ARTICLE

A Randomized Phase I Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Recombinant *Erwinia* Asparaginase (JZP-458) in Healthy Adult Volunteers

Tong Lin[1,*], Martha Hernandez-Illas[2], Andres Rey[2], Jack Jenkins[1], Reddy Chandula[1], Jeffrey A. Silverman[1] and Mi Rim Choi[1]

L-asparaginase has been an important component of acute lymphoblastic leukemia (ALL) therapy for over 40 years, and is standard therapy during ALL induction and consolidation treatment. L-asparaginases are immunogenic and can induce hypersensitivity reactions; inability to receive asparaginase has been associated with poor patient outcomes. There are L-asparaginases of varied bacterial origins, with the most commonly used being Escherichia coli (*E. coli*); therefore, to ensure that patients who develop hypersensitivity to *E. coli*-derived asparaginases receive an adequate therapeutic course, alternative preparations are warranted. JZP-458 is a recombinant *Erwinia* asparaginase produced using a novel *Pseudomonas fluorescens* expression platform that yields an enzyme with no immunologic cross-reactivity to *E. coli*-derived asparaginases. To evaluate the safety, tolerability, and pharmacokinetics (PK) of a single dose of JZP-458, a randomized, single-center, open-label, phase I study was conducted with JZP-458 given via i.m. injection or i.v. infusion to healthy adult volunteers. At the highest doses tested for each route of administration (i.e., 25 mg/m^2 i.m. and 37.5 mg/m^2 i.v.), JZP-458 achieved serum asparaginase activity (SAA) levels $\geq$ 0.1 IU/mL at 72 hours postdose for 100% of volunteers. Bioavailability for i.m. JZP-458 was estimated at 36.8% based on SAA data. All dose levels were well-tolerated, with no unanticipated adverse events (AEs), no serious AEs, and no grade 3 or higher AEs. Based on PK and safety data, the recommended JZP-458 starting dose for the pivotal phase II/III study in adult and pediatric patients is 25 mg/m^2 i.m. and 37.5 mg/m^2 i.v. on a Monday/Wednesday/Friday dosing schedule.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Inability to receive asparaginase secondary to hypersensitivity has been associated with poor patient outcomes, thus alternative asparaginase preparations are needed.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This study evaluated safety, tolerability, and pharmacokinetics of a single dose of i.m. or i.v. JZP-458, a recombinant *Erwinia* asparaginase with no immunologic cross-reactivity to Escherichia coli (*E. coli*)-derived asparaginases in healthy adult volunteers.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ At the highest doses tested (i.e., 25 mg/m^2 for i.m. and 37.5 mg/m^2 for i.v.), JZP-458 achieved serum asparaginase

activity levels $\geq$ 0.1 IU/mL at 72 hours postdose in each route for 100% of the healthy volunteers with complete asparagine depletion and no unanticipated adverse events (AEs), serious AEs, or grade $\geq$ 3 AEs. The recommended pivotal phase II/III JZP-458 starting dose for patients with acute lymphoblastic leukemia (ALL)/lymphoblastic lymphoma (LBL) who develop hypersensitivity to *E. coli*-derived asparaginases is 25 mg/m^2 i.m. and 37.5 mg/m^2 i.v. on a Monday/Wednesday/Friday dosing schedule.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ JZP-458 may become a treatment alternative for patients with ALL/LBL who develop hypersensitivity to *E. coli*-derived asparaginases.

Acute lymphoblastic leukemia (ALL) is the most common cancer among children and the most frequent cause of death from cancer before 20 years of age. In the past several decades, a substantial improvement in the survival of patients with ALL was achieved as a result of multi-agent chemotherapeutic regimens.[1] L-asparaginase has been

an important component of ALL therapy for over 40 years, and is standard therapy during ALL induction and consolidation in all pediatric regimens and most adult protocols.[2,3] L-asparaginase hydrolyzes the amino acid asparagine to aspartic acid and ammonia. Leukemic blast cells express only limited amounts of asparagine synthetase and are

[1]Jazz Pharmaceuticals, Palo Alto, California, USA; [2]QPS Miami Research Associates (Miami Clinical Research), Miami, Florida, USA. *Correspondence: Tong Lin (Tong.Lin@jazzpharma.com)

dependent on the availability of extracellular asparagine for growth. These cells may be selectively killed when L-asparaginase depletes the circulating endogenous asparagine pool.[2,3] The dependence of leukemic cells on exogenous asparagine supplies the rationale for asparaginase treatment.

The pharmacodynamic (PD) goal of asparaginase therapy is asparagine depletion. Asparagine levels are difficult to measure accurately when asparaginase is present in blood because the enzyme can continue to break down asparagine *ex vivo* if the sample is not immediately processed and stored on ice. Therefore, monitoring of serum asparaginase levels is more reliable than measurement of asparagine itself. In clinical practice, serum asparaginase activity (SAA) levels serve as a surrogate marker for asparagine depletion. Although the level of asparaginase activity required for complete asparagine depletion still remains under debate, nadir SAA levels ≥ 0.1 IU/mL have been used in various studies and treatment protocols and are the accepted threshold for demonstrating adequate asparagine depletion.[4,5]

Due to the short half-life, the administration schedule of some L-asparaginases is an important variable requiring dosing every 48–72 hours, a schedule that in clinical practice translates to a dosing schedule of Monday/Wednesday/Friday for 2 weeks, for a total of 6 doses. Clinical practice guidelines also recommend checking SAA levels after dosing to make any necessary adjustments to maintain nadir SAA levels ≥ 0.1 IU/mL. If the 48- or 72-hour postdose level is below the lower limit of quantification (LLOQ), this may indicate a need for higher or more frequent dosing. The route of administration of L-asparaginases is also an important component; in clinical practice, both the i.m. and i.v. routes are used routinely, depending on the treating oncologist's preference and/or institutional guidelines.[4]

L-asparaginases are immunogenic and can induce hypersensitivity reactions with high titers of neutralizing antibodies that may limit their therapeutic effect.[3,6] Previous studies reported hypersensitivity reactions in up to 30% of patients treated with asparaginases, leading to early discontinuation of asparaginase treatment in some of those patients.[7–9] Unfortunately, the inability to receive asparaginase due to hypersensitivity reactions is associated with poor patient outcomes.[10,11] High-risk and slow early responding standard-risk patients with ALL who do not complete their prescribed asparaginase course have a significantly inferior event-free survival (EFS) compared with patients who complete their prescribed course.[10,11] Additionally, some patients may develop antibodies to asparaginases that neutralize the asparaginase without leading to clinical hypersensitivity; this is known as silent inactivation.[3]

