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Comparison of native *E. coli* and PEG asparaginase pharmacokinetics and pharmacodynamics in pediatric acute lymphoblastic leukemia

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Abstract

Asparaginase is used routinely in frontline clinical trials for the treatment of childhood acute lymphoblastic leukemia (ALL). The goals of this study were to assess the pharmacokinetics and pharmacodynamics of asparaginase and to mathematically model the dynamics between asparaginase and asparagine in relapsed ALL. Forty children were randomized to receive either native or PEGylated (PEG) *Escherichia coli* asparaginase during reinduction therapy. Serial plasma asparaginase and asparagine, cerebrospinal fluid (CSF) asparagine, and serum anti-asparaginase antibody samples were collected. The asparaginase clearance was higher ($P=.001$) for native vs. PEG asparaginase. Patients with antibodies to PEG asparaginase had faster PEG clearance ($P=.004$) than antibody-negative patients. Patients who were positive for antibodies had higher CSF asparagine than those who were negative ($P=.04$). The modeling suggests that by modifying dosages, comparable asparagine depletion should be achievable with both preparations. At relapse, there were significant pharmacokinetic and pharmacodynamic differences due to asparaginase preparation and antibody status.

Introduction

Asparaginase is one of the major anticancer drugs used in the treatment of acute lymphoblastic leukemia (ALL).⁽¹⁻¹⁰⁾ This enzyme reduces the levels of asparagine by hydrolyzing it to aspartic acid and ammonia. In the United States, three preparations of asparaginase are available: native enzyme from *Escherichia coli* (Elspar), PEGylated (PEG) enzyme from *E. coli* (Oncospar), and native erwinia enzyme from *Erwinia chrysanthemi* asparaginase (Erwinase). The antileukemic action of asparaginase is based on systemic depletion of asparagine because leukemic cells are more reliant on exogenous asparagine for survival than are normal host cells. *In vitro*, it is necessary to deplete asparagine for 4 days to achieve optimal leukemic cell kill.⁽⁴⁾ Patients with higher cerebrospinal fluid (CSF) asparagine levels (> 1

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μmol/L) during asparaginase treatment were more likely to have isolated CNS relapse,(8) and patients with relapsed ALL with lower Day 14 plasma asparagine levels (< 3 μmol/L) were more likely to achieve a second complete remission.(10;11)

Several factors influence the activity of asparaginase. These include pharmacologic factors such as asparaginase clearance, immunologic factors such as antibodies to asparaginase, and endogenous factors such as formation of asparagine via asparagine synthetase. First, the pharmacokinetics of asparaginase differ dramatically due to preparation. Specifically, the half-lives of native *E. coli* (about 1.25 days) and erwinia asparaginase (less than 1 day), are significantly shorter than PEG asparaginase (about a week). This has led to very different doses and frequencies of doses for the different preparations.(12;13) The formation of antibodies to asparaginase is another important factor that leads to hypersensitivity reactions in 30-75% of patients receiving native asparaginase and 5-18% of patients receiving PEG asparaginase. (12;13) Antibodies to asparaginase tend to diminish its efficacy by neutralizing asparaginase activity (resulting in faster asparaginase clearance), and high levels of anti-asparaginase antibodies have also been correlated with a shorter duration of asparagine depletion. Silent hypersensitivity has been described as patients who are positive for antibodies to asparaginase but do not exhibit clinical allergy. Typically, as is the case in this study, the development of silent hypersensitivity does not elicit a change in asparaginase formulation. Silent hypersensitivity has been associated with compromised antileukemic effect (9) although the negative consequences of silent (or clinical) hypersensitivity differs based on the overall treatment regimen (14). Another form of resistance to asparaginase relates to the endogenous formation of asparagine. Patients who have a greater formation of asparagine systemically may require higher or more protracted courses of asparaginase to deplete asparagine over an extended period.(15;16)

In the current study, we randomized pediatric patients with relapsed ALL to receive either native or PEG asparaginase during reinduction therapy. The main goals of the study were to compare depletion of asparagine between these two groups, study the differences in pharmacokinetics, and investigate the effects of antibodies on the pharmacokinetics of asparaginase and the depletion of asparagine. In this process, we developed a mechanistic mathematical model of the pharmacokinetics and pharmacodynamics of asparaginase and asparagine to allow us to perform simulations to better understand the underlying dynamics of asparaginase and predict effective treatments.

