/MS detector in positive MRM (multiple reaction monitoring) mode. The extraction of ZYAN1 and

internal standard in acidified diluted blood samples and urine samples was carried out by protein precipitation using acetonitrile (100%). The calibration standards were prepared in diluted blood and in urine, with a calibration curve (CC) range of 2–5000 ng/mL and a lower limit of quantitation of 2 ng/mL. The quality control (QC) samples were analyzed intermittently with each batch of blood and urine samples at low, medium, and high levels and dilution QCs were within the acceptance limits of $\pm 15\%$.

3.2 Pharmacokinetic and Pharmacodynamic Assessments

During all parts, blood samples were collected for the assessment of pharmacokinetic (maximum concentration [C_{max}], time to C_{max} [t_{max}], area under the concentration–time curve [AUC] from time zero to time t [AUC_t], AUC from time zero to infinity [AUC_∞], elimination half-life ($t_{1/2}$), elimination rate constant [λz], clearance [CL], apparent volume of distribution [V_d], and accumulation index [for multiple-dose only]) and pharmacodynamic (hemoglobin, hematocrit, reticulocyte count, and EPO concentration) parameters.

3.3 Statistical Methods

Descriptive statistics were performed to assess pharmacokinetic, pharmacodynamic, and safety parameters. Pharmacodynamic parameters were evaluated using pretreatment and post-treatment clinical and laboratory findings. To determine whether there was a significant difference in the pharmacodynamic and safety variables post-dose compared with baseline, a paired t test with a two-sided tail for an $\alpha = 0.05$ significance level was performed. For the analysis of sex effect, data from fasted and fed subjects was used. To determine the effect of food in both male and female subjects, a t test with a two-sided tail for an $\alpha = 0.05$ significance level was performed. WinNonlin® Professional Software version 6.4 (Pharsight, Cary, NC, USA), SAS® version 9.4 (SAS Institute, Cary, NC, USA), and Microsoft Excel® (Microsoft Corp., Redmond, WA, USA) were used for pharmacokinetic analysis, randomization, and statistical analysis.

3.4 Study Stopping Rule

As this was a first trial of ZYAN1 in human subjects, for the safety of the participating subjects the protocol allowed for dose escalation or continued dosing within any dose group to be placed on hold if any of the following occurred:

- Hemoglobin rose more than 1 g/dL at any point of time after dosing in four of six subjects in any panel.

- A single serious AE (SAE) occurred in a volunteer on ZYAN1 during any panel that was possibly related to study drug; or two or more subjects experienced similar AEs of moderate severity in the same dosage group that were possibly related to the study drug; or pharmacokinetic saturation was reached or desired pharmacokinetic/pharmacodynamic effect was achieved.

4 Results

4.1 Subjects' Disposition

This phase I study was conducted from October 2014 to December 2015. Subjects were screened for inclusion and exclusion criteria and those found eligible were enrolled into the study after written informed consent was given by each of the participants.

A total of 100 subjects were enrolled into the study (56 in part I, 32 in part II, and 12 in part III) and included in the safety analysis. All of the subjects in part I ($n = 56$) and part II ($n = 32$) of the study who received a dose of the study drug (ZYAN1 or placebo) were included in the pharmacodynamic analysis. For part III, all subjects who received a dose of the study drug (ZYAN1) and completed

the study as per protocol in each of period 1 and period 2 ($n = 10$) were included in the pharmacodynamic analysis. In part III, ten of 12 subjects completed the study. One subject was withdrawn from the study due to non-compliance with the protocol and the other subject was withdrawn from the study due to an SAE of cellulitis that was not related to ZYAN1.