Alternative asparaginase preparations are needed to ensure that patients who develop hypersensitivity to Escherichia coli (*E. coli*)-derived asparaginases are able to complete their full treatment course. Asparaginase *Erwinia chrysanthemi* (ERW; crisantaspase) is an effective treatment option for patients with ALL who have developed hypersensitivity to *E. coli*-derived asparaginase.[3,12] However, since 2016, there has been a worldwide shortage of ERW due to ongoing manufacturing issues, which have resulted in disruptions in the ability to make the product available on a consistent basis.[13] Both JZP-458 and ERW are forms of *Erwinia* asparaginase or crisantaspase. JZP-458 is a recombinant *Erwinia* asparaginase derived from a novel *Pseudomonas fluorescens* expression platform. The primary amino acid sequence of JZP-458 is the same as *Erwinia* asparaginase, and the activity is comparable based on a broad range of *in vitro* measurements (Jazz Pharmaceuticals data on file). Therefore, similar to *Erwinia* asparaginase, JZP-458 is also expected to have no immunologic cross-reactivity to *E. coli*-derived asparaginases.[14] JZP-458 is being developed as a component of a multi-agent chemotherapeutic regimen to treat patients with ALL or lymphoblastic lymphoma (LBL) who develop hypersensitivity to *E. coli*-derived asparaginases.

JZP-458 was evaluated in a randomized, single-center, open-label phase I study. The study was designed to evaluate the safety, tolerability, and pharmacokinetics (PK) of a single dose of JZP-458 in healthy adult volunteers following either an i.m. injection or a 2-hour i.v. infusion. Data from this study will facilitate the selection of an appropriate starting dose and dosing regimen of JZP-458 for use in a pivotal phase II/III study in adult and pediatric patients with ALL or LBL who develop hypersensitivity to *E. coli*-derived asparaginases.

METHODS

This phase I, randomized, single-center, open-label study was conducted in the United States between November 19, 2018, and May 20, 2019. This study was approved by the IntegReview Institutional Review Board in Austin, Texas, and conducted at QPS Miami Research Associates (Miami Clinical Research) in Miami, Florida, in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All healthy volunteers provided written informed consent prior to enrollment.

Eligibility criteria
Eligible volunteers were men and nonpregnant, nonlactating women between the ages of 18 and 55 years with a normal body mass index (i.e., 19.0–30.0 kg/m^2) who were in good general health as determined by the investigator at screening and day −1 and were able to understand and comply with study-specific requirements. Main exclusions from the study included the history or presence of any illness, physical finding, laboratory examination, or electrocardiogram finding that, in the opinion of Jazz Pharmaceuticals and/or the investigator, might confound the results or conduct of the study or pose a risk to the healthy volunteer. This included any condition that might interfere with the distribution, metabolism, or excretion of drugs.

Study design
This study screened healthy adult volunteers for eligibility between 2 and 28 days prior to dosing (**Figure S1**). Eligible healthy volunteers checked in at the study center on day −1 for baseline assessments, then were admitted to the inpatient clinic and received a single dose of the study drug

872

on day 1. All volunteers remained in the inpatient clinic for PK and safety assessments until they were discharged on day 5. Safety follow-up telephone calls regarding adverse events (AEs) occurred on days 6 and 30.

This was an open-label study with a total enrollment of 30 healthy adult volunteers. There were 3 cohorts in the study: JZP-458 dose cohort 1, JZP-458 dose cohort 2, and ERW dose cohort. The study used an adaptive design for JZP-458, where the starting dose for cohort 1 was 25 mg/m^2, and the dose selection for cohort 2 was based on safety, tolerability, and PK data from cohort 1. An ERW dose cohort was also included in the study. Based on the enzymatic activity assay developed by Jazz Pharmaceuticals for JZP-458 that was used in an analytical comparability assessment, the starting dose of 25 mg/m^2 was expected to provide similar asparaginase activity to the approved dose of ERW at 25,000 IU/m^2, which was also administered in this study.[12] A sentinel dosing approach was followed for JZP-458 for the first 2 volunteers dosed in the study, who were randomized to the JZP-458 dose cohort 1 only. These 2 volunteers were randomized to either i.m. or i.v. JZP-458, 1 to each route of administration. One week separated the sentinel dosing volunteers from the dosing of the remaining volunteers in the initial cohorts (JZP-458 dose cohort 1 and ERW dose cohort), which was permitted by the protocol, as the safety and tolerability for the first 2 volunteers were deemed acceptable by the investigator and sponsor (no study-drug related AE $\geq$ grade 3).

Once the safety and tolerability were considered acceptable, the next 16 volunteers were randomized to JZP-458 dose cohort 1 and ERW dose cohort with 10 volunteers randomized to the JZP-458 dose cohort 1 and 6 volunteers to the ERW dose cohort. Within the JZP-458 dose cohort 1 and ERW dose cohort, the volunteers were randomized to i.m. or i.v. treatment groups in a 1:1 ratio. This randomization schema is equivalent to randomizing all 18 volunteers to i.m. JZP-458, i.v. JZP-458, i.m. ERW, or i.v. ERW in a 2:2:1:1 ratio, while ensuring that the first 2 randomized volunteers received i.m. JZP-458 and i.v. JZP-458 following the sentinel dosing approach. The volunteers were administered either a single i.m. injection or a single i.v. infusion over 2 hours.

The safety, tolerability, and PK data for all volunteers in the JZP-458 dose cohort 1 were evaluated by the investigator and sponsor, and it was determined to enroll additional volunteers in a second cohort. For JZP-458 dose cohort 2, 12 additional volunteers were randomized to i.m. or i.v. treatment groups in a 1:1 ratio; the dose levels were selected based on the JZP-458 cohort 1 safety, tolerability, and PK data.

Objectives

The primary objective was to assess the safety and tolerability of a single dose of JZP-458 (i.m. or i.v.) in healthy adult volunteers, assessed by the occurrence of treatment-emergent AEs and clinically significant changes in vital signs and laboratory tests. The secondary objective was to characterize the PK of a single dose of JZP-458 (i.m. or i.v.) in healthy adult volunteers, based on SAA data. Additional assessments included serum asparaginase concentration

(SAC) determinations for JZP-458, and the measurement of L-asparagine and L-glutamine levels to assess the PD effect of JZP-458 in healthy adults.

Pharmacokinetic/pharmacodynamic sample collection and bioanalytical method

Serial blood samples for PK/PD evaluation were collected from all healthy volunteers at prespecified timepoints up to 96 hours postdose. For i.m. dosing, samples were taken predose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72, and 96 hours after dosing. For i.v. dosing, samples were taken predose and at 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24, 36, 48, 72, and 96 hours after the start of the 2-hour infusion. Blood samples for PK analysis were collected into labeled 4 mL serum separator tubes and allowed to clot. Samples were centrifuged and supernatant serum was stored at −80°C. Blood samples for PD analysis were collected into 4 mL lithium heparin tubes. Samples were immediately centrifuged and supernatant plasma was stored at −80°C within 30 minutes.

