Hypersensitivity Reactions to Native E. coli L-asparaginase in Children With Acute Lymphoblastic Leukemia Treated in Trial ALL-BFM 2000: Impact of Treatment Schedule and Type of Glucocorticoid in Induction

Möricke, Anja ; Rizzari, Carmelo ; Alten, Julia ; Attarbaschi, Andishe ; Beier, Rita ; Biondi, Andrea ; Burkhardt, Birgit ; Bodmer, Nicole ; Boos, Joachim ; Cario, Gunnar ; Conter, Valentino ; Flotho, Christian ; Kulozik, Andreas ; Lanvers-Kaminsky, Claudia ; Mann, Georg ; Niggli, Felix ; Silvestri, Daniela ; von Stackelberg, Arend ; Stanulla, Martin ; Valsecchi, Maria-Grazia ; Schrappe, Martin ; Zimmermann, Martin

Letter

Open Access

Hypersensitivity Reactions to Native *E. coli* L-asparaginase in Children With Acute Lymphoblastic Leukemia Treated in Trial ALL-BFM 2000: Impact of Treatment Schedule and Type of Glucocorticoid in Induction

Anja Möricke[1,*], Carmelo Rizzari[2,*], Julia Alten[1], Andishe Attarbaschi[3,4], Rita Beier[5], Andrea Biondi[2], Birgit Burkhardt[6], Nicole Bodmer[7], Joachim Boos[6], Gunnar Cario[1], Valentino Conter[2], Christian Flotho[8], Andreas Kulozik[9], Claudia Lanvers-Kaminsky[6], Georg Mann[4], Felix Niggli[7], Daniela Silvestri[10], Arend von Stackelberg[11], Martin Stanulla[5], Maria-Grazia Valsecchi[10], Martin Schrappe[1], Martin Zimmermann[5]

Correspondence: Anja Möricke (a.moericke@pediatrics.uni-kiel.de).

Asparaginase (ASNase) has become one of the key components in the treatment of acute lymphoblastic leukemia (ALL) by exploiting the inability of leukemic cells to synthesize the otherwise nonessential amino acid asparagine.[1,2] Currently, 3 ASNase products are commercially available: The original native *Escherichia coli* (*E. coli*) enzyme, the polyethylene glycol-conjugated *E. coli* product (PEG-L-asparaginase [PEG-ASNase]) with a longer half-life and reduced immunogenicity, and a structurally different product derived from *Erwinia chrysanthemi* (Erwinase). Among a wide range of ASNase-related toxicities, hypersensitivity reaction (HSR) is the main reason for limiting its use and requires replacement with alternative ASNase preparations. The incidence of clinical HSR to ASNase varies with the type of ASNase product, its treatment schedule, and route of administration.[3–10]

We report a retrospective study evaluating the incidence of clinically evident HSR to ASNase in the ALL-Berlin/Frankfurt/Münster (BFM) 2000 study using ASNase in a complex multiagent treatment protocol. ALL-BFM 2000 was part of the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP)-BFM ALL 2000 cooperative trial (registered at http://clinicaltrials.gov as NCT00430118 for BFM [Germany, Austria, and Switzerland]).[11,12] In ALL-BFM 2000, native *E. coli* ASNase (medac, GmbH) was administered intravenously (IV) as first-line product during induction and reinduction, and in the high-risk (HR) arm during intensified consolidation, resulting in a postinduction ASNase-free interval of ~16 weeks for standard risk (SR) and medium risk (MR) patients, and 6 weeks for HR patients (Figure 1). PEG-ASNase (Oncaspar, IV) or Erwinase (intramuscular) was used as second- or third-line product after HSR to native *E. coli* ASNase or the respective second-line product (Figure 1). Details of the trial AIEOP-BFM ALL 2000 have been published earlier.[11,12]

ASNase-related HSR was not systematically captured in ALL-BFM 2000. Data for the present study were collected retrospectively in 2162 patients with newly diagnosed ALL (≥1 to <18 years of age) consecutively enrolled in Germany or Switzerland between August 1999 and March 2005. Patients were included if documentation of induction protocol IA plus at least the following ASNase-containing therapy phase was available and native *E. coli* ASNase was used as first-line ASNase product. Patients who changed the ASNase product or discontinued ASNase early were identified through regular chemotherapy documentation, and patient records were reviewed to identify

