

BRIEF REPORT

A phase I randomized, double-blind, single subcutaneous dose escalation study to determine the safety, tolerability, and pharmacokinetics of rezafungin in healthy adult subjects

Kenan Gu¹ | Dennis Ruff² | Cassandra Key² | Marissa Thompson³ |
Shoshanna Jiang³ | Taylor Sandison⁴ | Shawn Flanagan⁴

¹National Institute of Allergy and Infectious Diseases, Rockville, Maryland, USA

²ICON Early Phase Services Clinical Research Unit, San Antonio, Texas, USA

³Emmes Company, LLC, Rockville, Maryland, USA

⁴Cidara Therapeutics, San Diego, California, USA

Correspondence

Kenan Gu, Division of Microbiology and Infectious Diseases, NIAID/NIH, 5601 Fishers Lane, Room 7G28 (MSC 9826), Bethesda, MD 20892-9826, USA. Email: kenan.gu@nih.gov

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Abstract

Rezafungin is a novel echinocandin being developed for the treatment and prevention of invasive fungal infections. The objectives of this randomized, double-blind study in healthy adults were to determine the safety, tolerability, and pharmacokinetics of rezafungin after subcutaneous (s.c.) administration. The study design consisted of six sequential cohorts of eight subjects, except for the first cohort with four subjects. The subjects were randomized in a 3:1 ratio of rezafungin to placebo and were to receive a single dose of 1, 10, 30, 60, 100, or 200 mg. The most common adverse events (AEs) were increased alanine aminotransferase and sinus bradycardia (unsolicited) and erythema at the injection site (solicited). Unsolicited AEs were generally mild to moderate and not rezafungin-related. Although the study was terminated after the 10 mg dose cohort due to concerns of potential increased severity of injection site reactions, no predetermined dose escalation halting criteria were met. Following the 10 mg single s.c. dose of rezafungin ($n = 6$), the geometric mean (GM) maximum concentration (C_{\max}) was 105.0 ng/ml and the median time to C_{\max} was 144 h. The GM area under the concentration-time curve was 32,770 ng*h/ml. The median estimated terminal half-life was 193 h. The GM apparent oral clearance was 0.255 L/h and the GM apparent volume of distribution was 68.5 L. This study demonstrates that a single s.c. dose of rezafungin in healthy adult subjects: (1) did not result in serious AEs, death, or withdrawal from the study due to an AE; and (2) produced a pharmacokinetic profile with long exposure period postadministration. In an effort to reduce the occurrence of injection site reactions, a re-evaluation of the rezafungin s.c. formulation could be considered in the future.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Currently marketed antifungal agents of the echinocandin class are dosed by i.v. once daily and are therefore not practical for prolonged or outpatient prophylaxis. Rezafungin is an investigational echinocandin (currently in phase III trials studying once-weekly i.v. administration) that has excellent activity against clinically relevant *Candida* and *Aspergillus* spp. and *Pneumocystis jirovecii*, a prolonged half-life allowing for longer dosing intervals, and a stability/solubility profile that allows for the possibility of subcutaneous (s.c.) dosing.

WHAT QUESTION DID THE STUDY ADDRESS?

The objectives of this randomized, double-blind study in healthy adults were to determine the safety, tolerability, and pharmacokinetics (PKs) of rezafungin after s.c. administration.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study provided safety data regarding s.c. administration of rezafungin. Despite common solicited injection site reactions, s.c. administration of 10 mg of rezafungin did not result in serious adverse event (AEs), death, or withdrawals due to an AE in healthy adult subjects. The study also showed that rezafungin had a PK profile with a long exposure period.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

In an effort to reduce the occurrence of injection site reactions, these findings may lead to a future re-evaluation of the s.c. formulation of rezafungin.