The bioanalytical analysis for PK samples was performed by Charles River Laboratories (Skokie, IL). PK samples were assayed for SAA levels using a validated enzyme activity method in human serum over the range of 0.025 IU/mL to 0.15 IU/mL. In addition, PK samples were also assayed for SAC using a validated electrochemiluminescence immunoassay method in human serum over the range of 1.0 ng/mL to 128 ng/mL. PD samples were assayed for L-asparagine and L-glutamine concentrations by Syneos Health (Princeton, NJ), using a validated liquid chromatography tandem mass spectrometry method over the range of 0.025 µg/mL to 10.0 µg/mL for L-asparagine and 0.250 to 100 µg/mL for L-glutamine.

Pharmacokinetic and statistical analyses

PK of JZP-458 were primarily evaluated based on SAA data. The following PK parameters were evaluated using noncompartmental analysis in Phoenix WinNonlin version 6.3: C_{max} = maximum SAA; C_{48h} = SAA value at 48 hours; C_{72h} = SAA value at 72 hours; T_{max} = time to reach C_{max}; AUC_{0-t} = area under the SAA-time curve from time zero to time of last quantifiable SAA; AUC_{0-inf} = area under the SAA-time curve from time zero to infinity; CL = clearance; CL/F = apparent clearance; V_{ss} = estimate of the volume of distribution at steady state following i.v. dosing; V_z/F = apparent volume of distribution following i.m. dosing; $t_{1/2}$ = terminal elimination half-life; and F = bioavailability for the i.m. route, calculated as the arithmetic mean dose normalized AUC_{inf}(i.m.)/AUC_{inf}(i.v.) × 100.

Descriptive statistics (n, mean, SD, median, minimum, and maximum) were used to summarize continuous data, whereas counts and percentages were used to summarize categorical data. *Post hoc* analyses were performed to assess the relationship between SAA and SAC. Correlation and linear regression analyses were performed by study drug and across routes of administration, as well as by route of administration. In the linear regression modeling, SAC was the dependent variable and SAA was the independent variable. Last, no formal hypothesis testing was performed.

RESULTS

Baseline demographics

In total, 30 healthy adult volunteers were enrolled and randomized in the study. Of the 30 volunteers enrolled, all 30 completed the study, including the final scheduled safety follow-up telephone call on day 30. The overall baseline demographics included a mean ± SD age of 38.4 ± 8.30 years, weight of 77.04 ± 9.998 kg, and body surface area of 1.91 ± 0.150 m^2 (**Table S1**). In addition, 63% were men, 97% were Hispanic or Latino ethnicity, 83% were White, and 17% were Black/African American.

Pharmacokinetics analysis

The PK analysis set consisted of all 30 healthy volunteers (100%) enrolled in the study.
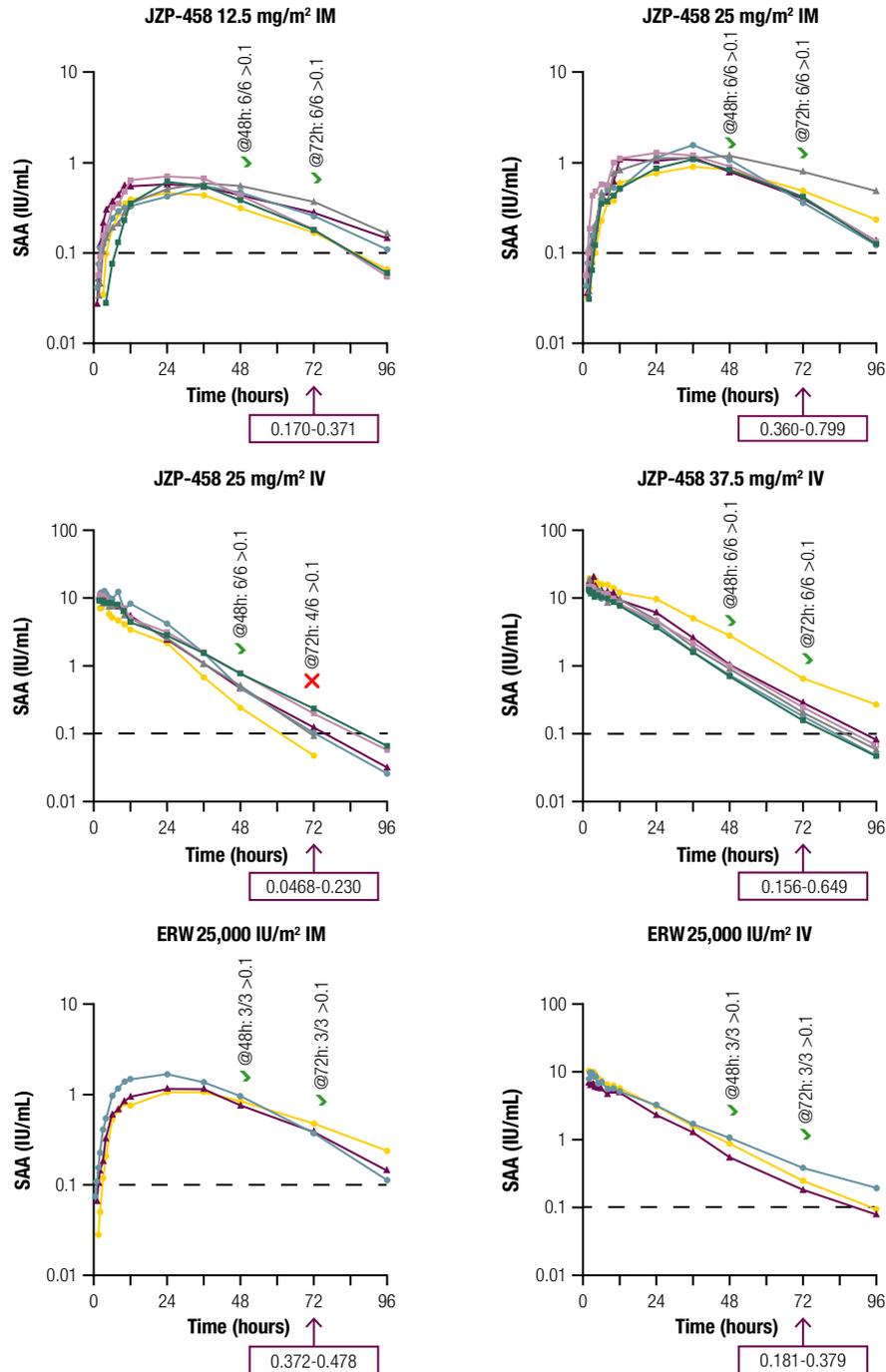
Figure 1 Individual SAA-time profiles. ERW, asparaginase *Erwinia chrysanthemi*; SAA, serum asparaginase activity. *Note*: Different colored lines represent individual healthy volunteers (*N* = 6 for each dosing cohort). Numbers in purple boxes represent SAA ranges at 72 hours postdose.