Results

Previous Asparaginase Treatment and Antibody Status

Of the 40 patients in this study, 36 received asparaginase in prior front-line ALL treatments (summarized in (17)): 30 only received native asparaginase, 1 only received PEG asparaginase, 4 received both native and erwinia asparaginase, and 1 received all three formulations. In addition, 1 patient did not receive prior asparaginase and 3 had unknown prior asparaginase drug history. Thirty-six patients were randomly assigned treatment with native vs PEG asparaginase (supplementary figure 1). Of these, 35 patients had antibody status measured. In addition, 4 patients were nonrandomly assigned to receive erwinia asparaginase due to hypersensitivity reaction in prior frontline ALL treatment. Three of these 4 developed antibodies to native and erwinia asparaginase. Of the randomized patients with evaluable antibody status at diagnosis of relapse, 13 were antibody positive for native asparaginase; of those 6 were also antibody positive for PEG asparaginase and another 4 of the 13 were also antibody positive for erwinia asparaginase. All but one of the 13 patients who were positive for antibody to native asparaginase prior to the start of reinduction had received it in their front-line therapy (the one had an unknown asparaginase drug history). Furthermore, 28 patients were randomized to receive either native or PEG asparaginase and received all their

asparaginase treatment without being switched to erwinia asparaginase due to a clinical allergic reaction. Of these, 14 developed antibodies to either native or PEG asparaginase prior to or during therapy. This group is considered to have silent hypersensitivity since they did not have clinical allergy and continued to receive their randomized asparaginase formulation.

Asparaginase pharmacokinetics

Asparaginase pharmacokinetic samples for native and PEG asparaginase were available in 33 of the 40 patients (4 patients were nonrandomly assigned to receive erwinia asparaginase due to previous hypersensitivity reactions to the other formulations, and 3 did not have pharmacokinetics samples) for the first pharmacokinetic course (Day 8) and in 26 of those 33 patients (4 patients were switched to erwinia asparaginase due to hypersensitivity reactions during the first 21 days of reinduction therapy, and 3 did not have pharmacokinetics samples) for the second pharmacokinetic course (Day 29) (Table 1, supplementary figure 1). Representative concentration vs time plots are shown in supplementary figure 2. The clearance of asparaginase was significantly higher ($P = .001$) at both times for native than for PEG asparaginase. Furthermore, the clearance increased from Day 8 to Day 29 with both formulations (native asparaginase, $P = .004$; PEG asparaginase, $P = .002$). In addition, the PEG asparaginase clearance was significantly higher ($P = .004$) and the time PEG asparaginase was above a threshold of 1 IU/mL was significantly shorter ($P = .03$) in patients who were positive for antibodies to PEG. Although there was no significant increase in the native asparaginase clearance or decrease in the time above the threshold of 1 IU/mL based on antibody status (possibly due to the small number of samples available), the trends were in the expected directions (higher median clearance on Day 29 and shorter time above the threshold for antibody-positive patients) (Table 1).

