Continuous PEGasparaginase Dosing Reduces Hypersensitivity Reactions in Pediatric ALL: A Dutch Childhood Oncology Group ALL11 Randomized Trial

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ABSTRACT

PURPOSE The primary objective of this randomized study was to determine whether a continuous dosing schedule (without the asparaginase-free interval) would result in less hypersensitivity reactions to PEGasparaginase (PEGasp) compared with the standard noncontinuous dosing schedule.

METHODS Eight hundred eighteen patients (age 1-18 years) with ALL were enrolled in the Dutch Childhood Oncology Group-ALL11 protocol and received PEGasp. Three hundred twelve patients stratified in the medium-risk arm were randomly assigned to receive 14 individualized PEGasp doses once every two weeks in either a noncontinuous or continuous schedule after the first three doses in induction (EudraCT: 2012-000067-25). Hypersensitivity reactions were defined as allergies, allergic-like reactions, and silent inactivation. Secondary end points were other asparaginase-related toxicities, asparaginase activity and antibody levels, and outcome.

RESULTS During induction, 27 of 818 patients (3.3%) experienced hypersensitivity reactions. After random assignment, 4 of 155 (2.6%) in the continuous treatment arm versus 17 of 157 (10.8%) patients in the noncontinuous treatment arm had hypersensitivity reactions ($P < .01$), of which two (1.3%) versus 13 (8.3%) were inactivating reactions ($P < .01$). The occurrence of inactivating hypersensitivity reactions was seven times lower in the continuous arm (odds ratio, 0.15 [0.032-0.653]). In addition, antibody levels were significantly lower in the continuous arm ($P < .01$). With exception of a lower incidence of increased amylase in the continuous arm, there were no significant differences in total number of asparaginase-associated toxicities between arms. However, the timing of the toxicities was associated with the timing of the asparaginase administrations. No difference in 5-year cumulative incidence of relapse, death, or disease-free survival was found between both treatment arms.

CONCLUSION A continuous dosing schedule of PEGasp is an effective approach to prevent antibody formation and inactivating hypersensitivity reactions. The continuous PEGasp schedule did not increase toxicity and did not affect the efficacy of the therapy.

ACCOMPANYING CONTENT

Data Supplement

Protocol

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INTRODUCTION

Asparaginase plays an important role in the treatment of pediatric ALL.[1-3] Hypersensitivity is one of its major side effects and hampers its use. The formation of antidrug antibodies accelerates clearance and neutralizes asparaginase with or without clinical symptoms of an allergy. The latter case is called silent inactivation and can only be diagnosed by monitoring asparaginase activity levels.[4,5]

A meta-analysis of the Ponte di Legno Toxicity Working group showed that hypersensitivity against PEGasparaginase (PEGasp) in induction, with concomitant corticosteroids, was rare and occurred in approximately 2% of the patients.[6] Reactions almost exclusively occurred after an asparaginase-free interval, resulting in a significantly higher postinduction rate of 8%. That study also showed that the number of asparaginase-free intervals is an important determinant for allergic reactions to PEGasp.

CONTEXT

Key Objective

Is it possible to reduce the rate of hypersensitivity reactions by switching from a standard noncontinuous PEGasparaginase (PEGasp) dosing schedule with an asparaginase-free interval to a continuous PEGasp dosing schedule without any intervals?

Knowledge Generated

A continuous PEGasp dosing schedule led to a seven-fold reduction in inactivating hypersensitivity reactions. The continuous dosing schedule was safe and did not change the efficacy of the ALL therapy.

Relevance *(S. Lentzsch)*

Continuous PEGasp administration for children with ALL should be preferred. A continuous PEGasp strategy will significantly increase the proportion of patients who complete all asparaginase treatment, which is especially important in countries with shortages of second-line Erwinia asparaginase.*

*Relevance section written by *JCO* Associate Editor Suzanne Lentzsch, MD, PhD.

The former Dutch Childhood Oncology Group (DCOG) ALL10 protocol used native Escherichia coli asparaginase in induction and 15 doses of PEGasp in medium-risk (MR) intensification.[7] Thirty percent of the patients had an allergy or silent inactivation in intensification, necessitating a switch to Erwinia asparaginase. In addition, we found that during the asparaginase-free interval, antidrug antibody levels increased and were significantly higher in patients experiencing an allergic reaction or having silent inactivation in intensification.[8] Continuing exposure to PEGasp in patients with silent inactivation reduced the antidrug antibody levels and resulted in recovery of therapeutic asparaginase activity levels.[9]

We therefore hypothesized that continuous dosing without an asparaginase-free interval will reduce the incidence of hypersensitivity. The DCOG ALL11 protocol is based on DCOG ALL10, but several changes were made for all patients to improve asparaginase therapy.[10] First, the less immunogenic PEGasp was used during induction and intensification to prevent allergic side effects. Second, a real-time therapeutic drug monitoring (TDM) program was used to individualize asparaginase dosing schedules. Third, a reduced starting dose of PEGasp (1,500 IU/m² once every two weeks instead of 2,500 IU/m² once every two weeks) was implemented because of the high PEGasp activity levels (mean trough level 899 U/L) found in ALL10, as levels >100 U/L fully deplete asparagine levels.[8] In the present randomized study, we analyzed whether a continuous dosing schedule (without an asparaginase-free interval) resulted in less hypersensitivity reactions to PEGasp compared with the standard noncontinuous dosing schedule (with an asparaginase-free interval), whereas patients received an equal total number of PEGasp doses. We also compared asparaginase-related toxicities, antidrug-antibody formation, and outcome and described individualized dosing using TDM of both schedules.