Table 1 Proportion of healthy volunteers with SAA levels at 48 and 72 hours postdose

Category	SAA level	JZP-458 12.5 mg/m^2 i.m. (N = 6)	JZP-458 25 mg/m^2 i.m. (N = 6)	JZP-458 25 mg/m^2 i.v. (N = 6)	JZP-458 37.5 mg/m^2 i.v. (N = 6)	ERW 25,000 IU/m^2 i.m. (N = 3)	ERW 25,000 IU/m^2 i.v. (N = 3)
≥ 0.1 IU/mL, *n* (%)	SAA at 48 hours	6 (100)	6 (100)	6 (100)	6 (100)	3 (100)	3 (100)
	SAA at 72 hours	6 (100)	6 (100)	4 (67)	6 (100)	3 (100)	3 (100)

ERW, asparaginase *Erwinia chrysanthemi*; SAA, serum asparaginase activity.

Serum asparaginase activity data. In this study, the starting dose for JZP-458 dose cohort 1 was 25 mg/m^2 for both i.m. and i.v. administrations. Individual SAA-time profiles were generated for all treatment groups (**Figure 1**). Predose SAA values were below the limit of quantitation for all volunteers and all treatments. The number and proportion of volunteers with SAA levels ≥ 0.1 IU/mL at 48 and 72 hours postdose are presented in **Table 1**.

The dose level of cohort 2 was determined based on the safety, tolerability, and PK SAA data from cohort 1. There were no unanticipated AEs, no reported serious AEs, and no grade 3 or higher AEs observed in cohort 1. The PK SAA data for JZP-458 in cohort 1 at 25 mg/m^2 is shown in **Figure 1**. For the i.m. dose of 25 mg/m^2, SAA values at 72 hours postdose were ≥ 0.1 IU/mL in 6 of 6 (100%) healthy volunteers. This suggested that an i.m. dose of 25 mg/m^2 is expected to maintain SAA levels ≥ 0.1 IU/mL throughout the treatment duration on a Monday/Wednesday/Friday dosing schedule in the pivotal phase II/III study. Therefore, in cohort 2, the i.m. dose level was decreased by 50% to 12.5 mg/m^2 to study the dose proportionality and safety profile at this dose. Alternatively, for an i.v. dose of 25 mg/m^2, SAA values at 72 hours postdose were ≥ 0.1 IU/mL in only 4 of 6 (67%) healthy volunteers. This suggested that the i.v. dose of 25 mg/m^2 was inadequate for maintaining SAA levels ≥ 0.1 IU/mL for 72 hours. Therefore, in cohort 2, the i.v. dose level was increased by 50% to 37.5 mg/m^2.

Following i.m. administration of JZP-458, SAA levels achieved ≥ 0.1 IU/mL in 6 of 6 (100%) healthy volunteers at 48 and 72 hours postdose for both the 12.5 mg/m^2 and 25 mg/m^2 dose levels. After i.v. administration of JZP-458, SAA levels achieved ≥ 0.1 IU/mL in 6 of 6 (100%) healthy volunteers at 48 hours and in 4 of 6 (67%) healthy volunteers at 72 hours postdose at the dose level of 25 mg/m^2, whereas SAA levels achieved ≥ 0.1 IU/mL in 6 of 6 (100%) healthy volunteers at both 48 and 72 hours postdose at the dose level of 37.5 mg/m^2. Data suggested that at the same dose level, i.m. route of administration was able to maintain higher levels of SAA when compared with i.v. administration. SAA data for healthy volunteers who received ERW are also presented in **Figure 1** and **Table 1**.

Mean and 95% confidence interval (CI) curves for SAA were also generated for JZP-458 for i.m. administration at 25 mg/m^2 and i.v. administration at 37.5 mg/m^2 based on observed data (*N* = 6 each; **Figure 2**). Data indicated that the lower bound of 95% CI achieved ≥ 0.1 IU/mL at 72 hours postdose for both i.m. administration at 25 mg/m^2 and i.v. administration at 37.5 mg/m^2 for JZP-458 (lower bound of 95% CI for i.m. and i.v. were 0.31107 IU/mL and 0.09476 IU/mL, respectively). These data facilitated dose recommendations for the pivotal phase II/III study.

PK parameters based on SAA were summarized for all treatment groups (**Table 2**). When administered i.m., JZP-458 was slowly absorbed based on SAA, with median T_{max} of 24 hours and 36 hours following administration of 12.5 mg/m^2 and 25 mg/m^2 doses, respectively. Mean $t_{1/2}$ values of 23.4 hours and 19.1 hours were estimated following administration of 12.5 mg/m^2 and 25 mg/m^2, respectively. When administered i.v., JZP-458 SAA levels declined with mean $t_{1/2}$ of 11.5 hours and 12.6 hours following administration of 25 mg/m^2 and 37.5 mg/m^2 doses, respectively. As expected, the $t_{1/2}$ of JZP-458 after i.m. administration was longer than i.v. infusion due to absorption rate-limited elimination kinetics. Furthermore, JZP-458 volume of distribution was approximately the same as the plasma volume following both i.m. and i.v. administrations, suggesting that JZP-458 was mostly confined to the central vascular compartment.

Dose proportionality assessment based on SAA showed that JZP-458 exposures increased with increasing doses based on SAA (**Table S2**). For both i.m. and i.v. administration, the increases in JZP-458 SAA exposures (C_{max} and AUC) were approximately dose-proportional for the dose ranges studied. The bioavailability for JZP-458 for the i.m. route of administration was also calculated and estimated at 36.8% for JZP-458 based on SAA data.

Serum asparaginase concentration data. Historically, asparaginase PK has been evaluated based on the SAA data. However, SAA is not considered a true measurement of the drug levels; therefore, an enzyme content assay was developed to measure JZP-458 drug levels (i.e., asparaginase concentrations) in human serum.

Mean SAC-time profiles were generated for all treatment groups (**Figure 3a,b**), and PK parameters based on SAC are summarized in **Table S3**. When administered i.m., JZP-458 was slowly absorbed based on SAC, with median T_{max} values of 30 hours for both 12.5 mg/m^2 and 25 mg/m^2 doses. Mean $t_{1/2}$ values of 28.9 hours and 25.4 hours were estimated for JZP-458 at 12.5 mg/m^2 and 25 mg/m^2, respectively. Following i.v. administration of JZP-458, SAC levels declined with mean $t_{1/2}$ of 12.0 hours and 12.7 hours following administration of 25 mg/m^2 and 37.5 mg/m^2 doses, respectively.

Dose proportionality and bioavailability were also assessed for JZP-458 based on SAC (**Table S4**). JZP-458 exposures increased with increasing dose based on SAC. For both i.m. and i.v. administration, the increases in JZP-458 exposures based on SAC (C_{max} and AUC) were approximately dose-proportional for the dose ranges studied. For the i.m. route of administration, bioavailability was estimated at 43.9% for JZP-458 based on SAC data.

