

TO THE EDITOR:

Dosing-related saturation of toxicity and accelerated drug clearance with pegaspargase treatment

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Polyethylene glycol conjugated asparaginase (pegaspargase) is used for acute lymphoblastic leukemia (ALL). Pharmacokinetics of pegaspargase is affected by anti-asparaginase antibodies,^{1,2} but other factors affecting asparaginase pharmacokinetics should be investigated. In a frontline ALL trial, we showed no difference in treatment outcome between pegaspargase 2500 U/m² and 3500 U/m².³

On St. Jude Children's Research Hospital Total XVI protocol (NCT00549848), patients received 3000 U/m² pegaspargase (Oncaspar) IV on day 3 of induction. After induction, pegaspargase differed by risk arm (supplemental Figure 1, available on the *Blood* Web site); 411 patients were randomized to receive 2500 or 3500 U/m² during continuation³ and nonrandomized patients were assigned to 2500 U/m² (supplemental Figure 2). Serum pharmacokinetics were assessed on 3 occasions: induction, week 7 of continuation, and week 17 of continuation treatment (supplemental Figure 1). The data were well fit by a nonlinear 1-compartment model (supplemental Methods). We found that 99.7%, 97.7%, and 99.8% of doses resulted in day 14 trough concentrations >0.1 U/mL at induction, week 7, and week 17, respectively (supplemental Figure 5; Figure 1A-B), with a median time >0.1 U/mL (threshold time or TT) for each dose estimated at more than 22 days for all occasions, risk groups, and dosages (supplemental Table 4; Figure 1C-D).

Patients randomized to 3500 U/m² did not have more pancreatitis, thrombosis, osteonecrosis, or serious hepatotoxicity than patients randomized to 2500 U/m² (Figure 2), nor did they receive fewer doses of pegaspargase (13.8 vs 13.3 doses among standard/high-risk [SHR] patients, $P = .43$; 4.5 vs 4.6 doses among low-risk [LR] patients, $P = .56$). At induction, there were no associations between systemic exposure and serum total bilirubin or creatinine, but greater exposure (lower initial maximum velocity [V_{max}], lower intrinsic clearance [CL_{int}], higher estimated day 14 serum asparaginase activity, and longer TT) was associated with higher serum alanine aminotransferase level, adjusting for age and sex ($P \leq .010$; supplemental Figure 7).

In the population pharmacokinetic model, both continuity of dosing (SHR vs LR) and dosage randomization affected pegaspargase pharmacokinetics ($P < 1.1 \times 10^{-16}$ for both; supplemental Table 2). Among patients randomized to receive 2500 U/m², SHR (continuous dosing) patients had higher V_{max} and CL_{int} than LR patients (intermittent dosing) during both weeks 7 and 17 ($P = 1.5 \times 10^{-29}$

and 2.1×10^{-22} for V_{max} and CL_{int} at week 7; $P = 1.7 \times 10^{-39}$ and 3.2×10^{-31} for V_{max} and CL_{int} at week 17; Figure 1E-H). The same difference by risk arm was observed in patients receiving 3500 U/m² ($P = 7.1 \times 10^{-26}$ and 1.6×10^{-19} for V_{max} and CL_{int} at week 7; $P = 9.4 \times 10^{-50}$ and 2.6×10^{-40} for V_{max} and CL_{int} at week 17; Figure 1E-H). Moreover, SHR patients had higher V_{max} and CL_{int} during week 17 than week 7 when comparing within the same dosage level ($P \leq 1.1 \times 10^{-6}$; supplemental Figure 8B,D). This difference in pharmacokinetics from week 7 to week 17 was not observed in LR patients at either dosage, whose asparaginase treatment was given intermittently (not continuously) ($P \geq .22$; supplemental Figure 8A,C). These results indicate that continuous dosing may accelerate clearance of pegaspargase over time.