METHODS

Patients and Treatment

From November 2012 to July 2020, children age 1-18 year with newly diagnosed ALL were enrolled in the DCOG ALL11 protocol (EudraCT 2012-000067-25; Dutch Trial Register NL3227). Details of this study are described elsewhere.[10] The study was approved by the Institutional Review Board. Informed consent was obtained from the parents or guardians and from patients 12 years and older according to the Dutch law. This study was conducted in accordance with the Declaration of Helsinki.

All patients received three PEGasp doses of 1,500 IU/m² once every two weeks intravenously at protocol days 12 and 26 (protocol IA) and 40 (protocol IB). In the postinduction phase, standard-risk patients received one dose, MR patients 14 doses, and high-risk patients two to five doses of PEGasp (Data Supplement, Fig S2, online only). No universal premedication was used. With the nationwide TDM program, 14 ± 2 days postadministration, trough serum asparaginase activity levels were measured with the aspartic acid β-hydroxamate test in real time for dose adjustments with a target serum level of 100-250 U/L.[11] Weekly serum levels were measured after the first PEGasp dose or the first dose after an asparaginase-free interval for early detection of silent inactivation. Patients with silent inactivation or allergic reaction to PEGasp received individualized Erwinia asparaginase treatment (starting dose of 20,000 IU/m² given three or four times a week). Six doses substituted one PEGasp dose.

MR patients without contraindication for receiving PEGasp were randomly assigned to receive 14 individualized PEGasp doses every 2 weeks at the start of MR intensification

(standard arm A: noncontinuous schedule) or to continue PEGasp every 2 weeks after the third dose in protocol IB (experimental arm B: continuous schedule; Fig 1).

End Points

Hypersensitivity

Primary end point was incidence of hypersensitivity. Three different types of hypersensitivity were described as defined previously[6]: (1) allergies: allergic reactions with inactivation of PEGasp, (2) allergic-like reactions: symptoms of an allergic reaction without inactivation of PEGasp, and (3) silent inactivation: inactivation of PEGasp without symptoms of an allergic reaction. Total hypersensitivity was defined as the sum of allergies, allergic-like reactions, and silent inactivations. Inactivating hypersensitivity was defined as the sum of allergies and silent inactivations. One event per patient was counted in the analysis.

For the first part of our hypersensitivity analysis covering the first three doses of PEGasp during induction, we included patients who had received at least one PEGasp dose. For the second part, the postinduction analysis, we included patients who had received at least one PEGasp dose according to the noncontinuous or continuous schedule. The severity of allergic reactions was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.[12]

Antidrug Antibodies

A bead-based assay (Protavio Ltd, Athens, Greece) was used to measure anti-PEG and anti–E. coli asparaginase antibodies (Data Supplement). Antibody levels were collected at five different time points before and after PEGasp random assignment (Fig 1). One sample was collected before PEGasp treatment to measure pre-existing anti-PEG levels. Antibody levels were compared over time between treatment arms.

Nonallergic Asparaginase-Related Adverse Events

Other asparaginase-related adverse events (AEs) CTCAE v4.03 grade ≥3, including major clinical toxicities, such as febrile neutropenia, infection, thromboembolic event, pancreatitis, and avascular necrosis, and minor laboratory toxicities, such as an increase in blood bilirubin, ALT, AST, amylase, glucose, and triglycerides, were collected during each treatment phase. The duration of each treatment phase was also monitored to determine any differences in treatment delays between treatment arms.

Outcome

Cumulative incidence of relapse (CIR), cumulative incidence of death (CID) in first remission (CR1), and disease-free survival (DFS) of both treatment arms were compared.

Intravenous Immunoglobulin Prophylaxis Random Assignment

Within the ALL11 study, there was a second random assignment to receive or not receive intravenous immunoglobulin (IVIG) prophylaxis, the results of which will be published separately. We evaluated the distribution of patients with or without IVIG prophylaxis across both treatment arms and analyzed whether IVIG prophylaxis affected the hypersensitivity rate.

Statistical Analysis

The power calculation for the PEGasp random assignment is available in the Data Supplement (Tables S1 and S2 and Fig S1). A two-sample test for equality of proportions with continuity correction was used to compare two observed

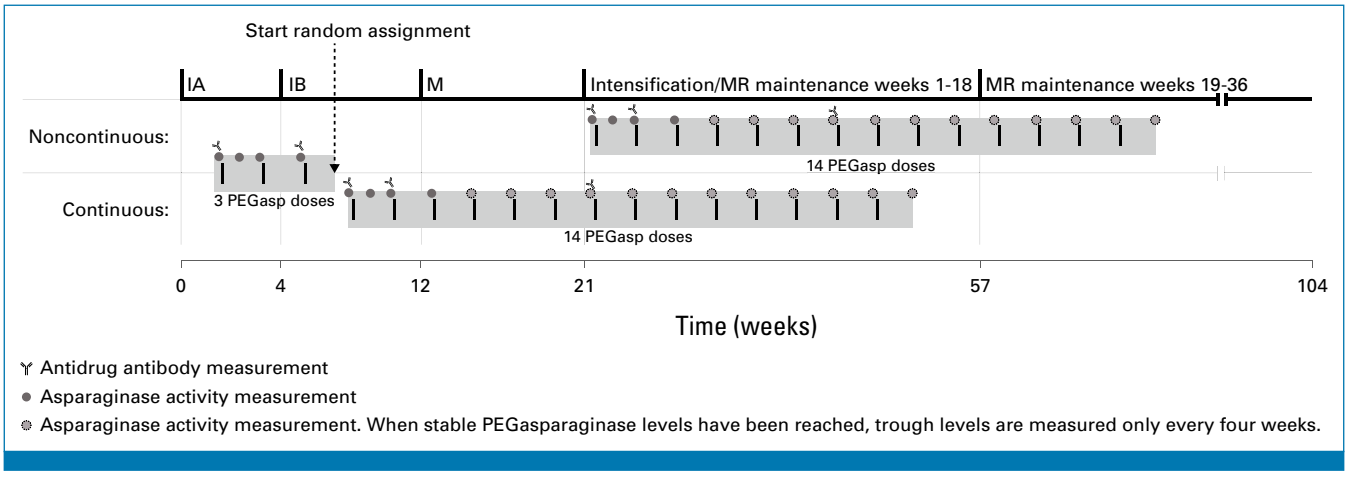

FIG 1. PEGasparaginase dosing and sample collection schedule. All samples for antibody and asparaginase activity measurements were collected before dose administration. MR, medium risk.