The relationship between SAA and SAC was further explored for JZP-458 (**Figure 3c**). When assessed across routes
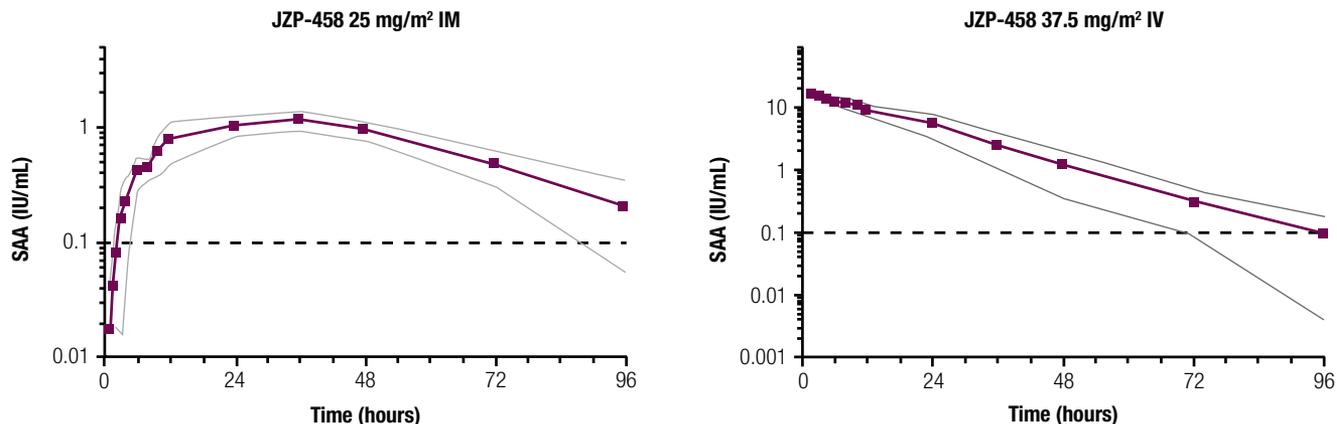
Figure 2 Mean (95% CI) SAA-time profiles. CI, confidence interval; SAA, serum asparaginase activity. *Note*: Gray lines represent 95% CIs (*N* = 6 for each dosing cohort).

of administration for JZP-458, a strong positive association was observed between SAA and SAC with a correlation coefficient greater than 0.95. Additionally, the equation from the linear regression model was SAC = 1407.9 × SAA. These data suggest that when SAA levels are at 0.1 IU/mL, the corresponding SAC would be ~ 141 ng/mL in this healthy adult population.

Pharmacodynamic data
Asparaginase hydrolyzes the amino acid asparagine into aspartic acid and ammonia. Plasma levels of asparagine were monitored throughout the treatment duration. Mean SAA vs. mean plasma asparagine concentration over time profiles are provided in **Figure 4**. Baseline (predose) mean plasma asparagine concentrations were similar for i.m. and i.v. groups; individual asparagine concentrations ranged from 5.09 µg/mL to 13.8 µg/mL for all volunteers, which is consistent with literature-reported values.[15] After JZP-458 administration (i.m. and i.v.), mean plasma asparagine

levels were rapidly depleted from the predose concentrations (cohort 1: 8.62 µg/mL and 8.96 µg/mL for i.m. and i.v., respectively; cohort 2: 6.42 µg/mL and 5.89 µg/mL for i.m. and i.v., respectively) to levels below the assay LLOQ (0.025 µg/mL) for both routes, and remained undetectable through the final sample collection time point at 96 hours. Data indicated that there was direct correlation between SAA and the reduction in plasma asparagine levels. At all JZP-458 dose levels, plasma asparagine levels were completely depleted with JZP-458 administration. At the highest JZP-458 doses tested (i.e., 25 mg/m^2 for i.m. and 37.5 mg/m^2 for i.v.) in this phase I healthy volunteer study, JZP-458 achieved SAA levels ≥ 0.1 IU/mL at 72 hours postdose for 100% of the healthy volunteers for each route, and resulted in a complete depletion of plasma asparagine levels through 96 hours postdose, the last time evaluated.

In addition to asparagine, asparaginase is also capable of hydrolyzing glutamine to glutamic acid and ammonia, but with much less efficiency. Due to this, glutamine plasma levels

Table 2 PK summary based on SAA

Treatment, mean (CV%)	C$_{max}$ (IU/mL)	C$_{48h}$ (IU/mL)	C$_{72h}$ (IU/mL)	T$_{max}$ (h)	t$_{1/2}$ (h)	AUC$_{0-t}$ (IU•h/mL)	AUC$_{0-inf}$ (IU•h/mL)	CL[a] (L/h)	V[a] (L)
JZP-458 12.5 mg/m^2 i.m. (*N* = 6)	0.6 (13.2)	0.4 (18.7)	0.2 (32.9)	24.0 (24.0–36.0)	23.4 (23.6)	33.3 (15.1)	36.9 (18.4)	0.4 (27.3)	14.1 (25.1)
JZP-458 25 mg/m^2 i.m. (*N* = 6)[b]	1.2 (18.8)	0.9 (17.3)	0.5 (33.6)	36.0 (24.0–48.0)	19.1 (21.8)	66.3 (15.6)	67.4 (9.0)	0.4 (11.1)	11.7 (20.9)
JZP-458 25 mg/m^2 i.v. (*N* = 6)	10.9 (10.2)	0.5 (37.7)	0.1 (52.3)	2.3 (2.0–3.5)	11.5 (12.8)	181 (20.5)	182 (20.4)	0.2 (25.7)	2.7 (22.4)
JZP-458 37.5 mg/m^2 i.v. (*N* = 6)	16.8 (18.1)	1.2 (66.3)	0.3 (63.8)	2.3 (2.0–3.5)	12.6 (11.2)	315 (29.1)	317 (29.5)	0.1 (25.0)	2.5 (16.6)
ERW 25,000 IU/m^2 i.m. (*N* = 3)	1.3 (25.7)	0.9 (11.6)	0.4 (14.3)	24.0 (24.0–36.0)	20.6 (26.1)	70.5 (15.3)	75.8 (11.7)	0.4 (20.8)	13.0 (32.2)
ERW 25,000 IU/m^2 i.v. (*N* = 3)	9.0 (21.0)	0.8 (31.1)	0.3 (37.6)	2.0 (2.0–2.5)	14.9 (12.0)	180 (14.6)	183 (15.1)	0.2 (13.9)	3.5 (8.1)

N is the number of healthy volunteers exposed. Mean (CV%) presented for all parameters except for T$_{max}$ values, which are reported as median and range. AUC$_{0-inf}$, area under the curve from time 0 extrapolated to infinity; AUC$_{0-t}$, area under the curve from time 0 to the time of last quantifiable SAA; C$_{48h}$, SAA at 48 hours; C$_{72h}$, SAA at 72 hours; CL, clearance; C$_{max}$, maximum SAA; CV, coefficient of variation; ERW, asparaginase *Erwinia chrysanthemi*; PK, pharmacokinetics; SAA, serum asparaginase activity; T$_{max}$, time at which C$_{max}$ is observed; t$_{1/2}$, terminal elimination half-life; V, volume of distribution.
[a]For i.m. treatments, CL = CL/F (apparent clearance) and V = Vz/F (apparent volume of distribution). For i.v. treatments, CL = CL and V = Vss (estimate of the volume of distribution at steady state).
[b]*n* = 5 for t$_{1/2}$, AUC$_{0-inf}$, CL, and V.