INTRODUCTION

Rezafungin is an investigational antifungal agent with the favorable efficacy and safety profiles of its echinocandin class. Currently marketed echinocandins are dosed by i.v. infusions once daily¹ and are therefore not practical for prolonged or outpatient prophylaxis. The prolonged half-life of rezafungin allows for i.v. dosing 400 mg loading dose, followed by 200 mg once weekly in phase II and phase III studies,² and its stability/solubility enables s.c. formulation³ with moderate to high bioavailability in rats and monkeys (up to ~80%). The smaller rezafungin dose requirement also supports the possibility of s.c. dosing volume.

To our knowledge, this is the first study to evaluate the safety, tolerability, and pharmacokinetics (PKs) of single ascending doses of rezafungin administered s.c. in healthy adults.

METHODS AND MATERIALS

Study design and treatments

This was a phase I, single-center, prospective, randomized, double-blind trial of single-ascending doses of rezafungin administered by s.c. injection to six cohorts of healthy adult subjects to evaluate its safety, tolerability, and PKs.

Rezafungin injection for s.c. use (100 mg/ml), containing rezafungin acetate, and mannitol, was manufactured by Patheon Ferentinoc. The placebo was 5% dextrose injection, USP, a sterile, nonpyrogenic solution of dextrose in water for injection.

Participants were admitted to the clinical research unit (CRU) for check-in for clinical evaluations the day before dosing. They were randomized and dosed on day 1 of the study and confined to the CRU for 7 days for continued assessments and sample collections. Follow-up visits or telephone calls were conducted on day 14 (± 1 day), day 21 (± 1 day), and day 30 (± 1 day).

Ethics

The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines as required by 21 Code of Federal Regulations. All study-related documents were approved by IntegReview Institutional Review Board. All subjects signed an informed consent form prior to participating in the study.

Subjects

A total of 44 subjects were planned to be enrolled into one of six cohorts: cohort 1 (1 mg) with four subjects and

cohorts 2 to 6 (10, 30, 60, 100, and 200 mg) with eight subjects each. Subjects in each cohort were randomized in a 3:1 ratio (rezafungin to placebo). Eligible participants were healthy men and women 18 to 45 years old with body mass index (BMI) of 18.5 to 35.0 kg/m², a minimum weight of 50 kg, and adequate venous access for blood collection. Exclusion criteria included history of hypersensitivity or allergic reactions to echinocandins, receipt of any live or killed vaccines or immunoglobulins within 14 days of dosing, or previous participation in any rezafungin trial. Subjects were excluded if they were positive for hepatitis B, hepatitis C, or HIV serology; had clinical laboratory values outside the reference ranges prior to dosing; had abnormal electrocardiogram (ECG) findings; or presented with any clinically significant condition in the opinion of the investigator.

Dose escalation criteria

Two sentinel subjects were to be admitted initially in each cohort (except for cohort 1) and randomized 1:1 to receive rezafungin or placebo. All sentinel subject safety data through day 30 was reviewed by the principal investigator prior to dosing the remainder of the cohort. Safety data for each cohort through day 30 was to be confirmed by review of objective predefined criteria before continuing to the next cohort.

Safety and tolerability assessments

Safety was assessed by occurrence of solicited and unsolicited adverse events (AEs), serious AEs (SAEs), and clinical evaluation (including clinical laboratory tests, ECGs, physical examinations, and vital signs) on select days from dosing through day 30. Tolerability was assessed by occurrence of solicited local reactogenicity symptoms (injection site evaluation) from dosing to day 30.

Solicited AEs, as specified in the case report form and recorded through the regular collection of data, comprised reactogenicity symptoms localized to the area of the s.c. dose, including pain, tenderness, pruritus, ecchymosis, induration/swelling, erythema, nodule, and ulceration. Unsolicited AEs were those not specified in the case report form and recorded as volunteered by subjects or other unsolicited manner. AEs were recorded from dosing to the final follow-up visit and graded based on severity as mild (grade 1), moderate (grade 2), or severe (grade 3) and assessed to be related or not related to the study product by the study physician. AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA) version 23.1.

Safety and clinical laboratory tests were conducted on blood and urine samples collected after subjects had fasted for at least 8 h and included hematology, coagulation, blood chemistry, and dipstick urinalysis.