Asparagine pharmacodynamics

Plasma and CSF asparagine samples were available in 32 and 24 patients, respectively (figure 1). Representative concentration vs time plots are shown in supplementary figure 2. There were differences in the plasma and CSF asparagine depletion rates due to drug formulation and antibody status. Specifically, patients who were positive for antibodies at any time during reinduction therapy had attenuated depletion of plasma asparagine ($P = .01$) and CSF asparagine ($P = .04$) compared to those who were negative for antibodies throughout reinduction therapy. Additionally, the time that asparagine was depleted below a threshold of 3 μ M (plasma) or 1 μ M (CSF) was shorter (48% and 88%, respectively, $P < .05$) in patients with antibodies to either native or PEG asparaginase during reinduction therapy than in those who remained negative throughout reinduction therapy. These results indicate that patients with silent hypersensitivity reactions (since this group of patients did not have clinical reactions, all of them continued to receive their randomized dose of asparaginase) had poorer depletion of CSF asparagine compared to antibody negative patients. When we considered the model-estimated pharmacodynamic parameters, we found that there was no significant difference between V_{max} , k_{in} , or K_{mCSF} in patients who were antibody-positive versus those who were negative at any point during reinduction therapy. This indicates that the effects of antibodies are probably on asparaginase pharmacokinetics rather than on pharmacodynamics.

We observed a trend toward greater depletion ($P = .1$) of CSF asparagine over the course of reinduction therapy in those who received native asparaginase than in those who received PEG asparaginase. Furthermore, we observed a significantly higher model-estimated V_{max} for native asparaginase than for PEG asparaginase (2 times higher, $P = .015$), consistent with patients receiving native asparaginase having greater CSF asparagine depletion (supplementary figure 3). Because most post-treatment plasma asparagine levels were below the level of detection, we were not able to compare plasma depletion rates between formulations.

Patients (n=4, data not shown) who had hypersensitivity reactions to either native *E. coli* or PEG asparaginase during the study were switched to erwinia asparaginase. All 4 patients were positive for antibodies to native *E. coli* and PEG asparaginase by Day 28, and 3 of the 4 patients were positive for antibodies to erwinia asparaginase by Day 28. This subgroup of patients had no significant reduction in their plasma or CSF asparagine from Day 8 to Day 29 ($P = .25$).

Finally, we observed no significant association between the remission induction rate and the asparaginase treatment arm, (17) although the study was not powered to detect such a difference.

Mechanistic model simulations

Using the estimated asparaginase pharmacokinetic and asparagine pharmacodynamic parameters in these patients we simulated changes in asparaginase dose and clearance (e.g., due to antibody effects) along with changes to asparagine pharmacodynamic parameters to see how they would affect the depletion of CSF asparagine. A depiction of the simulations subdivided by formulation is shown in figure 2A. In all the simulations only patients who had data for plasma asparaginase, plasma asparagine, and CSF asparagine were used (native asparaginase: n=9 (Ab- 6, Ab+ 3); PEG asparaginase: n=9 (Ab- 6, Ab+ 3)).

First, we considered the effects of increasing the asparaginase dose over a clinically relevant range (increasing PEG asparaginase from 2,500 IU/m² to 3,500 IU/m² per dose or increasing native asparaginase from 6,000 IU/m² to 10,000 IU/m² per dose). Increases in asparaginase dose are predicted to cause an increase in the time that CSF asparagine remains below a threshold of 1 μ M (figure 3). Next, we considered the effects of increased clearance due to the development of antibodies to asparaginase. Specifically, given the median clearance for each formulation that we observed in this group of children with relapsed ALL, a weekly dose of 3,500 IU/m² of PEG asparaginase would be needed to obtain a similar median time that CSF asparagine was below the threshold as with a thrice weekly dose of 6,000 IU/m² of native asparaginase (figure 2B). As expected, increases in asparaginase clearance caused a decrease in the duration that CSF asparagine was below the threshold.