(a) IM

(b) IV

JZP-458 12.5 mg/m² IM
JZP-458 25 mg/m² IM
ERW 25,000 IU/m² IM

JZP-458 25 mg/m² IV
JZP-458 37.5 mg/m² IV
ERW 25,000 IU/m² IV

(c)
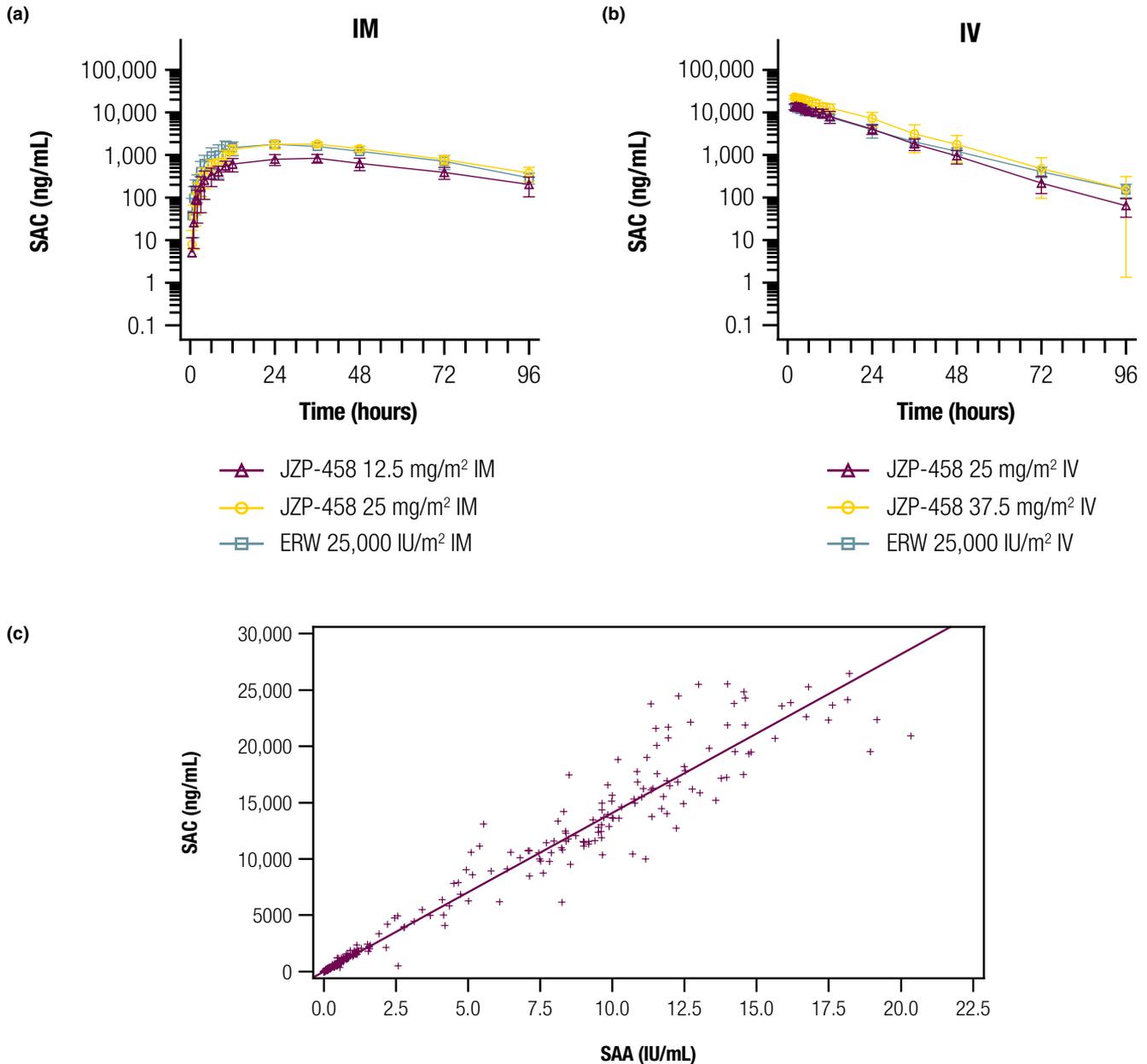
Figure 3 Mean SAC-time profiles and the correlation between SAC and SAA for JZP-458. (**a**) Mean SAC-time profiles for JZP-458 i.m. Note: LLOQ = 1.00 ng/mL. Values below the LLOQ were set to zero. (**b**) Mean SAC-time profiles for JZP-458 i.v. Note: LLOQ = 1.00 ng/mL. Values below the LLOQ were set to zero. (**c**) Correlation between SAC and SAA for JZP-458 i.m. and i.v. administration. Note: Regression line equation: SAC = 1407.9 × SAA; Pearson correlation coefficient = 0.9779. ERW, asparaginase *Erwinia chrysanthemi*; LLOQ, lower limit of quantitation; SAA, serum asparaginase activity; SAC, serum asparaginase concentration.

were monitored as well (**Figure S2**). Baseline (predose) mean plasma glutamine concentrations were similar for i.m. and i.v. groups; individual glutamine predose concentrations ranged from 60.4 µg/mL to 146 µg/mL for all volunteers, which is consistent with literature-reported values.[15] Data showed that mean plasma glutamine levels fell quickly following JZP-458 i.v. administration from the predose concentrations of 106.5 and 74.0 µg/mL for cohort 1 (25 mg/m² i.v.) and cohort 2 (37.5 mg/m² i.v.), respectively, to levels below the assay LLOQ (0.25 µg/mL) for ~ 12 hours, after which glutamine levels recover to approximately predose levels at the