4.2 Pharmacokinetic Results

4.2.1 Single Ascending-Dose Study

The single-dose mean concentration versus time profiles of ZYAN1 for the various doses employed in the study are presented in Fig. 2. It is apparent from Fig. 3 that all of the plasma profiles were parallel across the absorptive, distributive, and elimination phases of ZYAN1. The mean pharmacokinetic data for ZYAN1 are depicted in Table 1. Regardless of the orally administered single-dose levels of ZYAN1, the t_{max} was relatively rapid (median t_{max} of approximately 2.5 h). Both C_{max} and AUC parameters showed an almost dose-proportional increase over the studied 30-fold dose range of ZYAN1; there appeared to be a slight deviation from dose proportionality at higher oral doses of ZYAN1 (Fig. 3). The $t_{1/2}$ of ZYAN1 (range
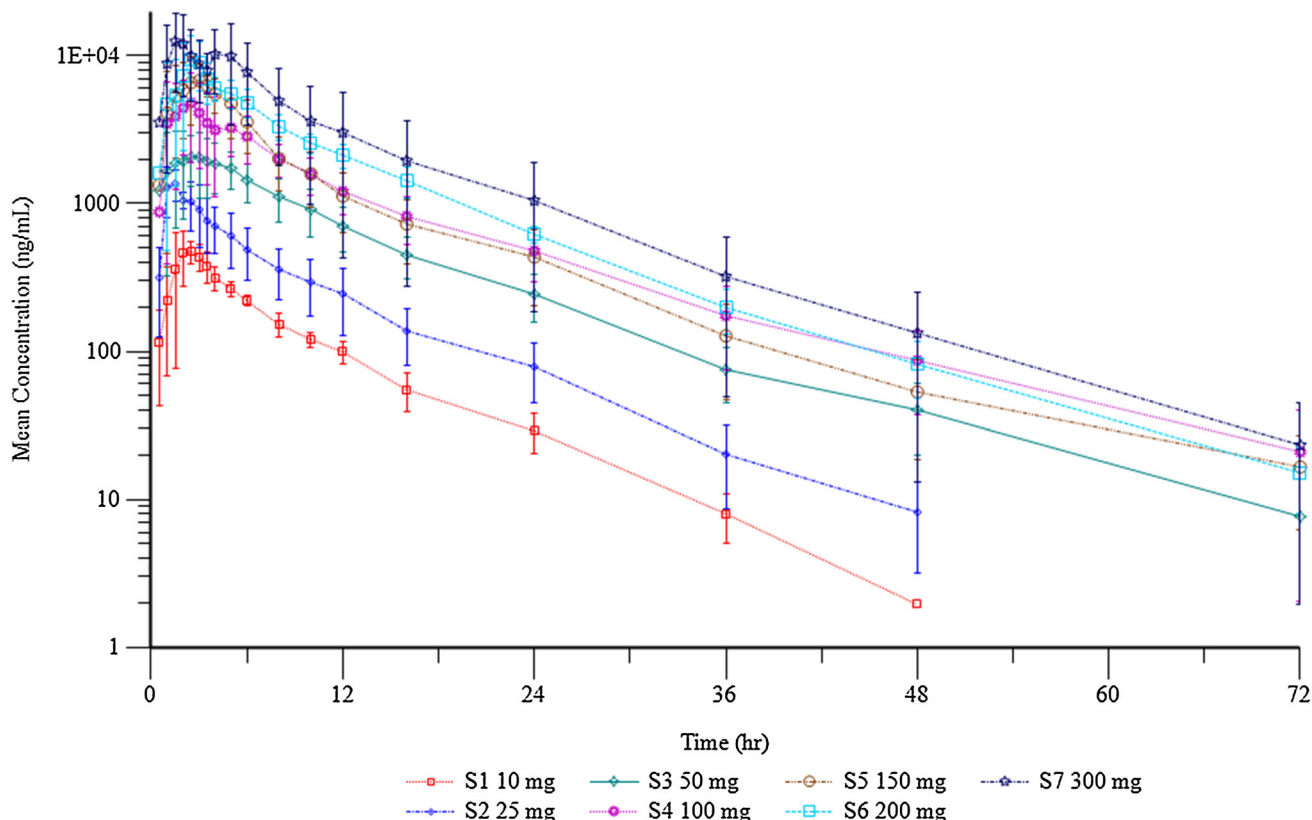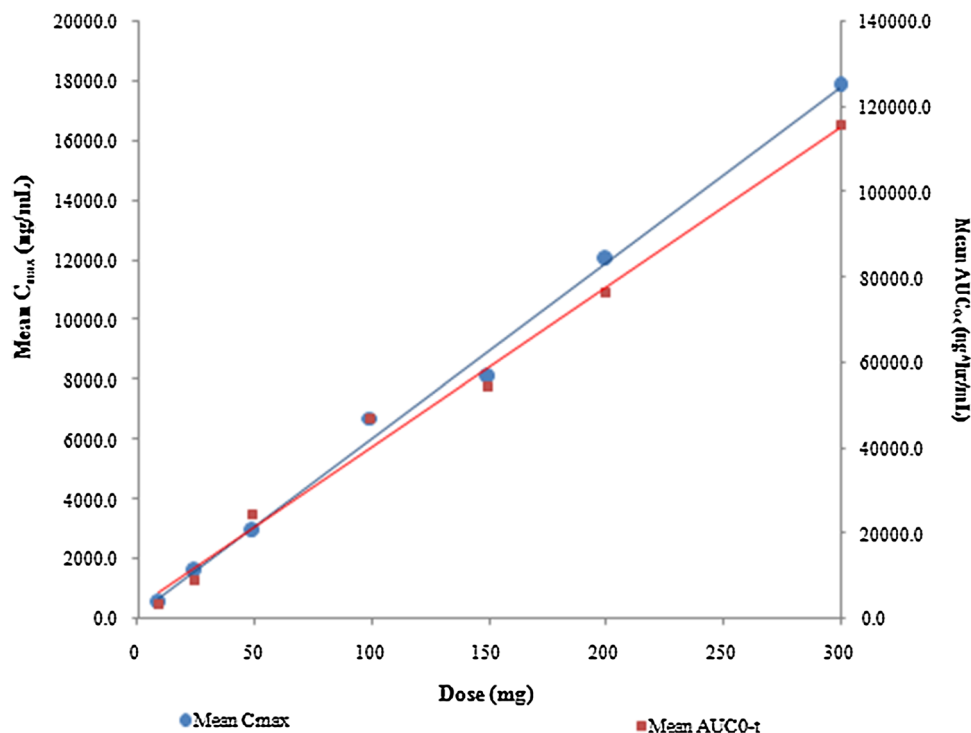

Fig. 2 Mean concentration vs. time graph with error bars for part I

Fig. 3 Dose linearity plot of maximum concentration (C_{max}) and area under the concentration–time curve from time zero to time t (AUC_t) for part I

6.9–11.3 h) was dose independent and the parallel decay in the log terminal phases of various doses is evident in Fig. 2. The somewhat shorter $t_{1/2}$ values observed at the lowest dose levels (i.e., 10 and 25 mg) was attributable to sample collection timepoints in the terminal phase of elimination of ZYAN1 (instead of 48 h, samples were collected for 72 h). After single oral ZYAN1 administration, the amount of ZYAN1 recovered in urine was 3.63 ± 1.02, 9.45 ± 1.76, 18.99 ± 3.36, 41.13 ± 12.06, 56.23 ± 17.26, 62.83 ± 18.11, and 82.66 ± 39.25 mg for the 10, 25, 50, 100, 150, 200, and 300 mg dose of ZYAN1, respectively. It was observed that percentage of ZYAN1 recovered in urine ranged from 27 to 42% after a single dose from 10 to 300 mg (Table 2). The apparent oral CL (range 2–2.9 L/h) and V_d (range 28–47 L) parameters showed remarkable consistency across the single doses of ZYAN1 administered in the study.

4.2.2 Multiple Ascending-Dose Study

The multiple-dose (Day 1, 3, and 5 dosing) mean concentration versus time profiles of ZYAN1 for the four doses used in the study are presented in Fig. 4. Based on the similarity of the plasma profiles of ZYAN1, the data unambiguously supported lack of accumulation after alternate-day dosing in any of the dosing cohorts. Additionally, if common timepoints such as pre-dose, 12 h, or 24 h post-dose are examined, there appears to be consistency in the concentrations of ZYAN1. The mean

pharmacokinetic data after multiple ascending doses of ZYAN1 are provided in Table 3. Almost identical mean C_{max} values were observed at the first and fifth day of dosing for the 150, 200, and 300 mg doses of ZYAN1 (Fig. 4). The median t_{max} of ZYAN1 was observed at around 1.5–3.0 h after administration of the 100, 150, 200, and 300 mg doses either on the first day or the fifth day of dosing, suggesting that similar absorption rates occur regardless of the dose size or the day of dosing. The mean $t_{1/2}$ of ZYAN1 after multiple doses was observed to be between 9.4 and 13 h for ZYAN1 after the last day of dosing. Furthermore, the percentage recovered in urine of ZYAN1 for the 100, 150, 200, and 300 mg dose ranged from 30 to 50% (Table 4). Based on the mean terminal $t_{1/2}$ value of approximately 10 h, it was expected that ZYAN1 was unlikely to show accumulation when orally administered on alternate days. Accordingly, no accumulation of the drug was observed after the first and third dose administration of ZYAN1 100, 150, 200, and 300 mg (Table 3).

4.2.3 Sex and Food Effect Study

The mean pharmacokinetic data for fasted and fed subjects are provided in Table 5.