Pegaspargase dosage also affected clearance. For both continuation weeks 7 and 17, patients randomized to receive 3500 U/m² had higher V_{max} and CL_{int} than those randomized to 2500 U/m² on the same risk arm (Figure 1E-H; supplemental Results). In addition, LR patients randomized to receive 2500 U/m² had lower V_{max} and CL_{int} during continuation week 7 than they did during induction, when they received the higher 3000 U/m² dosage (supplemental Figure 8A,C). Similarly, LR patients receiving 3500 U/m² had higher V_{max} and CL_{int} during continuation week 7 than they did during induction (supplemental Figure 8A,C).

Systemic exposure to pegaspargase was affected by dosage, dosing continuity, and clearance. Because of higher clearance with continuous dosing, SHR patients had lower day 14 activity and shorter TT during week 17 than week 7 ($P \leq 2.2 \times 10^{-8}$; supplemental Figure 8F,H). In contrast, no such difference was observed for LR patients ($P \geq .28$; supplemental Figure 8E,G). Moreover, SHR patients (continuous dosing) had shorter TT for continuation week 17 than LR patients (intermittent dosing) randomized to the same dosage arm ($P = 6.3 \times 10^{-8}$ for 2500 U/m² arm; $P = 1.3 \times 10^{-11}$ for 3500 U/m² arm; Figure 1D). As a result of faster clearance with the higher dosage, TT was not longer at 3500 U/m² than 2500 U/m² among LR or among SHR patients at continuation weeks 7 or 17 (supplemental Results; Figure 1; supplemental Figure 5).

Both hypersensitivity ($P < 2.2 \times 10^{-16}$) and anti-pegaspargase antibodies ($P = 9.7 \times 10^{-4}$) were associated with faster elimination (supplemental Table 1), but associations differed by risk arm, dosage randomization, and occasion. For continuation week 7, anti-pegaspargase positivity was associated with lower day 14