final sample collection timepoint at 96 hours postdose. For the i.m. route, mean plasma glutamine levels declined following i.m. administration of JZP-458, with the lowest glutamine level observed at 36 hours postdose with 79% and 47% glutamine depletion at 25 mg/m² and 12.5 mg/m², respectively, after which glutamine levels recovered to levels similar to predose at the last sample collection timepoint of 96 hours postdose. Complete depletion of L-glutamine was not observed; glutamine levels were moderately affected to only partial depletion, and data were more variable than those observed for L-asparagine.
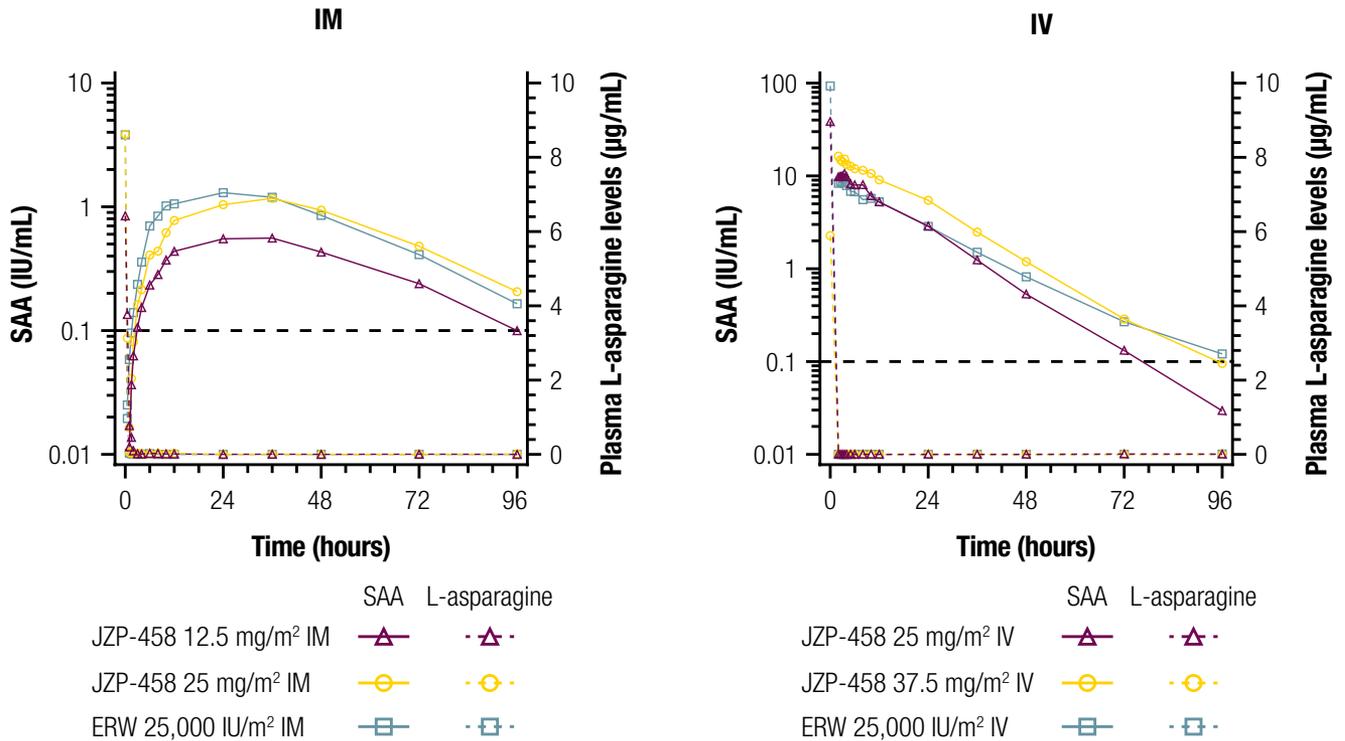
Figure 4 Mean SAA-time profiles and corresponding mean plasma L-asparagine levels. *Note*: LLOQ: asparaginase activity = 0.0250 IU/mL; L-asparagine = 0.0250 µg/mL. Values below the LLOQ were set to zero. ERW, asparaginase *Erwinia chrysanthemi*; LLOQ, lower limit of quantitation; SAA, serum asparaginase activity.

Safety and tolerability

The safety profile observed for JZP-458 in this phase I study was consistent with profiles of other asparaginases.[12,16–19] All dose levels of JZP-458 were well-tolerated; there were no unanticipated AEs, no serious AEs, and no grade 3 or higher AEs. The most common treatment-emergent AE occurring in ≥ 2 healthy volunteers in each dosing cohort was nausea (**Table 3**).

DISCUSSION

JZP-458, a recombinant *Erwinia* asparaginase with no expected immunologic cross-reactivity to *E. coli*-derived asparaginases, is being developed to ensure the availability of asparaginase therapy for patients with ALL or LBL who develop hypersensitivity to *E. coli*-derived asparaginases. In this randomized, single-center, open-label, phase I study, at the highest doses tested for each route of administration (i.e., 25 mg/m² for i.m. and 37.5 mg/m² for i.v.), JZP-458 achieved SAA levels ≥ 0.1 IU/mL at 72 hours postdose for 100% of the healthy adult volunteers in each route. The SAA levels observed in this study also indicated that JZP-458 is capable of complete depletion of plasma asparagine levels, which was confirmed by asparagine concentrations measured from this study. At all JZP-458 dose levels, plasma asparagine levels were completely depleted with JZP-458 treatment with both i.m. and i.v. routes of administration. Additionally, the safety profile for JZP-458 in this study was consistent with the profiles of other asparaginases.[12,16–19] All dose levels of JZP-458

were well-tolerated; there were no unanticipated AEs, no reported serious AEs, and no grade 3 or higher AEs.

Based on the cumulative PK and safety data, the recommended pivotal phase II/III JZP-458 starting dose is 25 mg/m² for the i.m. route of administration and 37.5 mg/m² for the i.v. route of administration on a Monday/Wednesday/Friday dosing schedule. These doses achieved SAA levels ≥ 0.1 IU/mL at 72 hours postdose for 6 of 6 (100%) healthy volunteers in this phase I study, and suggested that these doses are expected to maintain SAA levels ≥ 0.1 IU/mL throughout the treatment duration in the pivotal phase II/III study.

Completing asparaginase therapy is important for improved patient outcomes, as shown in previous studies. In the Dana-Farber Cancer Institute ALL Consortium Protocol 91-01 study, patients with asparaginase intolerance, defined as completion of ≤ 25 weeks of a planned total of 30 weeks of asparaginase therapy, had a significantly lower 5-year EFS when compared with patients who received ≥ 26 weeks of asparaginase therapy (73% vs. 90%, respectively; *P* < 0.01).[10] A recent Children's Oncology Group study demonstrated that high-risk and slow early responding standard-risk patients with ALL who did not complete their prescribed asparaginase doses had a significantly inferior EFS compared with patients who received all prescribed asparaginase doses.[11] Notably, patients with hypersensitivity reactions who completed their course of therapy with *Erwinia* asparaginase substitution showed similar EFS as those who completed their course of first-line asparaginase therapy.[11] These studies suggest that patients who complete their prescribed asparaginase doses, whether on