proportions. Odds ratio (OR) and 95% CIs were calculated using logistic regression models, with a two-sided *P* value <.05. We estimate a linear mixed-effects model to assess the change in antibody levels over time between the two arms. To estimate DFS from random assignment where the event is defined as relapse, secondary malignancy, or death, Kaplan-Meier's methodology was used. CIR in first remission for the two treatment arms was estimated by a competing-risk model[13] with death as the competing event. A computing risk model with relapse as the competing event was used to estimate CID in first remission.

Median follow-up time was assessed by the reverse Kaplan-Meier method.[14] To assess the difference in the CIR or CID, Gray's log-rank test was used.[15] To study the association between the number of nonallergic AEs (grade ≥3) and the independent factors, treatment phase and treatment arm, a generalized linear mixed-effects Poisson model was used; an interaction term between the two independent variables was also incorporated in the model. Analyses were performed by using intention-to-treat. Statistical analyses were performed in R software environment version 1.3.1093[16] and SPSS-Rel. 20.0.2012 (SPSS Inc, Chicago, IL). R libraries lme4, cmprsk, and survminer were used.

RESULTS

Patient and Treatment

Eight hundred nineteen patients with newly diagnosed ALL were treated according to the ALL11 protocol, of which 818 received at least the first PEGasp dose in induction. Five hundred seventy patients were stratified into the MR arm. In 23 MR patients, PEGasp treatment was truncated before receiving their first postinduction (see the CONSORT diagram in Fig 2). Of the 547 MR patients, 312 were randomly assigned and received at least one PEGasp dose: 157 and 155 patients in the noncontinuous and continuous asparaginase treatment arm, respectively. Patient characteristics of both arms did not differ (Data Supplement, Table S3).

The Data Supplement (Fig S3) shows the PEGasp doses and the corresponding trough asparaginase activity levels of nonallergic patients. The first three administrations were with a fixed dose of 1,500 IU/m² once every two weeks. The median trough PEGasp activity was 454 U/L (IQR, 344-553 U/L) in induction. After random assignment, individualized treatment with a target range of 100-250 U/L started. Overall, 2.6% of the serum levels of nonallergic patients were <100 U/L. Median PEGasp activity levels within the target range were reached after the ninth and 10th administration in the continuous and noncontinuous arm, respectively, with a median dose of 400 IU/m² (IQR, 400-500 IU/m²) once every two weeks. Median trough PEGasp activities were 194 U/L (158-234 U/L) and 192 U/L (154-238 U/L) in the continuous and noncontinuous arm, respectively.

End Points

Hypersensitivity

In induction, hypersensitivity reactions occurred in 27 patients (3.3%), of which eight (1.0%) were allergic-like reactions, 12 (1.4%) allergies, and seven (0.9%) silent inactivations (Data Supplement, Table S4). Fifteen of these 27 (55.6%) hypersensitivity reactions occurred on the first dose, two (7.4%) on the second dose, and 10 (37.0%) on the third dose.

After random assignment, four (2.6%) patients in the continuous treatment arm versus 17 (10.8%) patients in the noncontinuous treatment arm ($P < .01$) had hypersensitivity reactions, of which two (1.3%) versus 13 (8.3%) patients had inactivating hypersensitivity reactions ($P < .01$; Fig 3A; Data Supplement, Table S4). Of the 17 hypersensitivity reactions in the noncontinuous arm, 14 (82%) occurred on the first dose after the asparaginase-free period between induction and intensification. See the Data Supplement (Table S5) for the severity of allergic reactions. Patients in the continuous treatment arm were significantly less likely to get an inactivating hypersensitivity reaction (OR, 0.15 [0.032-0.653]).

Fifteen patients with inactivating hypersensitivity and one patient with an allergic-like reaction switched to Erwinia asparaginase. No subsequent inactivating hypersensitivity reactions to Erwinia asparaginase were found, but two of 16 (12.5%) patients developed an allergic-like reaction to Erwinia asparaginase; both were re-exposed and completed treatment.

Antibodies

Total antibody levels, including anti–E. coli asparaginase and anti-PEG, were significantly lower in the continuous treatment arm compared with the noncontinuous arm over time ($P < .01$; Fig 4). Without the asparaginase-free interval between doses 3 and 4, the antibody levels slightly increased by 1.1-fold, whereas antibody levels in the noncontinuous arm increased by 2.5-fold between doses 3 and 4 (during the asparaginase-free interval). The continuous arm showed lower total antibody levels, which were largely attributed to the significantly lower anti-PEG antibody levels over time ($P < .0001$), whereas anti–E. coli antibody levels did not differ significantly between the two arms. Ninety percent of the patients with an inactivating hypersensitivity reaction were positive for anti-PEG, and up to 60% for anti-E. coli asparaginase antibodies after the first postrandomization dose (Data Supplement, Fig S4). In the patients without an inactivating hypersensitivity reaction, 32% had anti-PEG and 13% had anti–E. coli asparaginase antibodies.