Next, we considered the effects of changes in asparagine depletion relative to plasma asparaginase AUC over 35 days. In this simulation, to compare individuals at the same exposure, each individual's dose was adjusted to yield a specified AUC. For example, patients receiving native asparaginase needed doses ranging between 7,580 and 18,325 to obtain a plasma asparaginase AUC of 35 IU/mL·day. We predicted that increases in AUC caused an increase in the time that CSF asparagine remained below a threshold of 1 μ M, and that at any given AUC, there will be more CSF asparagine depletion with the native asparaginase than with the PEG formulation (figure 4). Given an AUC with native asparaginase of 20 IU/mL·day (which is equivalent to a median native asparaginase dose of 6,000 IU/m²/dose $\times$ 12, thrice weekly for 4 weeks, in antibody-negative patients), an AUC with PEG asparaginase of 100 IU/mL·day (which is equivalent to a median PEG asparaginase dose of 3,500 IU/m²/dose $\times$ 4, weekly for 4 weeks, in antibody-negative patients) would be needed to obtain an equivalent depletion of CSF asparagine (figure 2B).

We also predicted how changes in r_{max} and K_m affected the duration of CSF asparagine depletion. Based on these patients with relapsed ALL, those who received PEG asparaginase were predicted to be more sensitive to changes in these parameters than those who received native asparaginase. Specifically, increases in r_{max} from 0.2 μ M/h to 2.0 μ M/h (10 $\times$ higher than the base level) were predicted to cause a 19% or 100% decrease in the median percent time that CSF asparagine was below the threshold of 1 μ M for native and PEG asparaginase, respectively (supplementary figure 4A). This indicates that one possible source of resistance to asparaginase could be increased asparagine synthetase activity. Changes in K_m from 12 to

120 μM (10 $\times$ the lower value given in the literature) were predicted to cause only a small median decrease (14%) in the time CSF asparagine was below the threshold with native asparaginase but a much larger decrease (64%) with PEG asparaginase (supplementary figure 4B).

Discussion

The pharmacokinetics of asparaginase observed in this study show several important differences within and between native and PEG asparaginase disposition, and these differences translate into different kinetics of asparagine depletion. First, while the asparaginase clearance in the current relapse study (native asparaginase: 1648-6865 mL/day/m²; PEG asparaginase: 33 to greater than 1162 mL/day/m²) differs from those in previously reported studies in newly diagnosed patients (native asparaginase: 1109-1280 mL/day/m²; PEG asparaginase: 180-252 mL/day/m²), (8;13;18) the magnitude of the differences observed between the two formulations are similar. Specifically, the clearance of native asparaginase was much higher than that of PEG asparaginase, consistent with the much higher and more frequent dosing of native asparaginase than PEG asparaginase. As is true in newly diagnosed patients, (8;9;18) the pharmacokinetics also differed by the presence of anti-asparaginase antibodies. Specifically, we observed an increase in asparaginase clearance in asparaginase antibody-positive patients. This more rapid clearance translated into a significant decrease in the time that asparagine was depleted below the threshold level.

We also observed differences in asparagine depletion due to formulation that were captured by the model parameter V_{max} , the maximal rate of asparagine depletion. These differences were consistent with previous findings of greater depletion of asparagine in relapsed patients who received native asparaginase than in those who received PEG asparaginase. (8;19;20) In an effort to account for the reduced depletion of asparagine by PEG asparaginase, our simulations suggest that a higher dose of PEG asparaginase (3,500 IU/m² weekly for 4 doses over 21 days) may increase the time of CSF asparagine depletion to be similar to that observed in patients who received native asparaginase at 6,000 IU/m² thrice weekly for 12 doses over 26 days. Furthermore, an increase in dose for antibody-positive patients (2,500 to 3,500 IU/m² weekly for 4 doses over 21 days for PEG asparaginase or 6,000 to 10,000 IU/m² thrice weekly for 12 doses over 26 days for native asparaginase) is predicted to result in asparagine depletion comparable to that of antibody-negative patients. A comparison of PEG asparaginase dosed weekly vs biweekly at 2500 IU/m²/dose showed that when adjusting for type of relapse and prior hypersensitivity, those receiving the weekly dose had a 78% lower rate of induction failure, a finding suggesting the advantage of a higher exposure to PEG asparaginase. (21) One possible consequence of increasing the asparaginase dose is increased toxicity (e.g. thrombosis, hypoalbuminemia, or pancreatitis). (21) To our knowledge, there are no data as to whether increased doses of asparaginase would increase the rate of hypersensitivity reactions in either antibody positive or negative patients. We are testing a higher dose of PEG asparaginase in a current randomized trial.