Table 3 Treatment-emergent adverse events

	JZP-458 12.5 mg/m² i.m. (*N* = 6)	JZP-458 25 mg/m² i.m. (*N* = 6)	JZP-458 25 mg/m² i.v. (*N* = 6)	JZP-458 37.5 mg/m² i.v. (*N* = 6)	ERW 25,000 IU/m² i.m. (*N* = 3)	ERW 25,000 IU/m² i.v. (*N* = 3)
TEAEs, *n* (%)						
Any TEAE	5 (83)	4 (67)	4 (67)	6 (100)	2 (67)	3 (100)
Grade 1	5 (83)	4 (67)	4 (67)	6 (100)	2 (67)	3 (100)
Grade 2	0	1 (17)	1 (17)	1 (17)	0	1 (33)
Grade ≥ 3	0	0	0	0	0	0
Serious TEAEs	0	0	0	0	0	0
Treatment-related TEAEs, *n* (%)[a]						
Nausea	2 (33)	4 (67)	3 (50)	6 (100)	2 (67)	3 (100)
Vomiting	1 (17)	2 (33)	2 (33)	2 (33)	0	2 (67)
Dyspepsia	5 (83)	0	0	0	1 (33)	0
Headache	0	1 (17)	0	0	0	1 (33)
Leukopenia	0	1 (17)	1 (17)	0	0	0
Decreased appetite	0	0	0	1 (17)	0	0
Diarrhea	0	0	0	1 (17)	0	0
Soft feces	0	0	1 (17)	0	0	0
Gastroesophageal reflux disease	0	1 (17)	0	0	0	0
Malaise	0	1 (17)	0	0	0	0
Paresthesia	0	0	0	1 (17)	0	0

ERW, asparaginase *Erwinia chrysanthemi*; TEAE, treatment-emergent adverse event.
[a]By preferred term using MedDRA dictionary, version 22.0; treatment-related TEAEs are shown in descending order of frequency.

first-line or second-line asparaginase, have better outcomes than those who discontinue early. These results highlight the need for alternative asparaginase preparations to ensure that patients who develop hypersensitivity to *E. coli*-derived asparaginases are able to complete their full treatment course.

CONCLUSIONS

At the highest doses tested for each route of administration (i.e., 25 mg/m² for i.m. and 37.5 mg/m² for i.v.), JZP-458 achieved SAA levels ≥ 0.1 IU/mL at 72 hours postdose in each route for 100% of the healthy adult volunteers in this phase I study, and resulted in complete asparagine depletion with no unanticipated AEs, serious AEs, or grade ≥ 3 AEs. Based on the cumulative PK and safety data from this study, the recommended phase II/III JZP-458 starting dose is 25 mg/m² for the i.m. route of administration and 37.5 mg/m² for the i.v. route of administration on a Monday/Wednesday/Friday dosing schedule.

Author Contributions. T.L., M.H.-I., A.R., J.J., R.C., J.A.S., and M.R.C. wrote the manuscript. T.L., J.J., R.C., J.A.S., and M.R.C. designed the research. M.H.-I. and A.R. performed the research. T.L., J.J., J.A.S., and M.R.C. analyzed the data.

Data sharing statement. All relevant data are provided within the manuscript and supporting files.

1. Hunger, S.P. & Mullighan, C.G. Acute lymphoblastic leukemia in children. *N. Engl. J. Med.* **373**, 1541–1552 (2015).
2. Pui, C.-H. & Evans, W.E. Treatment of acute lymphoblastic leukemia. *N. Engl. J. Med.* **354**, 166–178 (2006).
3. Pieters, R. *et al.* L-asparaginase treatment in acute lymphoblastic leukemia: a focus on *Erwinia* asparaginase. *Cancer* **117**, 238–249 (2011).
4. van der Sluis, I.M. *et al.* Consensus expert recommendations for identification and management of asparaginase hypersensitivity and silent inactivation. *Haematologica* **101**, 279–285 (2016).
5. Koprivnikar, J., McCloskey, J. & Fadel, S. Safety, efficacy, and clinical utility of asparaginase in the treatment of adult patients with acute lymphoblastic leukemia. *Onco. Targets Ther.* **10**, 1413–1422 (2017).
6. Rau, R.E. *et al.* Outcome of pediatric patients with acute lymphoblastic leukemia/lymphoblastic lymphoma with hypersensitivity to pegaspargase treated with PEGylated Erwinia asparaginase, pegcrisantaspase: a report from the Children's Oncology Group. *Pediatr. Blood Cancer* **65**, e26873 (2018).
7. Vrooman, L.M. *et al.* Erwinia asparaginase after allergy to E. coli asparaginase in children with acute lymphoblastic leukemia. *Pediatr. Blood Cancer* **54**, 199–205 (2010).
8. Egler, R.A., Ahuja, S.P. & Matloub, Y. L-asparaginase in the treatment of patients with acute lymphoblastic leukemia. *J. Pharmacol. Pharmacother.* **7**, 62–71 (2016).

9. Müller, H.J. *et al.* Pharmacokinetics of native Escherichia coli asparaginase (Asparaginase medac) and hypersensitivity reactions in ALL-BFM 95 reinduction treatment. *Br. J. Haematol.* **114**, 794–799 (2011).

10. Silverman, L.B. *et al.* Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. *Blood* **97**, 1211–1218 (2001).

11. Gupta, S. *et al.* Impact of asparaginase discontinuation on outcome in childhood ALL: a report from the Children's Oncology Group (COG). *J. Clin. Oncol.* 38, 1897–1905 (2020).

12. US Food and Drug Administration. ERWINAZE (asparaginase Erwinia chrysanthemi) Label <https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/125359s088lbl.pdf>. Accessed June 2, 2020.

13. US Food and Drug Administration. FDA drug shortages <https://www.accessdata.fda.gov/scripts/drugshortages/default.cfm>. Accessed March 23, 2020.

14. Willer, A. *et al.* Anti-Escherichia coli asparaginase antibody levels determine the activity of second-line treatment with pegylated E coli asparaginase: a retrospective analysis within the ALL-BFM trials. *Blood* **118**, 5774–5782 (2011).

15. Tong, W.H. *et al.* A prospective study on drug monitoring of PEGasparaginase and Erwinia asparaginase and asparaginase antibodies in pediatric acute lymphoblastic leukemia. *Blood* **123**, 2026–2033 (2014).

16. US Food and Drug Administration. Oncaspar® (pegaspargase) Label <https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/103411s5126lbl.pdf>. Accessed October 2, 2020.

17. European Medicines Agency. Spectrila public assessment report 2015 <https://www.ema.europa.eu/en/documents/assessment-report/spectrila-epar-public-assessment-report_en.pdf>. Accessed October 2, 2020.

18. Raetz, E.A. & Salzer, W.L. Tolerability and efficacy of L-asparaginase therapy in pediatric patients with acute lymphoblastic leukemia. *J. Pediatr. Hematol. Oncol.* **32**, 554–563 (2010).

19. Hijiya, N. & van der Sluis, I.M. Asparaginase-associated toxicity in children with acute lymphoblastic leukemia. *Leuk. Lymphoma* **57**, 748–757 (2016).