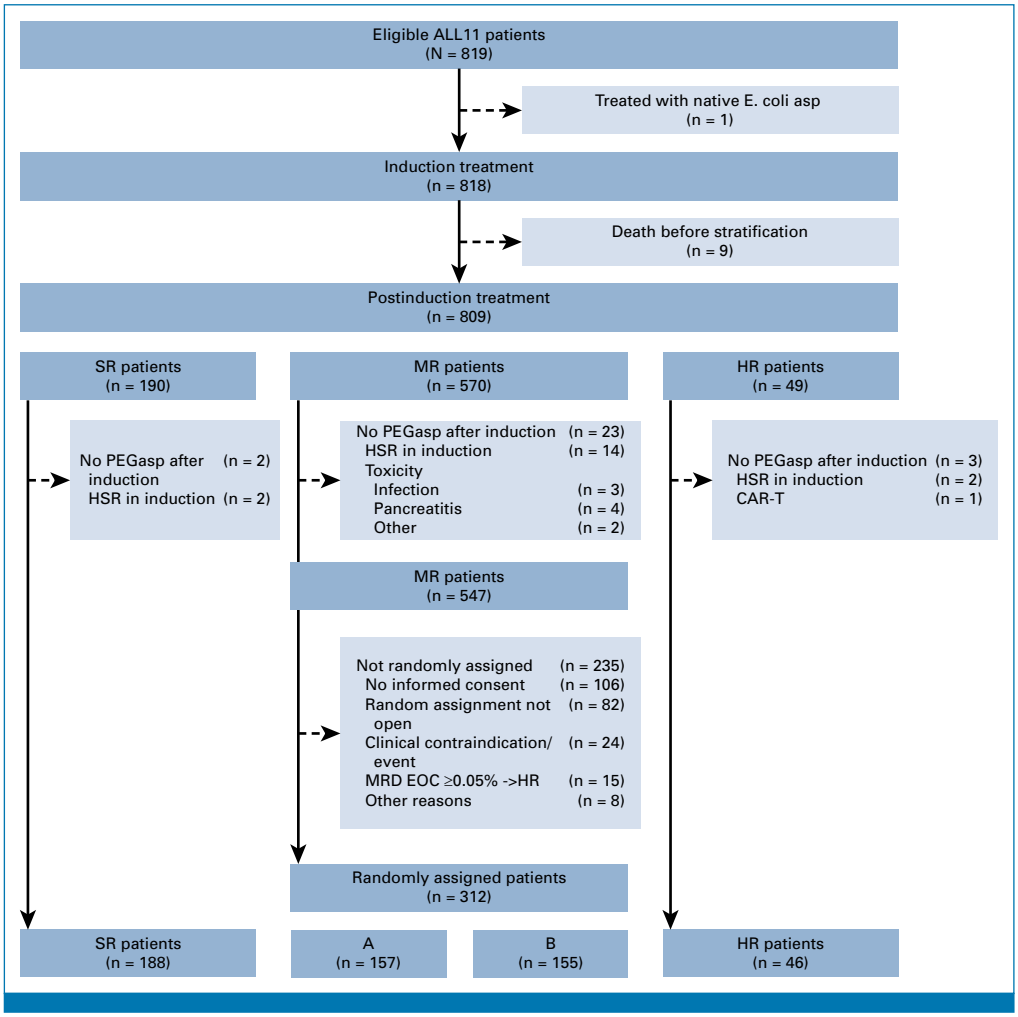

FIG 2. CONSORT flow diagram. HR, high-risk; HSR, hypersensitivity reaction; MR, medium risk; MRD EOC, minimal residual disease at the end of consolidation; SR, standard risk.

Nonallergic Asparaginase-Related Adverse Events

The total incidence of nonallergic asparaginase-related AEs ≥grade 3 is shown per treatment arm in Figure 3B. There were no statistically significant differences between the noncontinuous and continuous arm, with exception of a lower incidence of increased serum amylase in the continuous arm ($P < .05$).

The number of toxicities per treatment phase is shown in Table 1. Notably, the generalized Poisson model analysis showed that the occurrence of specific AEs was influenced by treatment phase and treatment arm (Data Supplement, Table S6). The treatment phase was associated with the occurrence of febrile neutropenia, a thromboembolic event, increased ALT, increased AST, hypertriglyceridemia, increased blood bilirubin, and increased serum amylase. In addition, the treatment arm was found to be associated with the occurrence of a thromboembolic event, pancreatitis, and increased bilirubin. Moreover, thromboembolic event, pancreatitis hypertriglyceridemia, and increased amylase were significantly associated with both the treatment arm and phase (interaction term).

In more detail, we observed (Table 1), for example, that thromboembolic events were higher in the continuous arm during protocol IB (n = 8; 5.2%) and protocol M (n = 6; 3.9%), compared with the noncontinuous arm (n = 3; 1.9% in protocol IB and n = 3; 1.9% in protocol M). Conversely, the continuous arm had lower occurrences of thromboembolic events during MR maintenance weeks 1-18 (n = 7; 4.5%) and weeks 19-36 (n = 3; 2.0%) compared with the noncontinuous arm (n = 11; 7.0% in weeks 1-18 and n = 13; 8.4% in weeks 19-36). A similar trend was observed for pancreatitis, with higher occurrence in the continuous arm during protocol IB (n = 4; 2.6%) and lower occurrence during MR maintenance weeks 1-18 (n = 1; 0.6%) and weeks 19-36 (n = 0; 0.0%), compared with the noncontinuous arm (n = 1; 0.6% in protocol IB, n = 8; 5.1% in MR maintenance weeks 1-18, and n = 3; 1.9% in MR maintenance weeks 19-36).