Halting criteria

Cohort escalation would be halted if: (1) two or more subjects in a cohort experienced the same grade 2 (or higher) drug-related AE in the same MedDRA-coded high-level group term through day 30; (2) two or more subjects within a cohort experienced postdose QTcF greater than 500 or change from baseline greater than 60 ms; or (3) any subject experienced skin ulceration of any grade at an injection site.

Pharmacokinetic assessments

PK profiles of rezafungin were to be assessed by measurement of drug levels in plasma after s.c. administration in each cohort (except for cohort 1).

Plasma for PK analysis was collected at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 96, 144, 312 (day 14), and 696 (day 30) h postdose. Rezafungin concentrations were determined by a validated liquid chromatography-tandem accurate mass spectrometry method with a lower limit of quantitation of 10 ng/ml. PK sampling was not conducted for cohort 1 participants for 1 mg dose, because it was anticipated that rezafungin concentrations would be undetectable in plasma at this dose level based on human i.v. dose concentration data,⁴ and this dose was to evaluate local tolerability. Bioanalytical data collection and analysis were completed with Analyst version 1.6.3/ICON LIMS (iBIO) version 1.7.11.

Statistical analyses

No formal sample-size calculations or formal hypothesis tests were conducted. When presented for a proportion, 95% confidence intervals were calculated using the Wilson Score method. Rezafungin PK parameters were estimated by noncompartmental analysis (NCA) using Phoenix WinNonlin version 8.2 or later (Certara, Princeton, NJ). The Lambda Z acceptance criteria used in the NCAs were as follows: rsq_{adjusted} greater than or equal to 0.90, span greater than or equal to 2.0 half-lives, and that the analysis include at least two timepoints after time to maximum plasma concentration (T_{max}). Terminal phase parameters will be excluded if they do not meet the Lambda Z acceptance criteria.

SAS version 9.4 or above or R version 3.2 or above were used to generate tables, figures, and listings.

RESULTS

The study was terminated after cohort 2 completion due to the number of subjects who experienced solicited AEs following s.c. administration. These events raised concerns of increased severity of injection site reactions with higher doses.

Disposition and demographics

Four subjects were enrolled and received study product in cohort 1 (three rezafungin and one placebo), and eight subjects were enrolled and received the study product in cohort 2 (six rezafungin and two placebo). Two randomized subjects in cohort 2 (one in the 10 mg dose group and 1 in the placebo group) terminated early from the study prior to receiving any study product and were excluded from safety and PK analyses.

Across all 14 randomized subjects, there were 13 men (93%) and one woman (7%). Half of the subjects were Hispanic or Latino (50%). The majority of the subjects were White (57%). The mean age for all subjects was 33.6 years (range: 23–45 years). At baseline, average height was 175.49 cm (range: 166.4–189.5 cm), average weight was 84.54 kg (range: 66.7–117.1 kg), and average BMI was 27.25 kg/m² (range: 22.7–32.6 kg/m²).

Safety and tolerability results

All 12 subjects that received the study product were included in the safety analysis population. There were no SAEs, deaths, or withdrawals from the study due to an AE, and no halting criteria were met.

Unsolicited AEs were generally mild and not rezafungin-related. The most common unsolicited AEs were increased sinus bradycardia (2 subjects [67%] of 3 who received rezafungin in cohort 1) and increased alanine aminotransferase (2 subjects [33%] of 6 who received rezafungin in cohort 2). A single subject in cohort 1 developed severe bradycardia, which was asymptomatic and determined to not be rezafungin-related. Five separate AEs, which occurred in three subjects in cohort 2, were considered mild but deemed related to rezafungin. One subject experienced three of these AEs: elevated white blood cell, monocyte, and basophil counts. The possible relationship to rezafungin was based on a temporal association to dosing. One subject had skin pigmentation at

the injection site. This was considered related to rezafungin. One subject had a mild headache deemed related to rezafungin given the temporal association to dosing. In all cases, the events were resolved.