The t_{max} was delayed under fed conditions with a median t_{max} value of 5 h compared with 2 h under fasting conditions. In addition, the ingestion of food decreased the C_{max} by 2.39 times as compared with the data obtained

Table 1 Pharmacokinetic data of single-dose escalation (part I)

Panel	ZYAN1 treatment (mg)	Pharmacokinetic variable							
		C_{max} (ng/mL)	AUC_t (h·ng/mL)	AUC_∞ (h·ng/mL)	t_{max} (h)	λz (1/h)	$t_{1/2}$ (h)	CL/F_obs (mL/h)	V_z/F_obs (mL)
1	10	566.467 ± 163.031	3597.269 ± 549.116	3659.531 ± 551.508	2.50 (1.50–3.00)	0.102 ± 0.019	6.976 ± 1.390	2784.592 ± 417.679	27,683.707 ± 4805.113
2	25	1634.500 ± 299.429	9177.789 ± 2359.163	9269.839 ± 2396.986	1.25 (1.00–2.50)	0.102 ± 0.016	6.917 ± 0.923	2870.759 ± 817.646	27,950.696 ± 5329.29
3	50	2959.333 ± 686.898	24,518.328 ± 5125.235	24,847.379 ± 5242.290	2.25 (1.00–5.00)	0.074 ± 0.024	10.434 ± 3.857	2084.748 ± 417.943	30,578.971 ± 10,323.282
4	100	6745.000 ± 1958.132	46,844.762 ± 13,259.837	47,320.651 ± 13,358.696	2.25 (1.00–5.00)	0.071 ± 0.023	10.685 ± 3.249	2271.507 ± 686.413	33,572.508 ± 10,971.959
5	150	8119.333 ± 1269.627	54,401.071 ± 12,592.181	54,672.481 ± 12,686.075	3.00 (2.50–5.00)	0.062 ± 0.008	11.357 ± 1.616	2891.042 ± 782.208	46,536.434 ± 9434.457
6	200	12,080.667 ± 3430.123	76,515.016 ± 6992.709	76,728.333 ± 7061.227	2.25 (1.50–5.00)	0.074 ± 0.01	9.502 ± 1.270	2625.566 ± 248.239	36,035.031 ± 6400.263
7	300	17,858.333 ± 2899.189	115,898.815 ± 45,549.563	116,226.587 ± 45,834.174	2.00 (1.00–5.00)	0.074 ± 0.011	9.484 ± 1.325	2835.924 ± 799.195	38,836.681 ± 12,903.706

All data are given as mean ± standard deviation, except for t_{max}, which is given as mean (range)

AUC_∞ area under the concentration–time curve from time zero to infinity, AUC_t area under the concentration–time curve from time zero to time t, CL/F_obs observed apparent total clearance of the drug from plasma after oral administration, C_{max} maximum concentration, $t_{1/2}$ elimination half-life, t_{max} time to maximum concentration, V_z/F_obs observed apparent volume of distribution during terminal phase after non-intravenous administration, λz elimination rate constant

under fasting conditions. The AUC values were decreased in fed conditions as compared with the fasting conditions after administration of ZYAN1. The mean $t_{1/2}$ of ZYAN1 was similar between fed vs. fasted treatments. The mean C_{max} for male and female subjects was not statistically significant but the extent of absorption appeared to be affected by sex.

4.3 Pharmacodynamic Results

This study was conducted in healthy volunteers with a normal hemoglobin range, and given the nature of the pharmacokinetic procedures there was blood loss during the study. Therefore, pharmacodynamic results that were solely based on key blood parameters may have to be interpreted with caution.

4.3.1 Single Ascending-Dose Study

In a single-dose panel at each dose level, changes in hemoglobin, hematocrit, reticulocyte count, and EPO were observed. A hemoglobin increase of >0.5 gm% was observed in most of the subjects receiving ZYAN1 tablets and the increase appeared to be more consistent starting from the oral dose of ZYAN1 100 mg and above. There was a dose response for mean serum EPO levels for doses of ZYAN1 from 100 mg up to 300 mg, which represented an almost proportional EPO increase with increasing ZYAN1 doses. The mean serum EPO C_{max} values versus time curve showed peak levels of EPO ranging from 6.6 mIU/L for ZYAN1 10 mg to 79.9 mIU/L for ZYAN1 300 mg (Fig. 5), while in subjects receiving placebo, the C_{max} of EPO observed was 9.6 mIU/L. The mean serum EPO t_{max} ranged from 10 to 72 h, with mean serum EPO returning to baseline levels within 24 h.

There was no apparent change in mean hematocrit, hemoglobin and reticulocyte counts over time or between various treatment doses (ZYAN1 vs. placebo) after single ascending doses of ZYAN1.

4.3.2 Multiple Ascending-Dose Study

In the multiple-dose phase of this study (part II), mean serum EPO concentrations increased following each dose of ZYAN1 (on Days 1, 3, and 5), with a dose-related response at ZYAN1 200 and 300 mg. We observed that the serum EPO C_{max} values ranged from 14.4 mIU/L for ZYAN1 100 mg to 60.5 mIU/L for ZYAN1 300 mg while it was 11.0 mIU/L for subjects who received placebo. The serum EPO profile after multiple dosing followed a similar profile to single dosing, with a lag in time of approximately 6–8 h to EPO C_{max} from pharmacokinetic t_{max} for ZYAN1 (Fig. 6).

Table 2 Descriptive statistics for urine data for part I

ZYAN1 treatment (mg)	Panel	Amount recovered (mg) [mean ± SD]	Percentage recovered (%) [mean ± SD]
10	1	3.63 ± 1.02	36.270 ± 10.245
25	2	9.45 ± 1.76	37.798 ± 7.059
50	3	18.99 ± 3.36	37.995 ± 6.718
100	4	41.13 ± 12.06	41.130 ± 12.064
150	5	56.23 ± 17.26	37.485 ± 11.508
200	6	62.83 ± 18.11	31.417 ± 9.056
300	7	82.66 ± 39.25	27.555 ± 13.083