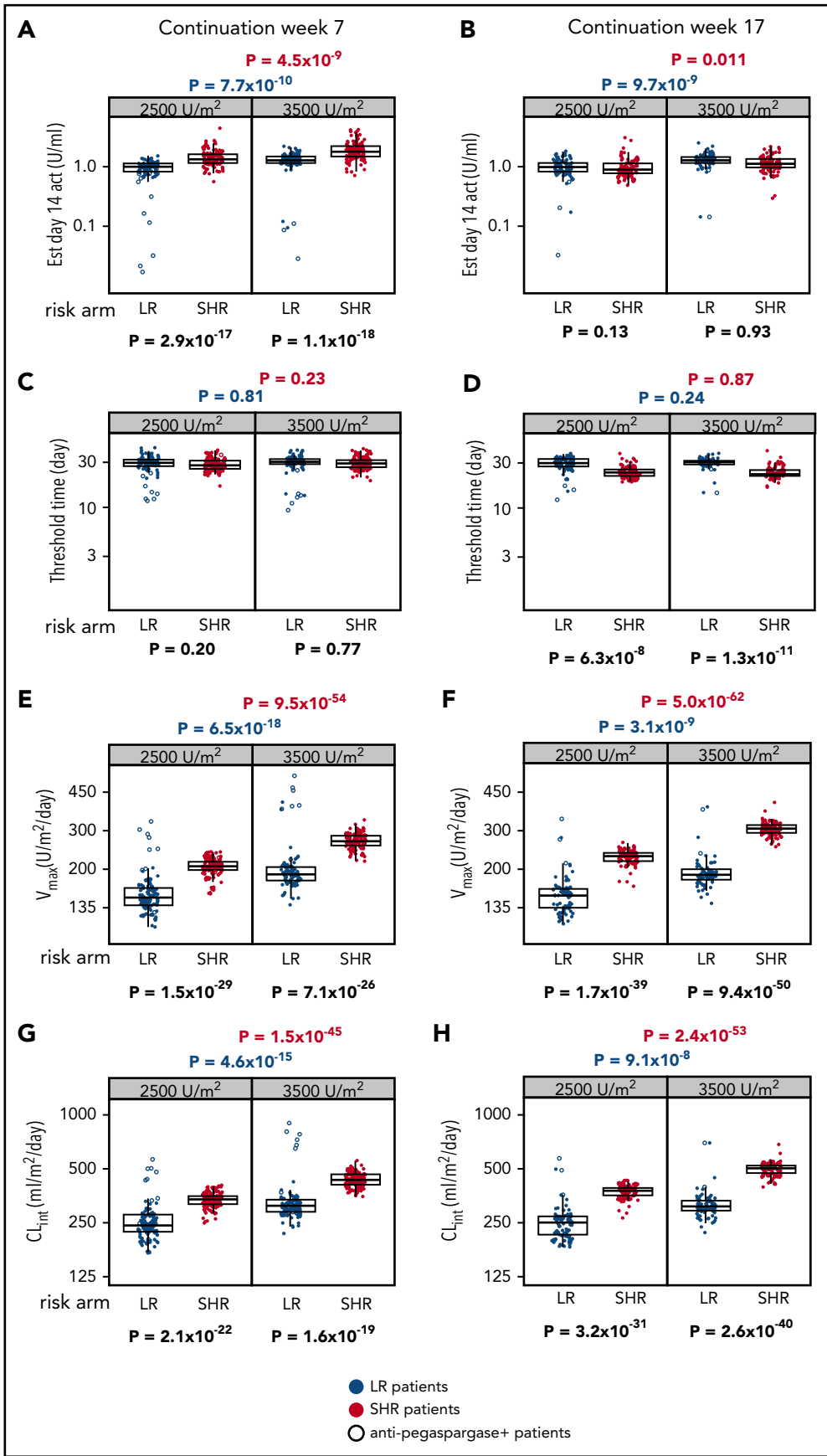


Figure 1. Individual post hoc pegaspargase pharmacokinetic estimates day 14 activity, threshold time, V_{max} , and CL_{int} during continuation week 7 and week 17 differed by risk arm and randomized pegaspargase dosage levels. There were 84, 89, 82, and 96 patients in low risk (LR, intermittent dosing) 2500 U/m², standard/high risk (SHR, continuous dosing) 2500 U/m², LR 3500 U/m², and SHR 3500 U/m² group, respectively, for week 7; and 63, 74, 66, and 79 in LR 2500, SHR 2500, LR 3500, and SHR 3500 group, respectively, for week 17. In each plot, red P values compare between different randomized dosage levels within SHR patients; blue P values compare between different randomized dosage levels within LR patients. P values below each plot in black compare between different risk arms randomized to receive the same dosage level. All P values were generated using linear mixed-effect model adjusting for antibody status and clinical reaction. Only randomized patients were included in these analyses.