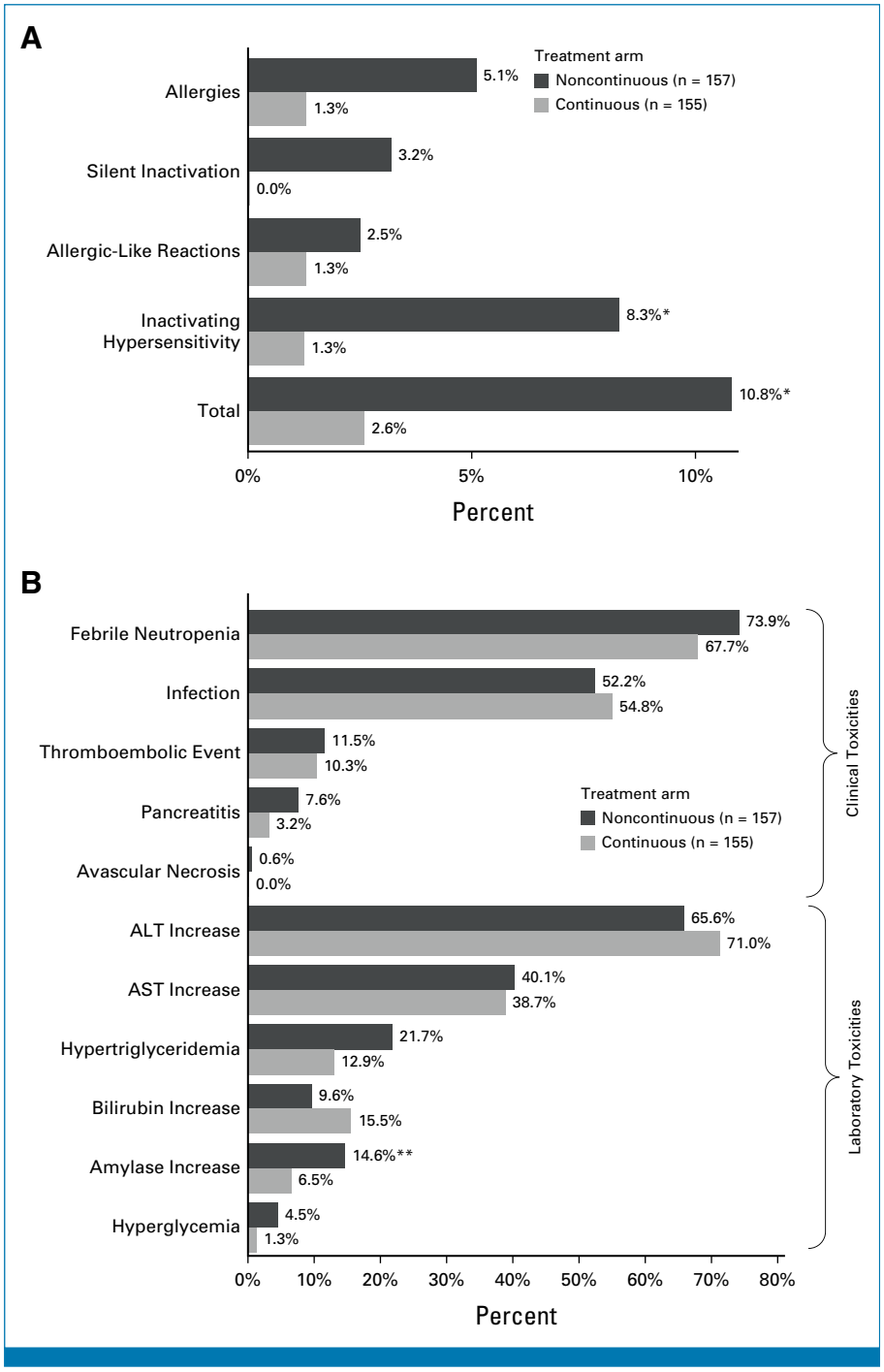

FIG 3. (A) Rate of hypersensitivity and (B) other clinical and laboratory toxicities (CTCAE ≥grade 3) after random assignment. Inactivating hypersensitivity reactions are the sum of allergies and silent inactivations. Hypersensitivity is the sum of allergies, silent inactivation, and allergic-like reactions. Rate of allergies, silent inactivations, and allergic-like reactions were not tested because of small numbers. *P < .01; **P < .05. CTCAE, Common Terminology Criteria for Adverse Events.

The median duration of treatment phases was comparable between the two arms, with the exception of a longer duration of protocol M in the continuous versus noncontinuous arm (median 10.3 weeks *v* 9.4 weeks, respectively, instead of the planned 9 weeks; P < .01; Data Supplement, Table S7). Asparaginase treatment was truncated in 10 (6.4%) patients in the noncontinuous treatment arm. The main reason for truncation was pancreatitis (n = 6), followed by intolerance (nausea) to Erwinia asparaginase (n = 2), hepatotoxicity (n = 1), and increased amylase (n = 1). In the continuous