SD standard deviation
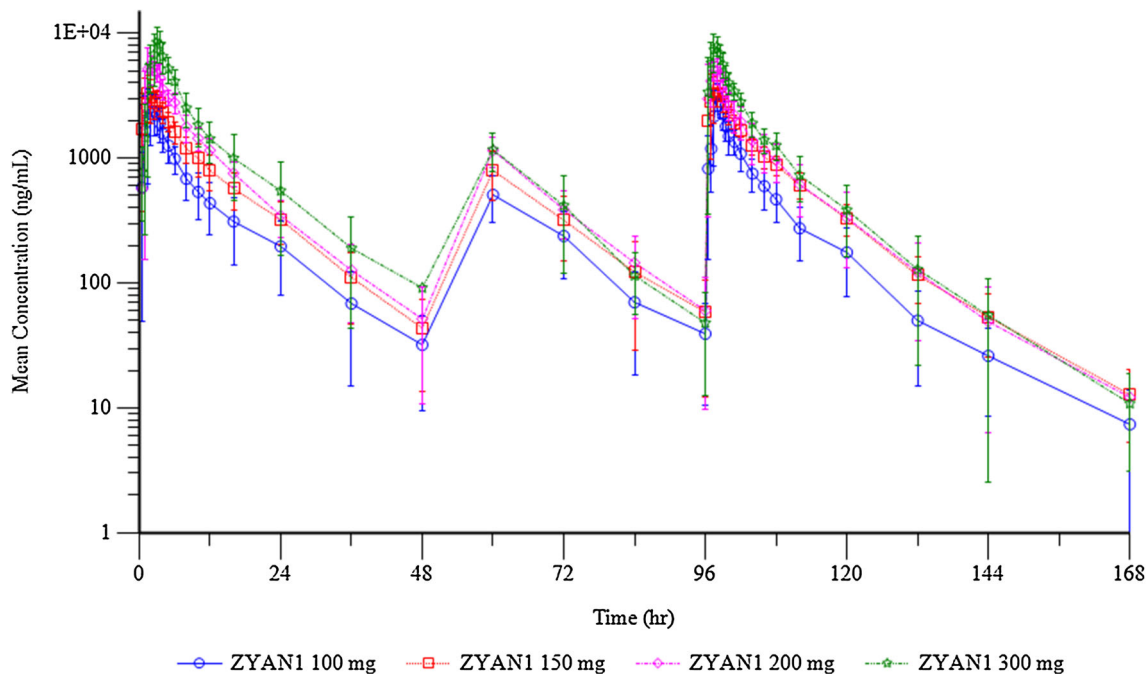
Fig. 4 Mean concentration vs. time graph with error bars for part II

It was observed that a higher number of subjects who received ZYAN1 showed a hemoglobin increase of >0.5 gm% on any timepoint than in the placebo group. A hemoglobin increase >1.0 gm% on any timepoint was also seen in more subjects who received ZYAN1 than in those who received placebo. There was no apparent change in mean hematocrit, hemoglobin, and reticulocyte count over time or between treatments (ZYAN1 vs. placebo) and there was no apparent dose response following ZYAN1 administration.

4.3.3 Sex and Food Effect Study

There was no apparent difference in mean serum EPO concentrations in subjects under fasted conditions compared with fed conditions (Fig. 7). In addition, there was no apparent difference in mean serum EPO concentrations between male and female subjects under either fasted or

fed conditions. Similarly, there were no apparent differences in mean hematocrit, hemoglobin, and reticulocyte count over time under fasted conditions compared with fed conditions, and no apparent difference between male and female subjects under either fasted or fed conditions.

4.4 Safety Evaluation

4.4.1 Single Ascending-Dose Study

A total of 20 AEs were reported in the single ascending-dose study. Of the AEs deemed possibly (?) related to study treatment, nine were reported in six subjects (14%) who received ZYAN1 and three were reported in three subjects (21%) who received placebo, while the rest of the AEs were not related to investigational product. All AEs deemed to be possibly related to study treatment (?) were grade 1 in severity. The reported AEs, mostly one episode

Table 3 Pharmacokinetic data for multiple-dose escalation (plan II)

ZYAN1 treatment (mg)	Panel	Day	N	Pharmacokinetic variable								
				C_{max} (ng/mL)	t_{max} (h)	AUC_t (h·ng/mL)	AUC_∞ (h·ng/mL)	$t_{1/2}$ (h)	λz (1/h)	CL (mL/h)	V_z/F_obs (mL)	Accumulation Index
100	1	1	6	6549.000 ± 1567.979	2.00 (1.00–3.00)	36,885.130 ± 11,488.927	38,817.512 ± 13,184.682	8.811 ± 1.869	0.082 ± 0.020	2834.521 ± 775.188	34,916.562 ± 8463.050	1.027 ± 0.018
		3	6	1016.400 ± 414.178	12.00 (12.00–12.08)	19,151.827 ± 8822.088	NP	NP	NP	NP	NP	NP
		5	6	6566.667 ± 1525.703	2.00 (1.50–2.50)	39,690.467 ± 11,051.227	40,102.984 ± 11,276.275	12.968 ± 6.654	0.065 ± 0.029	2744.020 ± 798.472	47,571.130 ± 18,996.205	1.106 ± 0.128
150	2	1	6	8185.667 ± 1215.600	1.50 (1.00–3.50)	59,780.215 ± 8539.865	62,811.249 ± 10,222.543	8.390 ± 2.431	0.088 ± 0.025	2487.695 ± 399.669	29,315.063 ± 6213.656	1.025 ± 0.026
		3	6	1591.833 ± 682.126	12.00 (12.00–12.17)	28,752.690 ± 13,537.466	NP	NP	NP	NP	NP	NP
		5	6	8657.000 ± 1190.575	1.75 (0.50–3.00)	65,307.036 ± 9629.912	65,706.064 ± 9748.640	10.205 ± 2.013	0.070 ± 0.015	2398.514 ± 378.402	34,943.264 ± 6986.821	1.043 ± 0.026
200	3	1	6	13,203.333 ± 2762.033	2.00 (1.50–3.50)	85,040.673 ± 12,019.503	88,180.063 ± 13,633.711	7.573 ± 2.103	0.097 ± 0.024	2344.752 ± 361.388	25,167.492 ± 5973.588	1.017 ± 0.019
		3	6	2309.333 ± 621.395	12.00 (12.00–12.05)	39,369.648 ± 12,307.240	NP	NP	NP	NP	NP	NP
		5	6	12,388.333 ± 1607.376	1.75 (0.50–2.50)	77,994.986 ± 21,218.895	78,368.940 ± 21,616.249	9.534 ± 1.467	0.074 ± 0.012	2758.333 ± 677.108	36,889.057 ± 5339.335	1.033 ± 0.018
300	4	1	6	19,286.667 ± 4086.434	3.00 (1.50–3.500)	118,269.238 ± 28,910.037	123,020.455 ± 32,873.290	7.968 ± 1.106	0.088 ± 0.011	2608.681 ± 641.704	29,476.605 ± 6045.099	1.017 ± 0.011
		3	6	2341.167 ± 787.913	12.06 (12.00–12.62)	40,533.695 ± 18,002.386	NP	NP	NP	NP	NP	NP
		5	6	16,450.000 ± 3422.484	1.75 (1.00–2.00)	105,472.355 ± 19,487.237	105,772.678 ± 19,542.028	9.445 ± 1.819	0.076 ± 0.015	2992.073 ± 697.789	41,797.611 ± 17,743.925	1.033 ± 0.022

All data are given as mean ± standard deviation, except for t_{max} which is given as mean (range)