The group of antibody positive patients analyzed in this study had silent hypersensitivity reactions (since we did not consider those who were switched to erwinia asparaginase due to clinical allergic reactions in the analysis). Therefore, this study indicates that patients with relapsed ALL and silent hypersensitivity have lower exposure to asparaginase and lower depletion of CSF asparagine. These results support testing an approach that would use asparaginase antibody status to alter therapy either by increasing the asparaginase dose (as suggested by the modeling simulations) or switching to an alternative therapy when positive asparaginase antibodies are present.

The simulations in this study also showed that increases in the endogenous production of asparagine ($r_{\max}$ in the model) over a physiological range could lead to resistance to asparaginase (measured as a shorter period of CSF asparagine depletion), especially in patients who received PEG asparaginase. Several studies (both *in vitro* and *in vivo*) have explored the relationship between asparaginase resistance and asparagine synthetase activity, with varying results. *In vitro*, depletion of asparagine can cause a transient increase in asparagine synthetase activity subsequently leading to resistance to asparaginase.(22-26) In addition, it has been shown that in *TEL-AML1*-negative patients, increased asparagine synthetase expression was linked to asparaginase resistance.(15) However, in *TEL-AML1*-positive patients and other populations, the relationship between asparagine synthetase and asparaginase resistance did not hold.(15-16;26) Recently it has been shown that bone marrow-derived mesenchymal cells can increase the asparagine availability to leukemia cells thus contributing to asparaginase resistance.(27)

We also considered how changes in the model parameter describing asparaginase affinity (K_M) over a physiological range affected the depletion of asparagine. Although changes in this parameter had less effect on the predicted depletion of asparagine for patients receiving native asparaginase compared to those receiving PEG asparaginase, tested over the range of asparaginase affinity parameters from the literature (12 to 29 μM), there was less than a 10% change in the depletion of CSF asparagine for either formulation.

It should be acknowledged that the differences in predicted asparagine depletion between the two asparaginase formulations are based upon model parameters estimated in a relatively small number of patients studied in the relapse setting. Parameter estimates might change if a larger number of patients were studied. Moreover, whether these formulation-specific differences in pharmacodynamic parameters would be recapitulated in naïve, newly diagnosed patients is not known.

Our mathematical model provides a practical method of analyzing pharmacokinetic and pharmacodynamic data from clinical trials. In addition, it offers an innovative approach to understanding the underlying dynamics of asparaginase, determining the mechanisms that most affect asparagine depletion, and allowing easy investigation of multiple treatment scenarios *in silico*. The findings from this modeling study are currently being used to aid in our understanding and interpretation of asparaginase treatment in ongoing protocols.

Patients, materials, and methods

Patients

Forty children with relapsed ALL were enrolled in protocol R16 at St. Jude Children's Research Hospital.(17) The institutional review board approved the study, and informed consent was obtained from parents/guardians or patients. This study was compliant with the regulations of the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

Treatment regimen and sample collection

Patients were randomized to receive either native *E. coli* or PEG asparaginase during reinduction therapy (supplementary figure 1). Native asparaginase was given intramuscularly thrice weekly for 12 doses over 26 days (days 8 to 34 of reinduction) at a dose of 10,000 IU/m², and PEG asparaginase was given intramuscularly weekly for 4 doses over 21 days (days 8, 15, 22, and 29 of reinduction) at a dose of 2,500 IU/m². Patients who had a hypersensitivity reaction to either native or PEG asparaginase were switched to erwinia asparaginase given intramuscularly thrice weekly for 12 doses over 26 days (days 8 to 34 of reinduction) at a dose of 10,000 IU/m² or an appropriate proportion of the schedule if a hypersensitivity reaction

occurred during the remission reinduction period. The remainder of the reinduction therapy has been described elsewhere.(17)