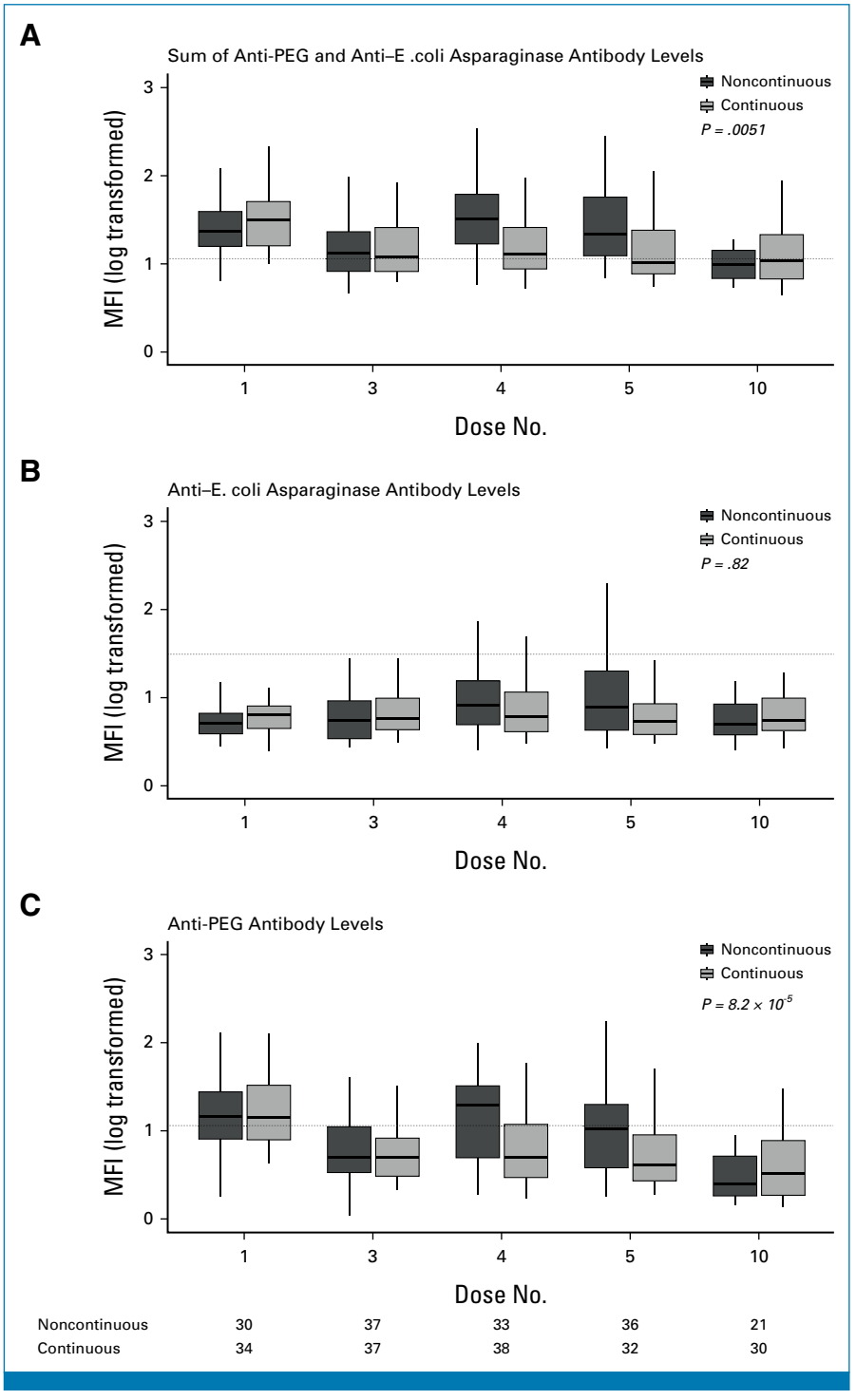

FIG 4. (A) Sum of anti-PEG and anti–E. coli asparaginase antibody levels, (B) anti–E. coli antibody levels, and (C) anti-PEG antibody levels in the noncontinuous and continuous arms. Dashed lines are thresholds to determine if samples were positive or negative. We used a linear mixed-effects model to assess the change in antibody levels over time between the two arms. MFI, mean fluorescence intensity; PEG, polyethylene glycol.

TABLE 1. Overview of Reported Toxicities per Protocol Phase

	Protocol IB		Protocol M		MR Maintenance Weeks 1-18		MR Maintenance Weeks 19-36	
Parameter	Noncontinuous	Continuous	Noncontinuous	Continuous	Noncontinuous	Continuous	Noncontinuous	Continuous
Patients, No.	157	155	157	155	157	154	155	151
PEGasp doses, No.	1[a]	3	0	4	10	8	4	0
Hypersensitivity, No. (%)	0 (0.0)	3 (1.9)	0 (0.0)	0 (0.0)	16 (10.2)	1 (0.6)	1 (0.6)	0 (0.0)
Major, No. (%)								
Febrile neutropenia	81 (51.6)	74 (47.7)	22 (14.0)	35 (22.3)	41 (26.1)	40 (26.0)	36 (23.2)	18 (11.9)
Infection	41 (26.1)	43 (27.7)	14 (8.9)	29 (18.7)	31 (19.7)	35 (22.1)	28 (18.1)	24 (15.7)
Thromboembolic event	3 (1.9)	8 (5.2)	3 (1.9)	6 (3.9)	11 (7.0)	7 (4.5)	13 (8.4)	3 (2.0)
Pancreatitis	1 (0.6)	4 (2.6)	0 (0.0)	0 (0.0)	8 (5.1)	1 (0.6)	3 (1.9)	0 (0.0)
Avascular necrosis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
Minor, No. (%)								
ALT increase	39 (24.8)	23 (14.8)	8 (5.1)	25 (16.1)	68 (43.3)	72 (46.8)	62 (40.0)	57 (37.7)
AST increase	17 (10.8)	11 (7.1)	5 (3.2)	9 (5.8)	28 (17.8)	37 (24.0)	31 (20.0)	21 (13.9)
Hypertriglyceridemia	6 (3.8)	5 (3.2)	0 (0.0)	10 (6.5)	30 (19.1)	10 (6.5)	17 (11.0)	0 (0.0)
Bilirubin increase	12 (7.6)	20 (12.9)	0 (0.0)	3 (1.9)	2 (1.3)	3 (1.9)	3 (1.9)	0 (0.0)
Amylase increase	2 (1.3)	4 (2.6)	0 (0.0)	1 (0.6)	18 (11.5)	5 (3.2)	7 (4.5)	0 (0.0)
Hyperglycemia	2 (1.3)	2 (1.3)	0 (0.0)	0 (0.0)	6 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)