AUC_∞ area under the concentration–time curve from time zero to infinity, AUC_t area under the concentration–time curve from time zero to time t, CL clearance, C_{max} maximum concentration, $t_{1/2}$ elimination half-life, t_{max} time to maximum concentration, λz elimination rate constant, NP not possible, V_z/F_obs observed apparent volume of distribution during terminal phase after non-intravenous administration, NP not possible

Table 4 Descriptive statistics for urine data for part II

ZYAN1 treatment (mg)	Panel	Day	Amount recovered (mg) [mean ± SD]	Percentage recovered (%)[mean ± SD]
100	1	1	50.13 ± 14.99	50.132 ± 14.998
		3	41.00 ± 11.07	41.001 ± 11.071
		5	50.14 ± 12.16	50.137 ± 12.160
150	2	1	52.77 ± 6.85	35.178 ± 4.566
		3	54.64 ± 13.35	36.427 ± 8.900
		5	44.23 ± 8.18	29.488 ± 5.453
200	3	1	64.95 ± 25.50	32.474 ± 12.749
		3	66.09 ± 8.74	33.044 ± 4.371
		5	75.76 ± 12.76	37.880 ± 6.381
300	4	1	82.97 ± 35.69	27.656 ± 11.899
		3	92.22 ± 41.92	30.741 ± 13.972
		5	99.15 ± 34.40	33.050 ± 11.466

SD standard deviation

Table 5 Descriptive pharmacokinetic data statistics by food condition for part III

Variable	Condition ($n = 10$)	
	Fasting	Fed
C_{max} (ng/mL)	10,352.800 ± 3076.528	4322.000 ± 1317.961
AUC_t (h·ng/mL)	71,331.577 ± 25,663.234	59,005.375 ± 16,846.249
AUC_∞ (h·ng/mL)	71,696.758 ± 25,837.440	59,561.279 ± 17,228.977
t_{max} (h)	2.000 (1.000–3.500)	5.000 (1.000–12.000)
λz (1/h)	0.067 ± 0.015	0.066 ± 0.017
HL_Lambda_z (h)	11.048 ± 3.378	11.328 ± 3.691
CL/F_obs (mL/h)	2479.865 ± 1333.719	2801.105 ± 1109.706
V_z/F_obs (mL)	37,927.046 ± 18,082.141	45,070.176 ± 19,928.748

All data are given as mean ± standard deviation, except for t_{max}, which is given as mean (range)

AUC_∞ area under the concentration–time curve from time zero to infinity, AUC_t area under the concentration–time curve from time zero to time t, *CL/F_obs* observed apparent total clearance of the drug from plasma after oral administration, C_{max} maximum concentration, t_{max} time to maximum concentration, V_z/F_obs observed apparent volume of distribution during terminal phase after non-intravenous administration, λz elimination rate constant, *HL_Lambda_z* terminal half-life

each, were rhinorrhea, tooth pain, sore throat, dizziness, headache, chest pain, diarrhea, upper respiratory tract infection, lethargy, nausea, throat irritation, increase in aspartate aminotransferase (AST), hyperkalemia, and back pain. No AEs were classified as grade 3.

4.4.2 Multiple Ascending-Dose Study

A total of 32 AEs were reported in the multiple ascending-dose study. Of the AEs possibly related to study treatment, 16 were reported in 11 subjects (46%) who received ZYAN1 and eight were reported in five subjects (63%) who received placebo. These treatment-related AEs were all grade 1 in severity, with the exception of dizziness in two subjects, which was classified as grade 2 in severity. The reported AEs were headache, dizziness, upper respiratory tract infection, nausea, diarrhea, phlebitis, sinus

tachycardia, dyspepsia, supra ventricular extrasystoles, abdominal pain, contusion, back pain, and nasal congestion. No AEs were classified as grade 3.

4.4.3 Sex and Food Effect Study

A total of 11 AEs were reported in this crossover study at a dose of ZYAN1 150 mg. One subject reported a SAE (grade 3) of cellulitis after 2 days of dosing; the subject was withdrawn from the study. The SAE was deemed not to be related to the study drug and resolved sequentially. Overall, in part III of the study, six AEs were classified as grade 1 in severity, four were classified as grade 2 in severity (headache [three AEs, one possibly related, two not related] and musculoskeletal pain [one AE, not related]), and one AE was classified as grade 3 (cellulitis, not related).
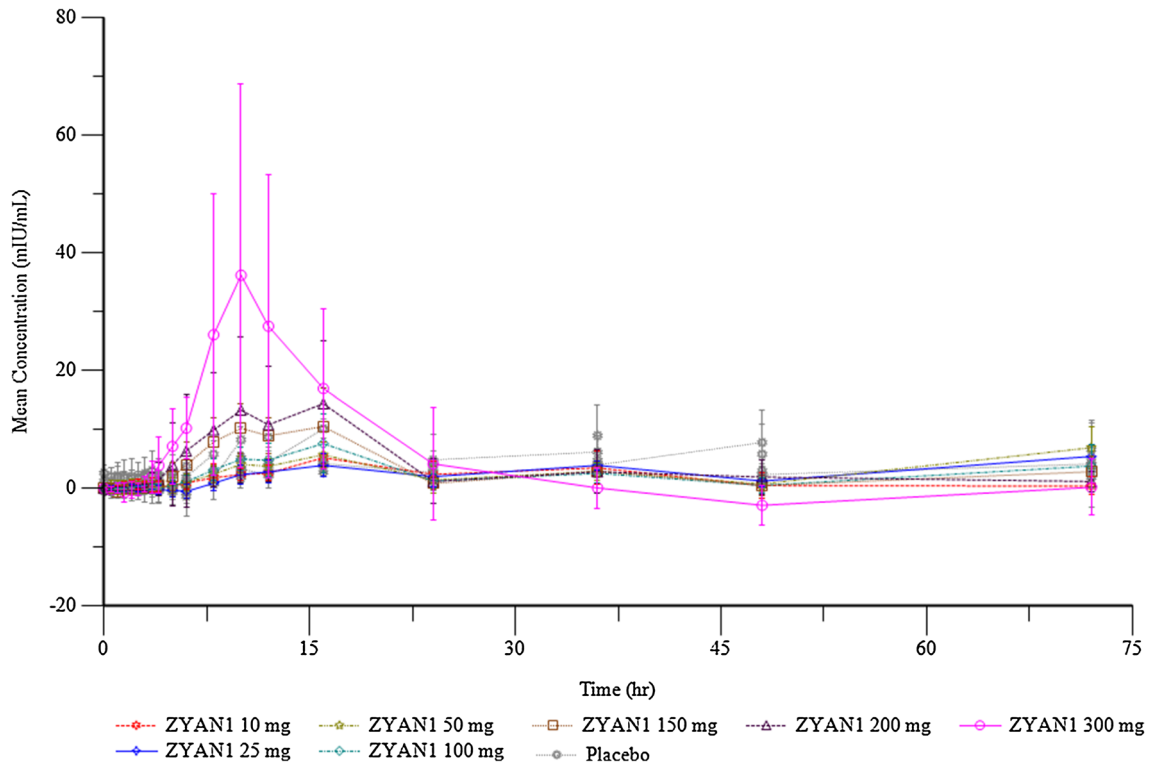
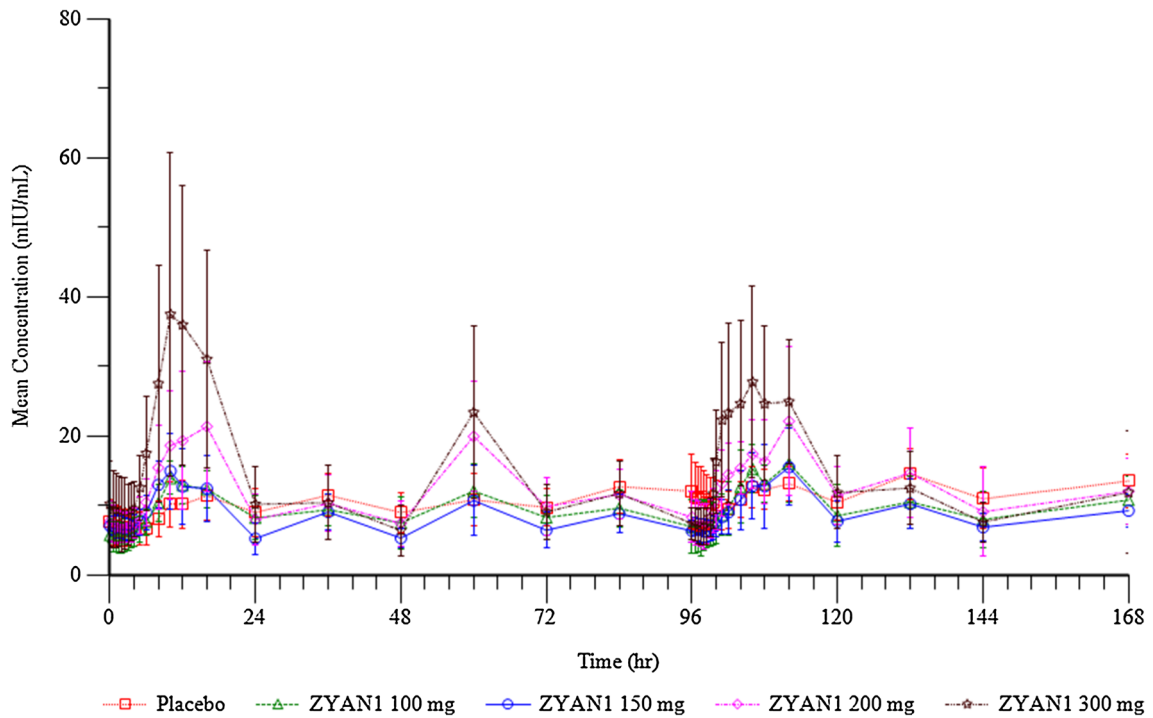**Fig. 5** Mean concentration of erythropoietin for part I (baseline corrected)