Plasma samples for asparaginase pharmacokinetic studies were collected before and after the doses given on Day 8 and Day 29 of reinduction therapy. For native *E. coli* and erwinia asparaginase, 4 to 6 samples were collected over a 3-day period. For PEG asparaginase, 4 to 6 samples were collected over a 7-day period. Anti-asparaginase antibody levels for all three preparations of asparaginase were measured on Days 8, 22, 29, and 37. Asparagine concentrations were measured in both the plasma and CSF on the same days. The CSF samples were collected in conjunction with lumbar punctures for triple intrathecal therapy (methotrexate, hydrocortisone, and cytarabine).

Asparaginase activity

Asparaginase activity in patient serum was analyzed using a kinetic spectrophotometric assay similar to previously described assays.(18;28) Details are provided in the supplement.

Asparagine concentrations

Methods for determining the plasma and CSF asparagine concentrations have been previously described.(20;29)

Anti-asparaginase activity

Methods for determining anti-asparaginase activity have been previously described.(14;20;30) We considered a sample to be positive to either native *E. coli*, PEG, or erwinia asparaginase if its optical density reading at a 1:400 dilution was greater than the 99% confidence interval of the negative controls for the respective formulation. Otherwise, the sample was considered negative.

Pharmacokinetic and pharmacodynamic models

To estimate the pharmacokinetics of asparaginase, we used a first-order one-compartment pharmacokinetic model with first-order absorption. The absorption compartment described the kinetics of asparaginase between its intramuscular delivery site and the plasma compartment. It was assumed that asparaginase has 100% bioavailability. We used nonlinear mixed effects modeling (via NONMEM V software; University of California at San Francisco(31)), subdividing by drug preparation (native *E. coli*, PEG, or erwinia asparaginase), to determine the population parameters. Because there might be a significant difference in the pharmacokinetics between the two courses of asparaginase due to asparaginase antibody levels affecting drug clearance, we allowed for a different clearance on the set of measured concentrations in each patient on Day 8 and Day 29. The individual pharmacokinetic parameters were estimated via a Bayesian estimation approach using the population estimates as priors. We also estimated the time (in days) from Day 8 to Day 37 that the asparaginase levels were above a threshold level of 1 IU/mL.

Mechanistic model of asparaginase/asparagine pharmacodynamics

We derived a model (supplementary figure 5) to account for the endogenous formation of asparagine (ASN), the effects of asparaginase (ASP) on plasma asparagine (ASN), and the dynamics of CSF asparagine (ASN_{CSF}).(13;32;33) The endogenous formation of asparagine was modeled as a function of the rate of systemic asparagine synthesis (r_{max}) and was assumed to be a saturable process with a baseline steady state level of plasma asparagine (ASN_0). This is modeled by the first term on the right-hand side of Equation 1. The pharmacodynamic effects of asparaginase on hydrolyzing plasma asparagine were assumed to follow Michaelis-Menten kinetics (V_{max} and K_m). This is modeled by the last term on the right-hand side of Equation 1.

Since asparaginase does not penetrate into the CSF,(34) the only processes we accounted for when modeling CSF asparagine were influx (k_{in} , K_{mCSF}), which was assumed to be saturable, and efflux (k_{out}) (Equation 2):

$$\frac{dASN}{dt} = r_{max} \left(1 - \frac{ASN}{ASN_0} \right) - \frac{k_{in} ASN}{K_{mCSF} + ASN} + k_{out} ASN_{mCSF} - \frac{V_{max} ASP ASN}{K_m + ASN} \quad (\text{Equation 1})$$

$$\frac{dASN_{CSF}}{dt} = \frac{k_{in} ASN}{K_{mCSF} + ASN} - k_{out} ASN_{CSF} \quad (\text{Equation 2})$$

Additionally we required the CSF steady state assumption of $k_{out} = k_{in} \frac{ASN_0}{ASN_{CSF0} (K_{mCSF} + ASN_0)}$, where ASN_{CSF0} is the steady state level of CSF asparagine.