Solicited reactogenicity symptoms is presented in Table 1. These findings were mild to moderate in intensity and considered to be related to rezafungin. None of the changes were deemed serious and there were no erosions or skin ulcers associated with these injection site changes. Resolution or reduction in nodule size generally occurred over 2–4 weeks, and no treatment of the injection area was necessary.

Pharmacokinetic results

PK data were available from all six subjects in cohort 2 who received rezafungin (10 mg) s.c. dose and presented in Table 2.

The median T_{max} was 144 h (range: 48 to 144 h). The geometric mean (GM) maximum plasma concentration was 105.0 ng/ml (coefficient of variation [CV] 28%). Rezafungin concentrations were still detected in five of six subjects through the last PK sample timepoint on day 30 (696 h postdose). The GM of the area under the concentration-time curve from the time of dosing to the last measurable concentration (nominal time 696 h postdose; $AUC_{[0-last]}$) was 32,770 ng*h/ml (CV 30%). The estimated GM AUC to infinity ($AUC_{(0-inf)}$) was 39,230 ng*h/ml (CV 24%). The estimated median terminal half-life ($t_{1/2}$) was 193.0 h (range: 140 to 219 h). The GM apparent oral clearance (CL/F) was 0.255 L/h (CV 24%). The GM volume of distribution (V_z/F) was estimated to be 68.5 L (CV 38%).

Figure 1 presents the geometric mean plasma concentration-time profile of rezafungin following s.c. administration.

DISCUSSION

The study was terminated after cohort 2 (10 mg) due to concerns of tolerability at higher doses. However, no dose escalation halting criteria were met, no subjects withdrew due to AEs, and no systemic AEs occurred that were deemed intolerable for subjects given 1 mg or 10 mg rezafungin subcutaneously. No biopsies of the injection site were taken from subjects who presented with solicited local injection site findings, but given the character and location of the nodules and the absence of skin erosion or ulcers, it is speculated that rezafungin, as formulated for s.c. administration in this study, resulted in a local panniculitis. The injection site reactions in nonclinical species were equivocal and tolerability

TABLE 1 Number and percentage of subjects experiencing solicited reactogenicity symptoms by symptom and dose group

Symptom	Any dose (N = 9)			1 mg (N = 3)			10 mg (N = 6)			Placebo (N = 3)		
	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI
Any symptom	7	78	45, 94	1	33	6, 79	6	100	61, 100	–	–	–
Pain	3	33	12, 65	1	33	6, 79	2	33	10, 70	–	–	–
Tenderness	4	44	19, 73	–	–	–	4	67	30, 90	–	–	–
Pruritus (itching)	1	11	2, 43	–	–	–	1	17	3, 56	–	–	–
Ecchymosis (bruising), functional grade	2	22	6, 55	–	–	–	2	33	10, 70	–	–	–
Ecchymosis (bruising), measurement grade	1	11	2, 43	–	–	–	1	17	3, 56	–	–	–
Induration (hardness)/swelling, functional grade	1	11	2, 43	–	–	–	1	17	3, 56	–	–	–
Induration (hardness)/swelling, measurement grade	1	11	2, 43	–	–	–	1	17	3, 56	–	–	–
Erythema (redness), functional grade	6	67	35, 88	–	–	–	6	100	61, 100	–	–	–
Erythema (redness), measurement grade	6	67	35, 88	–	–	–	6	100	61, 100	–	–	–
Nodule, functional grade	5	56	27, 81	–	–	–	5	83	44, 97	–	–	–
Nodule, measurement grade	4	44	19, 73	–	–	–	4	67	30, 90	–	–	–
Ulceration, functional grade	–	–	–	–	–	–	–	–	–	–	–	–
Ulceration, measurement grade	–	–	–	–	–	–	–	–	–	–	–	–

Note: N = Number of subjects in safety population.

Abbreviation: CI, confidence interval.