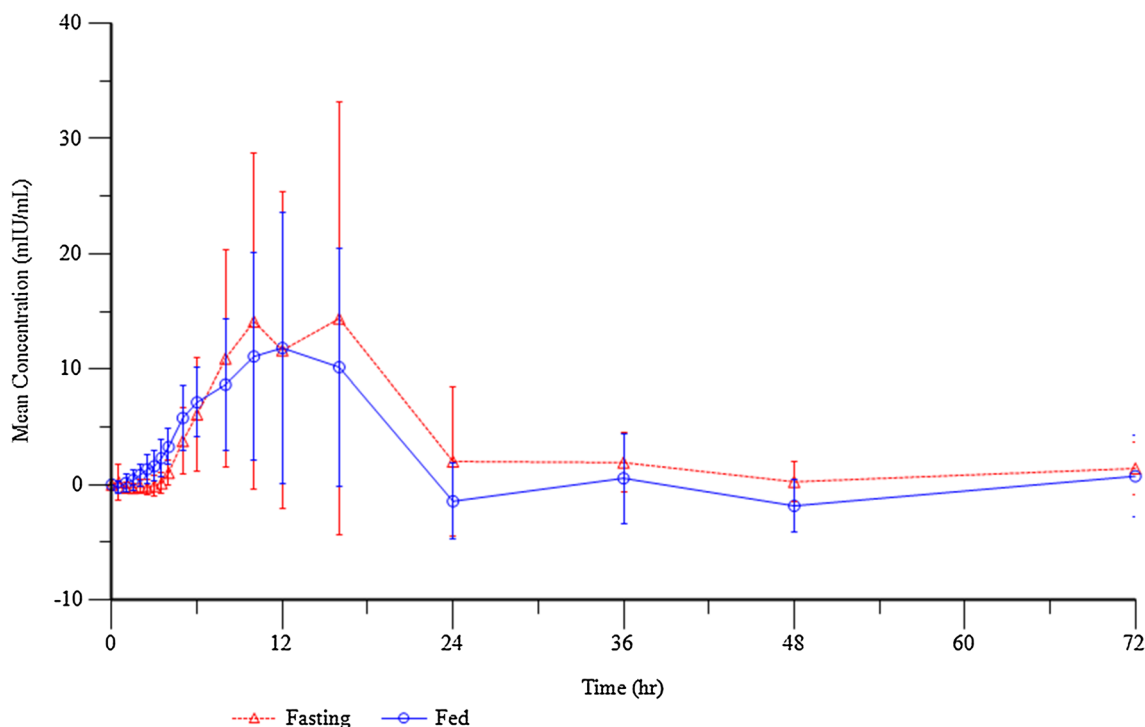**Fig. 6** Mean concentration of erythropoietin for part II (baseline corrected)

Fig. 7 Mean concentration of erythropoietin for part III (baseline corrected)

In addition, among the three studies, general safety was indistinguishable between active dose (ZYAN1) and placebo subjects, as assessed by clinical laboratory tests, vital signs, ECG assessments, and cardiac telemetry in the single ascending-dose, multiple ascending-dose, and food effect studies. The AEs observed in the study are summarized in Table 6.

5 Discussion

There has been considerable interest in PHD inhibitors for the treatment of anemia, with a few compounds, namely roxadustat (FG-4592), molidustat (BAY85-3934) and vadadustat, already being evaluated in late-phase clinical trials [26]. Therefore, we were interested in evaluating the safety, tolerability, pharmacokinetics, and pharmacodynamics of ZYAN1, a potent orally administered HIF-PHD inhibitor, to develop a comprehensive phase I package to aid in the planning of phase II development of this drug.