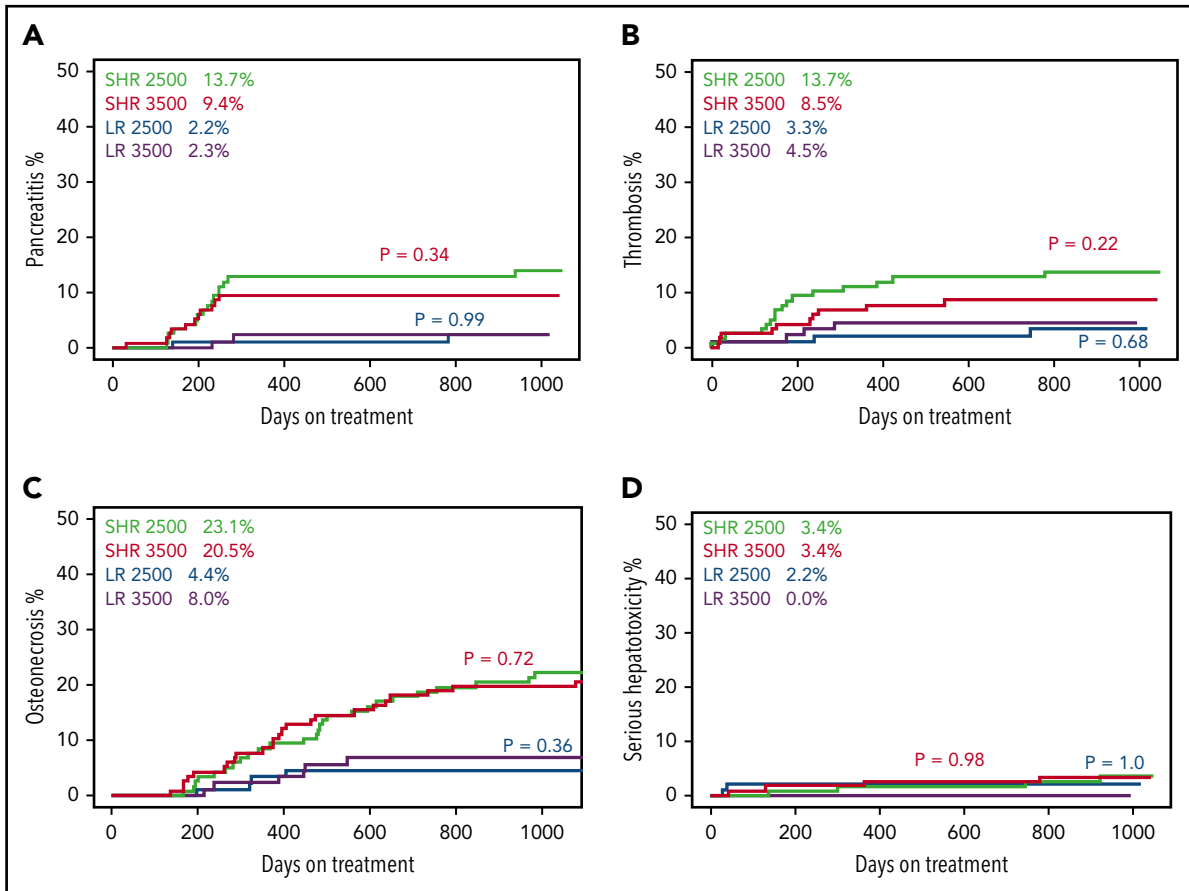


Figure 2. Randomization to 2500 vs 3500 U/m² pegaspargase dosage did not affect incidence of pancreatitis, thrombosis, osteonecrosis, and serious hepatotoxicity. (A) Grade ≥2. (B) Grade ≥2. (C) Grade ≥2. (D) Grade ≥3. Green lines are standard/high risk (SHR) patients randomized to receive pegaspargase 2500 U/m² (n = 117), red lines are SHR patients randomized to receive pegaspargase 3500 U/m² (n = 116), blue lines are low risk (LR) patients randomized to receive pegaspargase 2500 U/m² (n = 88), purple lines are LR patients randomized to receive pegaspargase 3500 U/m² (n = 90). Red P values compare between SHR 2500 U/m² and SHR 3500 U/m² patients. Blue P values compare between LR 2500 U/m² and LR 3500 U/m² patients. Percentage values show the cumulative incidence of the corresponding toxicity in each risk/dosage group. Only randomized patients were included in these analyses.

activity ($P = 2.4 \times 10^{-20}$), and shorter TT ($P = 7.8 \times 10^{-21}$; supplemental Figure 9A) adjusting for hypersensitivity and dosage among LR patients (intermittent dosing); this was also true for continuation week 17 among LR patients (supplemental Results). For continuation weeks 7 and 17 among SHR patients (continuous dosing), none of the post hoc parameters were associated with antipegaspargase positivity adjusting for hypersensitivity and dosage randomization ($P \geq .17$, supplemental Results; supplemental Figure 9B). No other factors, including age, sex, genetic ancestry, or ALL lineage, were associated with pharmacokinetics.