TABLE 2 Summary statistics of rezafungin PK parameters in plasma—10 mg dose group

Parameter (units)	N	Mean	Standard deviation	Minimum	Median	Maximum	Geometric mean	Geometric CV%
C_{\max} (ng/ml)	6	108.3	29.50	72.6	105.4	154	105.0	28
T_{\max} (h)	6	120.0	40.16	48.0	144.0	144	112.1	47
$AUC_{0-\text{last}}$ (ng*h/ml)	6	33,950	9989	22,400	31,950	49,400	32,770	30
$AUC_{0-\text{inf}}$ (ng*h/ml)	5	40,140	9626	29,100	37,100	53,200	39,230	24
$t_{1/2}$ (h)	5	188.2	29.30	140	193.0	219	186.2	17
CL/F (L/h)	5	0.2608	0.06142	0.188	0.2700	0.344	0.2550	24
V_z/F (L)	5	71.88	22.52	38.0	78.40	95.6	68.50	38

Notes: Lambda Z acceptance criteria were as follows: $r_{\text{sq_adjusted}} \geq 0.90$, span ≥ 2.0 half-lives, and at least two timepoints after T_{\max} .

Points used in the NCA were manually selected and include T_{\max} for some participants.

Abbreviations: $AUC_{0-\text{inf}}$, area under the concentration-time curve to infinity; $AUC_{0-\text{last}}$, area under the concentration-time curve from the time of dosing to the last measurable concentration; CL/F, apparent clearance; C_{\max} , maximum plasma concentration; CV%, percent coefficient of variation; PK, pharmacokinetic; $t_{1/2}$, terminal half-life; T_{\max} , time to maximum plasma concentration; V_z/F , apparent volume of distribution.

needed to be assessed clinically. Assessment of unsolicited AEs from this study suggests that rezafungin was safe in terms of the lack of SAEs and withdrawals due to AEs. There were no ECG, laboratory, or subjective unsolicited complaints.

PK results from this study demonstrated a slow rezafungin absorption rate, with peak rezafungin plasma concentration observed around 7 days postdose. Absorption appeared extensive following s.c. dose administration. The PK profile of rezafungin demonstrated a long exposure

period (nearly 1 month). After loosening the terminal phase elimination constant (λ_z) acceptance criteria from the analysis plan prespecified requirements for a span greater than or equal to three half-lives and at least three timepoints after T_{\max} to span greater than or equal to two half-lives and at least two timepoints after T_{\max} in addition to hand selecting the sample timepoints included in the calculation to include the T_{\max} sample, terminal phase PK parameters ($AUC_{0-\text{inf}}$, $t_{1/2}$, CL/F, and apparent V_z/F) were able to be estimated.