NOTE. This table provides an overview of the reported adverse reactions per protocol phase in each treatment arm. The first row of this table presents the number (percentage) of hypersensitivity reactions, encompassing clinical allergies, allergic-like reactions, and silent inactivations, without grading. The subsequent rows represent the number (percentage) of patients with major clinical toxicities and/or minor laboratory toxicities (grade ≥3) reported as highest grade per protocol phase. Because of small numbers in most categories, only descriptives are shown.
Abbreviations: MR, medium-risk; PEGasp, PEGasparaginase.
[a]One dose was given on day 40 at the end of induction (phase IA), just before random assignment.

treatment arm, asparaginase treatment was truncated in eight (5.2%) patients. The main reason for truncation was also pancreatitis (n = 5), followed by thromboembolic events (n = 3).

Outcome

The estimated median follow-up time was 57.2 months (95% CI, 51.9 to 61.3). No difference in 5-year CIR, CID, or DFS was found between both treatment groups. The 5-year CIR was 4.0% (SE, 1.8%) for noncontinuous treatment versus 5.6% (SE, 2.1%) for continuous treatment (Fig 5A). The 5-year CID in CR1 was 0.6% (SE, 0.6%) for noncontinuous treatment versus 1.9% (SE, 1.1%) for continuous treatment (Fig 5A). The 5-year DFS was 95.3% (SE, 1.9%) for noncontinuous treatment versus 91.9% (SE, 2.4%) for continuous treatment (Fig 5B).

IVIG and Hypersensitivity Reactions to Asparaginase

Patients with (n = 73; 53.3%) and without (n = 64; 46.7%) IVIG prophylaxis were equally distributed across the continuous and noncontinuous asparaginase treatment arms (Data Supplement, Table S8). Thirty-one of 64 (48.4%) patients in the continuous group and 42 of 73 (57.5%) patients in the noncontinuous group were treated with IVIG prophylaxis. The use of IVIG prophylaxis did not affect the incidence of hypersensitivity reactions since three of the 73 (4.1%) treated with IVIG prophylaxis had a hypersensitivity reaction versus four of the 64 (6.2%) of the control group (P = .858).

DISCUSSION

Among children and adolescents with newly diagnosed ALL, a continuous dosing schedule of PEGasp resulted in a seven times lower incidence of inactivating hypersensitivity reactions and lower antidrug antibody levels compared with a noncontinuous dosing schedule with an asparaginase-free interval of 3 months. A continuous dosing schedule did not result in more toxicity. Concomitant administration of PEGasp in protocol IB and during high-dose methotrexate (HDMTX) was feasible and safe. No difference in CIR, CID, or DFS was found between both treatment arms.

Reducing the hypersensitivity reactions to PEGasp is of importance from both a patient and cost perspective. Studies showed that patients with truncations of asparaginase treatment or inadequate asparaginase activity levels have a worse outcome.[1-3,17] This emphasizes the importance of completing the planned asparaginase therapy. Thus, patients with an allergic reaction to or silent inactivation of PEGasp have to switch to Erwinia asparaginase, which has a less convenient dosing schedule. This switch is not always performed because of higher costs[18] or (global) supply shortages of Erwinia asparaginase[19] or because of the fact