In summary, in this trial that randomized patients to receive 3500 U/m² vs a standard dosage of 2500 U/m², we did not observe any difference in toxicities between the 2 dosages (Figure 2). Interestingly, a prior study of pegaspargase dosages (with highest dosage of only 1500 U/m²) individualized based on trough plasma levels also did not find toxicity associated with dosage.⁴ This is consistent with and could be explained by the surprising effects of pegaspargase dosage and dosing continuity on pharmacokinetics, which resulted in a lack of difference in number of days with asparaginase activity >0.1 U/mL (TT) by dosage randomization (supplemental Figure 5; Figure 1C-D). Although alanine aminotransferase levels during induction were associated with asparaginase exposure (supplemental Figure 7),

as we reported previously,⁵ this did not translate into more clinically significant hepatotoxicity and were not observed after remission induction. The lack of difference in TT by dosage was caused by accelerated clearance with higher dosage or continuous dosing (Figure 1; supplemental Figure 8). This effect of dosage and dosing continuity on clearance is termed “accelerated blood clearance” (ABC) and has been reported in animals treated with PEGylated reagents.^{6,7} We observed for the first time an ABC phenomenon in humans. Whether ABC occurs for other PEGylated therapeutics in humans is not known. To our knowledge, this is the first report of faster clearance of pegaspargase, or any PEGylated medications, when administered at higher dosages.

Here, we used a nonlinear model that reasonably described pegaspargase pharmacokinetics; nonlinear models have been reported by others.^{8,9} Our volume of distribution was comparable to a previous report.¹⁰ This concordance indicates that population modeling, using limited rather than extensive sampling, could be used in future pegaspargase studies.

In conclusion, our data show that dosages >2500 U/m² are not necessary, consistent with our treatment outcome.³ Several ALL trial groups are investigating even lower dosages of pegaspargase.⁴

Given the effect of continuity of dosing and nonlinearity of elimination, pharmacokinetic evaluations should accompany new dosing strategies for this drug.

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Authorship

Contribution: Y.L., J.C.P., and M.V.R. developed the pharmacokinetic model, analyzed and interpreted the data, and wrote the manuscript; S.J., C.-H.P., H.I., C.C., M.V.R., and W.E.E. developed the protocol; S.J., C.-H.P., and H.I. recruited patients and interpreted the data; C.C. and W.Y. analyzed the data; and J.J.Y., S.E.K., and W.E.E. interpreted the data.

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Footnotes

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TO THE EDITOR:

Combinatorial treatment with menin and FLT3 inhibitors induces complete remission in AML models with activating FLT3 mutations

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The interaction between menin and the mixed lineage leukemia 1 (MLL1) protein plays an important role in aggressive acute leukemia with translocations of the *MLL1* (*KMT2A*) gene^{1,2} and with mutations in the nucleophosmin (*NPM1*) gene.³ We and others have recently shown that small-molecule inhibitors of the menin-MLL1 interaction can effectively block leukemia progression in these leukemia subtypes.⁴⁻¹¹ This resulted in phase 1 clinical trials with menin inhibitors, including KO-539 (registered at www.clinicaltrials.gov

as #NCT04067336), in acute myeloid leukemia (AML) patients. Activating mutations in FLT3 kinase, including internal tandem duplications (FLT3-ITDs), are found in a substantial fraction of AML patients, leading to poor clinical outcomes.¹²⁻¹⁷ Because overexpression or mutations in FLT3 often cooccur with *MLL1* translocations (16%) or *MLL1* partial tandem duplications (>50%)^{15,18-21} and *NPM1* mutations (>40%),^{16,22,23} we hypothesized that the antileukemic activity of menin inhibitors may synergize with