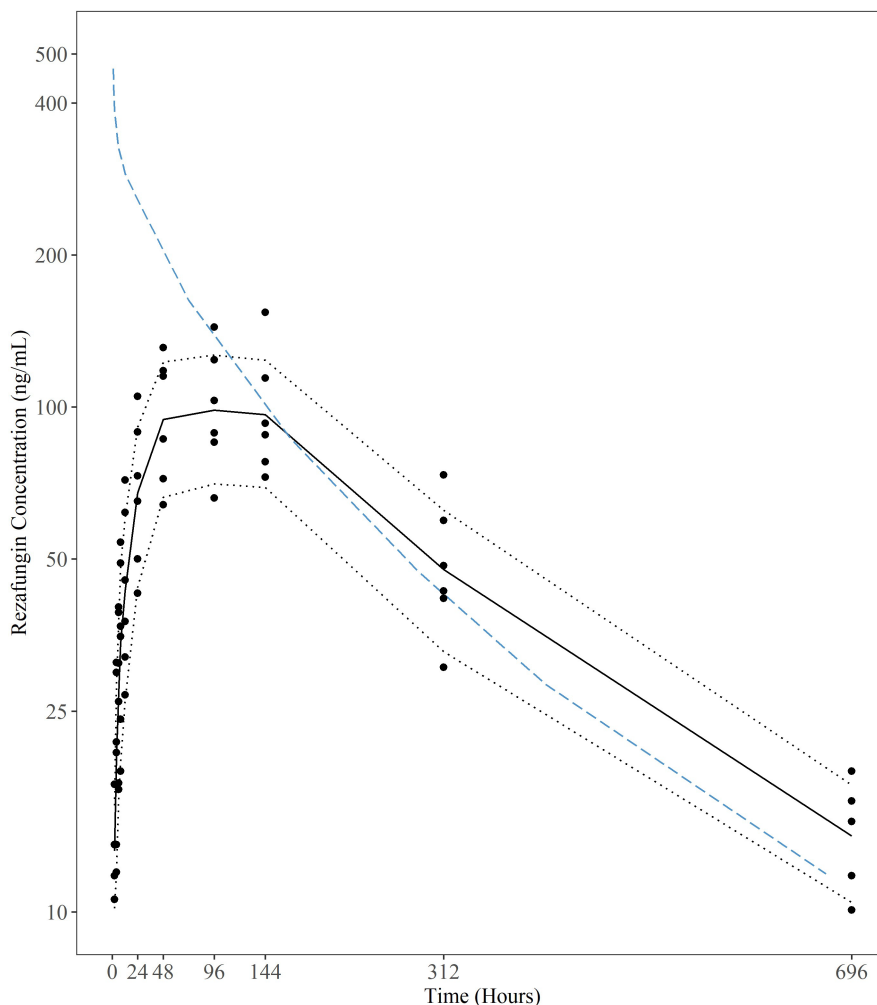


FIGURE 1 Pharmacokinetic results: semi-log geometric mean plasma concentration-time profile. The semi-log mean plasma concentration-time profile was demonstrated by the geometric mean and percent coefficient of variation of plasma concentrations over time of six subjects that received 10 mg of rezafungin (cohort 2) subcutaneously. Plasma for pharmacokinetic analysis was collected from subjects at predose and several timepoints through 696 h (29 days). The rezafungin concentrations were determined by a validated liquid-chromatography tandem mass spectrometry (lower limit of quantitation of 10 ng/ml). The i.v. concentration-time curve of dose adjusted i.v. results (400 mg i.v. divided by 40 mg) are presented for comparison with the s.c. results obtained from this study. —, SC GM; ·····, SC CV%; - - -, IV GM*. *Copyright © 2022 Ong et al.⁶ This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license

Echinocandins, which have been in clinical use globally for over 20 years, are only available via i.v. injection.⁵ No clinical studies have been conducted previously with s.c. rezafungin. Phase I and II clinical trials of i.v. rezafungin have been completed and provide insight into the potential for efficacy and systemic safety of s.c. rezafungin.⁶ In a human clinical study,⁴ the plasma PKs of rezafungin was well-characterized following single i.v. doses of 50, 100, 200, and 400 mg. T_{max} was observed at the end of infusion at ~1 h after the start of rezafungin infusion for all doses. Total body clearance was slow (~0.2 L/h) throughout the dose levels with a long apparent $t_{1/2}$ ($t_{1/2}$ ranged from 127–146 h). The rezafungin T_{max} following s.c. injection occurred ~6 days later. Rezafungin elimination following s.c. injection appeared

quite long, similar to that of the i.v. injection with a long half-life⁷ as shown in Figure 1. After approximately a month of 10 mg rezafungin s.c. administration, the rezafungin concentrations were still detected above 10 ng/ml in human systemic circulation. By comparing $AUC_{(0-last)}$ results between s.c. and i.v. administration routes, the absolute bioavailability of the s.c. formulation relative to previously reported clinical i.v. results could be estimated at ~65%.⁷

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CONFLICT OF INTEREST

Shawn Flanagan and Taylor Sandison are employees and stockholders of Cidara Therapeutics, Inc. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

K.G., S.F., T.S., M.T., and S.J. wrote the manuscript. K.G., D.R., S.F., and T.S. designed the research. D.R. and C.K. performed the research. M.T., S.J., and K.G. analyzed the data.

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