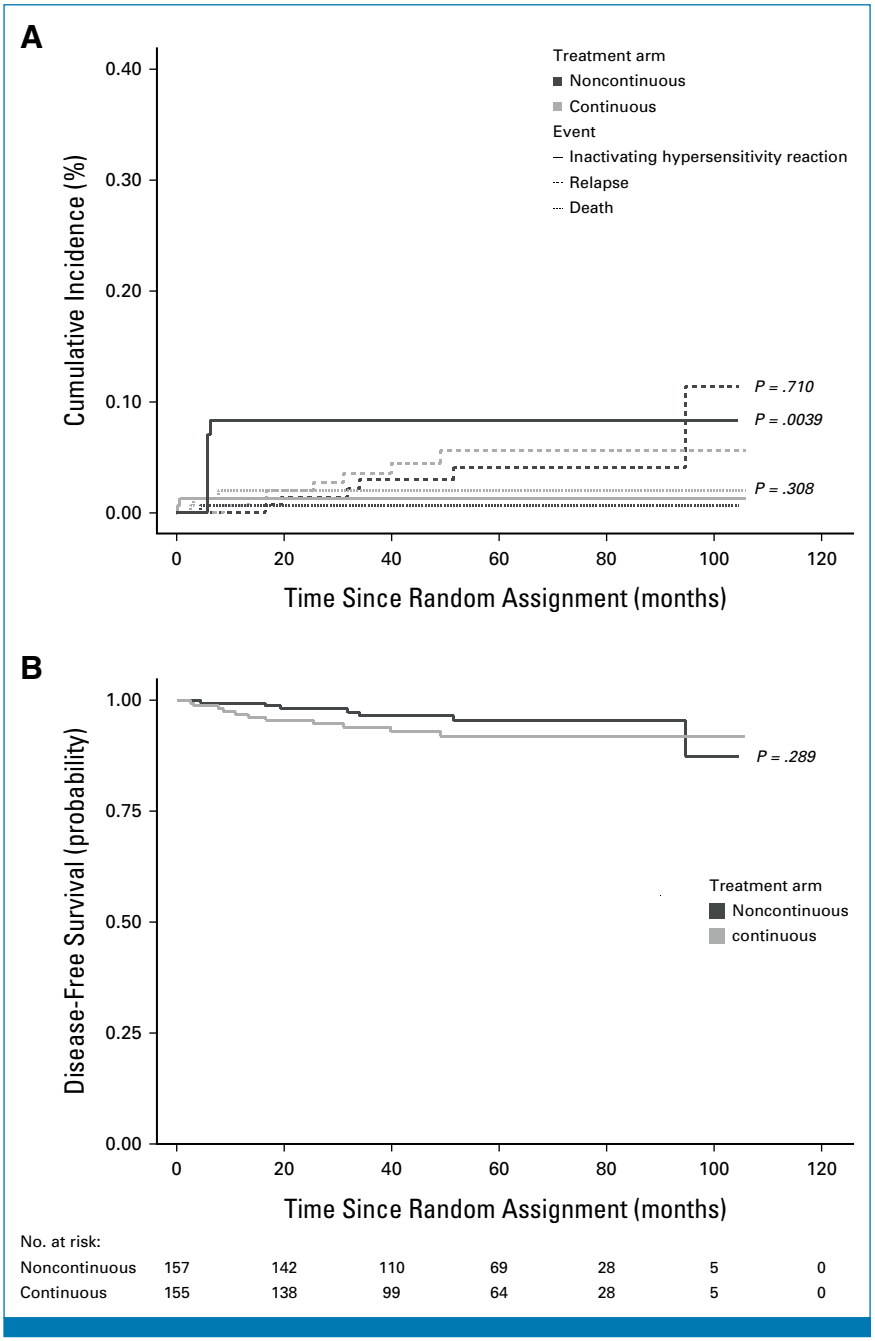

FIG 5. (A) Cumulative incidence of inactivating hypersensitivity reactions (solid line), relapse (dashed line), and death (dotted line) in CR1 and (B) disease-free survival in noncontinuous (dark grey) and continuous arms (light grey).

that TDM is not performed and silent inactivation of the drug is not diagnosed.

A recent meta-regression analysis clearly showed that the higher number of asparaginase-free intervals is the most significant risk factor for developing allergic reactions.[6] Our study confirmed our hypothesis that a continuous dosing schedule without an asparaginase-free interval is an effective approach to reduce antibody formation and prevent hypersensitivity reactions. The hypersensitivity rates in induction and noncontinuous dosing schedule were comparable with incidences reported in other protocols using PEGasp as first-line treatment.[6,20,21] After an PEGasp-free interval, inactivating hypersensitivity reactions occurred exclusively after the first and second dose, which is consistent with previous reports.[8,21,22] This observation suggests that the number of subsequent doses is of less importance for developing inactivating hypersensitivity reactions when

asparaginase treatment is continued without additional breaks.

To our knowledge, the NOPHO2008 trial is the only study that also randomly assigned intermittent versus continuous dosing of PEGasp. In contrast to our findings, no differences in enzyme inactivation (approximately 5.5%) or clinical allergies (approximately 2%) between both treatment arms were found.[23] Important differences with our study that may explain these apparent contradictory results are that they compared 10 continuous doses with three intermittent doses, which were administered every 6 weeks. Patients were randomly assigned after five continuous doses, which started after induction. The cumulative clinical allergy risk was 13%, and since reactions occurred after a median of two doses,[22] most patients prone to developing a hypersensitivity reaction already had one before random assignment. Finally, the asparaginase-free interval of 6 weeks was rather short.

The continuous dosing schedule appeared to be safe. CID in CR1 was comparable in both arms, and there were no significant differences in total number of asparaginase-associated toxicities, except for the frequency of increased serum amylase, which was even lower than that in the noncontinuous group. However, the timing of the toxicities was associated with the timing of the asparaginase administrations.

The continuous PEGasp treatment arm included four HDMTX courses. In vitro studies have shown that asparagine depletion by asparaginase could inhibit MTX polyglutamination.[24-26] So, asparaginase could potentially decrease MTX efficacy, which is primarily determined by intracellular levels of long-chain MTX polyglutamates. We therefore compared intracellular MTX polyglutamate levels in a subgroup of patients in both treatment arms and demonstrated that the impact of continuous asparaginase on MTX efficacy was less pronounced in vivo, with ongoing MTX polyglutamination and formation of long-chain MTX glutamates.[27] Moreover, in the current study, we found that the timing of asparaginase treatment did not change the efficacy of the therapy reflected by comparable CIR and DFS between both arms.

We showed that a continuous dosing schedule resulted in less antibody formation and thereby reduced the incidence of hypersensitivity reactions. The majority (90%) of patients with inactivating hypersensitivity reactions to PEGasp had anti-PEG antibodies. However, 32% of the patients without inactivating hypersensitivity also tested positive, making the use of antibodies not a suitable alternative for TDM to detect inactivation of asparaginase.

The postrandomization hypersensitivity rate of 10.8% in the noncontinuous PEGasp arm was much lower than that reported in previous studies using native E. coli asparaginase or PEGasp after native E. coli asparaginase in induction (30%-75%).[8,17,28-30]

Another advantage of a continuous dosing schedule is that it simplifies the interpretation of TDM, especially distinguishing allergic-like and real allergic reactions. Individualized dosing with dose adaptations is easier as well with continuous dosing, and fewer week levels need to be taken because there will be no additional doses at risk for silent inactivation.

We conclude that a continuous dosing schedule of PEGasp significantly reduces the inactivating hypersensitivity rate and antibody formation compared with a noncontinuous schedule. The continuous schedule of asparaginase treatment did not lead to more toxicity but changed the timing of toxicity. Finally, the continuous schedule did not change the efficacy of the therapy.

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EQUAL CONTRIBUTION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Continuous PEGasparaginase Dosing Reduces Hypersensitivity Reactions in Pediatric ALL: A Dutch Childhood Oncology Group ALL11 Randomized Trial

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