In totality, this phase I evaluation comprised 100 healthy volunteers, assigned to single ascending-dose panels, multiple ascending-dose panels, and a food/sex effect panel. In order to avoid the potential risk of an overt drug exposure in a larger number of subjects, each dose panel of eight subjects was subdivided into two blocks (applicable for parts 1 and II). In the first block, two subjects were enrolled (one placebo + one ZYAN1), while in the second block, six subjects were enrolled (one placebo + five

ZYAN1). The dosing of the second block of each panel was initiated 24 h post-dose and when the safety analysis of the first block was available.

Due to the nature of the study design, the pharmacokinetics of ZYAN1 were assessed under various conditions with extensive blood sampling to provide guidance for further development of the drug. The pharmacodynamics of ZYAN1 were monitored based on the assessment of hemoglobin, hematocrit, reticulocyte count, and EPO level. For safety assessment, AE and SAE reports, clinical examination, vitals, ECG, and clinical laboratory assessment were considered.

The single ascending-dose study of ZYAN1 suggested an almost dose-proportional increase in the exposure (i.e., C_{max} and AUC) in relation to a 30-fold range of orally administered ZYAN1. The absorption of ZYAN1 appeared to be relatively rapid and consistent among the various single doses. As the ZYAN1 doses increased, the V_d remained relatively consistent, suggesting a lack of non-linear distribution of the drug at higher doses. The apparent CL of ZYAN1 was similar across the dose levels, suggesting a lack of saturation in the elimination and/or excretory process of ZYAN1. Due to comparable CL and V_d, the $t_{1/2}$ did not vary as a function of dose.

The multiple ascending-dose pharmacokinetics of ZYAN1 mimicked the single-dose data with no accumulation of drug as judged by C_{max} and AUC values between Day 1 and Day 5 dosing of the drug. In addition, the $t_{1/2}$

Table 6 Summary of adverse events

ZYAN1 Panel (mg)	Adverse events	
	Test (n = 78)	Placebo (n = 22)
SAD 10	Nil	Nil
SAD 25	Rhinorrhea, right lower wisdom tooth pain, sore throat	Nil
SAD 50	Dizziness, headache, chest pain, Diarrhea, upper respiratory tract infection	Nil
SAD 100	Dizziness, headache, lethargy, nausea, throat irritation, increase in AST, hyperkalemia	Dizziness, headache
SAD 150	Nil	Nil
SAD 200	Back pain	Nil
SAD 300	Nil	Headache
MSD 100	Headache, dizziness	Upper respiratory tract infection
MAD 150	Headache, dizziness	Headache, dizziness
MAD 200	Nausea, headache, dizziness, diarrhea, phlebitis	Sinus tachycardia, dyspepsia, dizziness
MAD 300	Supra ventricular extrasystoles, abdominal pain, diarrhea, contusion, back pain, dizziness, headache, nasal congestion, phlebitis	Nil
Food and sex effect 150	Palpitation, cellulitis, back pain, musculoskeletal pain, head discomfort, headache (2), folliculitis, cellulitis	Headache

AST aspartate aminotransferase, *MAD* multiple ascending-dose, *SAD* single ascending-dose

values after multiple doses were found to be in a similar range to that observed for the single dose. It was also observed that the amount and percentage recovered in urine was comparable after every dose of ZYAN1 in the multiple-dose study and also similar to the observations seen with the respective single dose of ZYAN1.

The exploratory food effect study showed that both the rate and extent of absorption of ZYAN1 was altered by concomitant food intake. Similarly, the analysis between sexes suggested that female subjects had a higher exposure than males. However, as it was a small subject group, the data represents qualitative difference at best, but definitive conclusions cannot be inferred.

Our results showed that following a single dose of ZYAN1, mean serum EPO C_{max} values increased over time, with peak levels ranging from 6.6 mIU/L for ZYAN1 10 mg to 79.9 mIU/L for ZYAN1 300 mg. The initiation of EPO rise was observed as early as after 6 h of ZYAN1 administration and an increase of almost 2- to 4-fold was observed compared with baseline in the 150, 200, and 300 mg panels. The EPO response was not markedly different from placebo for doses up to ZYAN1 150 mg. There was no apparent difference observed for EPO between males and females or between fasted and fed conditions. With a single dose of ZYAN1, the effect on the biomarker to support the mechanism of action of our compound was observed [27, 28].

We observed a hemoglobin increase of >0.5 gm% in most of the subjects after a ZYAN1 single dose, with a more consistent rise with 100 mg and higher dosing.

Similar hemoglobin response was seen in multiple-dose panels even though the subjects had also undergone some study-related blood loss. This may suggest the ability of ZYAN1 to participate in erythropoiesis.

There was no apparent change in mean hematocrit, hemoglobin, and reticulocyte count over time or between treatments (ZYAN1 vs. placebo), and there was no apparent dose response following ZYAN1 administration.

In parts I, II, and III of the study, there were no clear differences in safety as assessed by clinical laboratory tests, vital signs, ECG assessments, and cardiac telemetry in subjects who had received either single or multiple doses of ZYAN1 compared with those who received placebo, and no dose-related trends in these safety assessments in subjects who received ZYAN1.

In parts I and II of the study, there were no apparent dose-related trends for any of the commonly occurring AEs. Common AEs of headache and dizziness were reported in both part I (single dose) and part II (multiple doses) of the study, the majority of which were deemed related to the study drug (including subjects who received placebo). Moreover, these commonly occurring AEs are similar to those seen with other PHD inhibitor compounds and no new AEs were reported [26].

While the urinary excretion of intact ZYAN1 was confirmed in healthy subjects (average 36%), supporting the importance of renal function, it also suggested the role of non-renal CL mechanisms for the elimination of ZYAN1. In this context, the pharmacokinetics of vadadustat were not found to be significantly altered in CKD patients

(stages 3 and 4) despite the role of renal CL mechanisms (approximately 59% of the radioactive dose) in the disposition of 14C-vadadustat in healthy subjects [29]. To underscore this observation, we have found that both acute kidney failure and chronic kidney failure models in rats did not alter the pharmacokinetics of ZYAN1 as compared with those in normal controls rats [30]. It was also noted that complete removal of both kidneys in rats led to a 2-fold increase in the exposure of ZYAN1, suggesting the role of non-renal CL pathways for the elimination of ZYAN1 [30].

The study was conducted in healthy volunteers mainly to evaluate safety, tolerability, pharmacokinetics, and pharmacodynamics. Therefore, safety and efficacy data derived from this study should be viewed with caution while also considering the patient group. Moreover, the study involved blood loss in the form of collection of blood samples for the pharmacokinetics, pharmacodynamics, and safety assessment, which perhaps had some influence with the efficacy parameters evaluated in the study.