Several model parameters were fixed using previously published data: r_{max} (0.2 $\mu\text{M}/\text{h}$; literature range: 0.06-6.0 $\mu\text{M}/\text{h}$, (13:33)); K_m (29 μM ; literature values: 12 and 29 μM , (13:33)); ASN_0 and ASN_{CSF0} (both set to each individual's measured pre-asparaginase asparagine concentration). The parameters V_{max} , k_{in} , and K_{mCSF} were estimated in each individual using the maximum likelihood estimation method as implemented in ADAPT II pharmacokinetic fitting software(35), using the fixed plasma asparaginase pharmacokinetic parameters that had been estimated for each individual. We also estimated the time that plasma asparagine was below 3 μM and that CSF asparagine was below 1 μM for each individual.

Simulations

Simulations were performed to determine the sensitivity of the model-predicted plasma and CSF asparagine disposition to changes in model parameters and to determine the effects of various doses of asparaginase. The simulations were performed using the pharmacokinetic and pharmacodynamic parameters that had been estimated for each individual.

Statistical methods

The Kruskal-Wallis test was used to compare differences within individuals, and the Wilcoxon test was used to compare differences between individuals in asparaginase pharmacokinetic parameters. Differences in the depletion of plasma or CSF asparagine were determined by comparing (via the Mann-Whitney U test) the area under the curve (AUC) on the serial asparagine-vs.-time plot as determined by the trapezoidal rule (i.e., the larger the area, the less the depletion of asparagine). In all cases, differences were considered significant if $P < .05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Measured plasma and CSF asparagine (ASN) concentrations in patients vs. time. Top row: plasma levels. Bottom row: CSF levels. Left column: patients dosed with native asparaginase. Right column: patients dosed with PEG asparaginase. Solid lines: patients negative for asparaginase antibodies at all times tested during induction therapy. Dashed lines: patients positive for asparaginase antibodies at any time during induction therapy. LLOQ (lower limit of quantification): 0.029 μM .

Figure 2.

Simulated CSF asparagine (ASN) vs. time (days). The shaded regions represent the range over all patients in the respective groups. (A) The red region is a 10,000 IU/m²/dose of native asparaginase. The solid (antibody negative) and dashed (antibody positive) lines represent the median result in simulations of patients who received native asparaginase. The blue region is a 2,500 IU/m²/dose of PEG asparaginase. The dot-dashed (antibody negative) and dotted (antibody positive) lines represent the median result in simulations of patients who received PEG asparaginase. The symbols are the patient samples. squares: Native ASP Ab-; stars: Native ASP Ab+; circles: PEG ASP Ab-; crosses: PEG ASP Ab+. (B) The red region is a 6,000 IU/m²/dose of native asparaginase. The solid (antibody negative) and dashed (antibody positive)

lines represent the median result in simulations of patients who received native asparaginase. The blue region is a 3,500 IU/m²/dose of PEG asparaginase. The dot-dashed (antibody negative) and dotted (antibody positive) lines represent the median result in simulations of patients who received PEG asparaginase.

Figure 3.

Simulated time CSF asparagine (ASN) < 1 μ M vs. asparaginase clearance. Lines represent the median result and the shaded regions represent the quartile range of all patients. Horizontal box represents the median and range of the measured asparaginase clearances. A: native asparaginase dosed thrice weekly for 4 weeks. The red region is 10,000 IU/m²/dose and the blue region is 6,000 IU/m²/dose. B: PEG asparaginase dosed weekly for 4 weeks. The red region is 3,500 IU/m²/dose and the blue region is 2,500 IU/m²/dose.

Figure 4.

Simulated time CSF asparagine (ASN) < 1 μ M vs. plasma asparaginase AUC over 35 days (IU/mL Day). To simulate each individual at the same exposure, the asparaginase dose in each individual was adjusted to target the specified AUC. Lines represent the median result and the shaded regions (red: native asparaginase. blue: PEG asparaginase) represent the quartile range of all patients. The Horizontal boxes represent the range of plasma asparaginase AUCs obtained from the patients in this study.

Table 1

Asparaginase pharmacokinetic and pharmacodynamic parameters.

Formulation	P value, Formulation ¹	Pharmacokinetic Parameter	Median (Range)	P value, Day ²	Ab Status ³	Median (Range)	n ⁴	P value, Ab Status ⁵
Native <i>E. coli</i> asparaginase		CL Day 8 (mL/day/m ²)	2941 (1768, 4994)	0.004	Ab-	3039 (1768, 4994)	9	0.4
		CL Day 29 (mL/day/m ²)	4207 (2622, 6865)		Ab+	2730 (1648, 3577)	8	
					Ab-	3756 (2622, 6865)	6	
					Ab+	4379 (3007, 6755)	5	
PEG asparaginase	0.001	Threshold time ⁶ (days)	8 (0, 24)	0.002	Ab-	16 (0, 22)	9	0.5
					Ab+	3 (1, 24)	8	
		CL Day 8 (mL/day/m ²)	89 (34, 280)		Ab-	85 (34, 232)	8	
		CL Day 29 (mL/day/m ²)	137 (33, ND)		Ab+	138 (72, 280)	8	
Native <i>E. coli</i> asparaginase	0.015	CL Day 29 (mL/day/m ²)			Ab-	115 (33, 156)	6	0.004
					Ab+	1162 (95, ND)	9	
		Threshold time ⁶ (days)	24 (0, 29)		Ab-	27 (0, 29)	8	
					Ab+	17 (4, 27)	8	
Native <i>E. coli</i> asparaginase	0.015	V _{MAX} (μM/days)/(IU/mL)	122 (65, 304)		Ab-	107 (65, 304)	6	0.7
					Ab+	135 (82, 201)	3	
					Ab-	54 (40, 167)	6	
					Ab+	85 (17, 109)	3	
Native <i>E. coli</i> asparaginase	0.3	K _{mCSF} (μM)	3.6 (0.25, 10)		Ab-	9.5 (1, 10)	6	0.5
					Ab+	0.7 (0.25, 10)	3	
					Ab-	1.0 (0.25, 2.2)	6	
					Ab+	1.0 (1.0, 10)	3	
Native <i>E. coli</i> asparaginase	0.3	k _{in} (1/days)	4.8 (0.6, 10)		Ab-	2.7 (0.6, 5.5)	6	1.0
					Ab+	10 (9.5, 10)	3	
					Ab-	5.7 (0.6, 10)	6	
					Ab+	0.7 (0.6, 1.1)	3	
Native <i>E. coli</i> asparaginase		Not Determined ⁷			Ab-		1	
					Ab+		4	

¹ comparison between native and PEG asparaginase clearance or pharmacodynamic parameter

² comparison between Day 8 and Day 29 asparaginase clearance

³ Ab-: The patient was negative for antibodies to native and PEG asparaginase at all times tested during induction therapy. Ab+: The patient was positive for antibodies to either native or PEG asparaginase at some time after the start of induction therapy.

⁴ number of patients.

⁵ comparison between asparaginase clearance or pharmacodynamic parameter in antibody-positive and antibody-negative patients

⁶ Estimated time (days) that asparaginase concentrations were > 1 IU/mL.

⁷ No determined due to no depletion of asparagine.

ND—Not Determined: the asparaginase activity was not detectable in 4 patients, indicating a very high asparaginase clearance (but one that could not be estimated).