As discussed in Sect. 1, hepcidin, a key regulator of iron absorption and remobilization, is normally down-regulated by erythropoiesis, anemia, and hypoxia. HIF stabilization, via the PHD inhibitor, is associated with hepcidin suppression, which increases intestinal iron absorption and also increases enzymes of iron transportation [26]. As current therapeutic options for the management of anemia of CKD have certain limitations, development of HIF-PHD inhibitors to favorably modify the HIF-PHD pathway can be helpful for the management of anemia with CKD patients. However, based on the observed results of this study, ZYAN1 is a safe and effective ESA for the treatment of ACDs. The proposed dose range of 100–200 mg every 2 days appears to be reasonable for the phase II development of ZYAN1 in this patient population.

6 Conclusion

Overall, ZYAN1 was well-tolerated when administered as single oral doses (or multiple oral doses with alternate-day dosing) in healthy male subjects. Serum EPO levels showed dose response in the ZYAN1 oral doses evaluated. Based on the $t_{1/2}$, pharmacodynamic activity, and lack of drug accumulation, an every 2 days dosing regimen of ZYAN1 is appropriate for phase II study. The proposed dose range of ZYAN1 for phase II study is 100–200 mg every 2 days.

References

1. Spinowitz BS, Kausz AT, Baptista J, Noble SD, Sothinathan R, Bernardo MV, et al. Ferumoxytol for treating iron deficiency anemia in CKD. J Am Soc Nephrol. 2008;19:1599–605.
2. Agarwal N, Prchal JT. Anemia of chronic disease (anemia of inflammation). Acta Haematol. 2009;122(2–3):103–8.
3. Hayes JM. Chemotherapy induced anemia: treating it as a disease not just as a condition. Adv Stud Pharm. 2007;5(5):125–32.
4. Haase VH. Regulation of erythropoiesis by hypoxia-inducible factors. Blood Rev. 2013;27(1):41–53.
5. Wang J, Pantopoulos K. Regulation of cellular iron metabolism. Biochem J. 2011;434(Pt 3):365–81.
6. Nemeth E, Genz T. Anemia of inflammation. Hematol Oncol Clin N Am. 2014;28(4):671–81.
7. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochim Biophys Acta. 2012;1823(9):1434–43.
8. Karuppagounder SS, Ratan RR. Hypoxia-inducible factor prolyl hydroxylase inhibition: robust new target or another big bust for stroke therapeutics? J Cereb Blood Flow Metab. 2012;32(7):1347–61.
9. Maxwell PH, Eckardt K. HIF prolyl hydroxylase inhibitors for the treatment of renal anaemia and beyond. Nat Rev Nephrol. 2016;12:157–68.
10. Jain MR, Joharapurkar AA, Pandya V, Patel V, Joshi J, Kshirsagar S, et al. Pharmacological characterization of ZYAN1, a novel prolyl hydroxylase inhibitor for the treatment of anemia. Drug Res. 2016;66(2):107–12.
11. Tsagalis G. Renal anemia: a nephrologist's view. Hippokratia. 2011;15(Suppl 1):39–43.
12. Haase VH. Hypoxic regulation of erythropoiesis and iron metabolism. Am J Physiol Renal Physiol. 2010;299(1):F1–13.

13. Melnikova I. Anaemia therapies. Nat Rev Drug Discov. 2006;5(8):627–8.

14. Balarajan Y, Ramakrishnan U, Ozaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. Lancet. 2011;378(9809):2123–35.

15. Muchnik E, Kaplan J. HIF prolyl hydroxylase inhibitors for anemia. Expert Opin Invest Drugs. 2011;20(5):645–56.

16. Rosenberger C, Mandriota S, Jurgensen JS. Expression of hypoxia-inducible factor-1alpha and -2alpha in hypoxic and ischemic rat kidneys. J Am Soc Nephrol. 2002;13(7): 1721–32.

17. Takubo K, Goda N, Yamada W. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. Cell Stem Cell. 2010;7(3):391–402.

18. Forristal CE, Winkler IG, Nowlan B, Barbier V, Walkinshaw G, Levesque JP. Pharmacologic stabilization of HIF-1alpha increases hematopoietic stem cell quiescence in vivo and accelerates blood recovery after severe irradiation. Blood. 2013;121(5): 759–69.

19. Beuck S, Schaenzer W, Thevis M. Hypoxia-inducible factor stabilizers and other small-molecule erythropoiesis-stimulating agents in current and preventive doping analysis. Drug Test Anal. 2012;4(11):830–45.

20. Bakris GL, Yu KH, Leong R, Shi W, Lee T, Saikali K, et al. Effects of a novel anemia treatment, FG-4592 - an oral hypoxia-inducible prolyl hydroxylase inhibitor (HIF-PHI) on blood pressure and cholesterol in patients with chronic kidney disease: LB-OR-06. J Clin Hypertens. 2012;14(7):489.

21. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol. 1992;12(12):5447–54.

22. Wang GL, Semenza GL. Purification and characterization of hypoxia-inducible factor 1. J Biol Chem. 1995;270(3):1230–7.

23. Rosen MD, Venkatesan H, Peltier HM, Bembenek SD, Kanelakis KC, et al. Benzimidazole-2-pyrazole HIF prolyl 4-hydroxylase inhibitors as oral erythropoietin secretagogues. ACS Med Chem Lett. 2010;1(9):526–9.

24. Data on file, Cadila Healthcare Ltd., 2014.

25. Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Veterinary Medicine, May 2001.

26. Besarab A, Provenzano R, Hertel J, Zebaneh R, Klaus SJ, Lee T, et al. Randomized placebo-controlled dose-ranging and pharmacodynamics study of roxadustat (FG-4592) to treat anemia in nondialysis-dependent chronic kidney disease patients. Nephrol Dial Transplant. 2015;30(10):1665–73.

27. Bernhardt WM, Wiesener MS, Scigalla P, Chou J, Schmieder RE, Gunzler V, et al. Inhibition of prolyl hydroxylases increases erythropoietin production in ESRD. J Am Soc Nephrol. 2010;21:2151–6.

28. Lee FS, Percy MJ. The HIF pathway and erythrocytosis. Annu Rev Pathol. 2011;6:165e92.

29. Buch A, Maroni B, Hartman C. Dose exposure relationship of vadadustat is independent of the level of renal function [poster no. #SA-PO537]. In: American Society of Nephrology Kidney Week; 3–8 Nov 2015; San Diego.

30. Patel H, Joharapurkar AA, Pandya VB, Patel VI, Kshirsagar SG, Patel P, et al. Influence of acute and chronic kidney failure in rats on the disposition and pharmacokinetics of ZYAN1, a novel prolyl hydroxylase inhibitor, for the treatment of chronic kidney disease-induced anemia. Xenobiotica. 2017;19:1–8. doi:10.1080/00498254.2016.1278287.