[1]Department of Pediatrics I, Pediatric Hematology/Oncology, ALL-BFM Study Group, Christian Albrechts University Kiel and University Hospital Schleswig-Holstein, Campus Kiel, Germany
[2]Pediatric Hematology-Oncology Unit, Department of Pediatrics, University of Milano-Bicocca, MBBM Foundation, Monza, Italy
[3]Department of Pediatric Hematology and Oncology, St. Anna Children's Hospital, Medical University of Vienna, Austria
[4]St. Anna Kinderspital and Children's Cancer Research Institute, Vienna, Austria
[5]Department of Pediatric Hematology/Oncology, Hannover Medical School, Hannover, Germany
[6]Department of Paediatric Hematology and Oncology, University Hospital Muenster, Germany
[7]University Children's Hospital, Zurich, Switzerland
[8]Division of Pediatric Hematology and Oncology, Medical Center, Faculty of Medicine, University of Freiburg, Germany
[9]Department of Pediatric Oncology, Hematology and Immunology, University of Heidelberg, Germany
[10]Bicocca Center of Bioinformatics, Biostatistics and Bioimaging, School of Medicine and Surgery, University of Milan-Bicocca, Monza, Italy
[11]Pediatric Hematology and Oncology, Charité Medical Center, Humboldt University, Berlin, Germany
*AM and CR have contributed equally to this work.
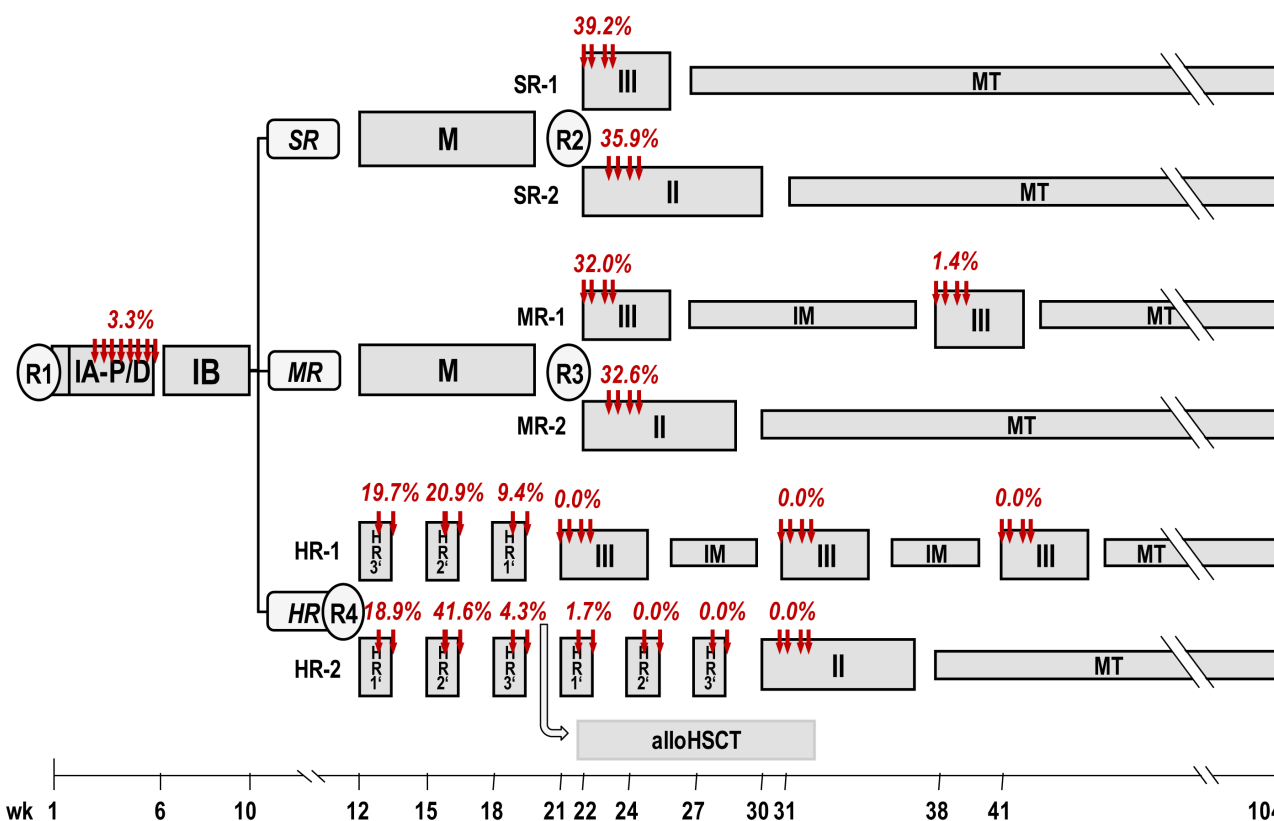Supplemental digital content is available for this article.

ALL-BFM 2000

Figure 1. Outline of the ALL-BFM 2000 protocol including the randomized studies (R1–4) with incidences of hypersensitivity reactions (HSR) to native *E. coli* ASNase. Native *E. coli* ASNase product (medac, GmbH) was administered IV as 1-h infusion per dose during induction protocol IA (5000 IU/m²/dose every 3 d for a total of 8 doses starting on day 12), protocol III (10,000 IU/m²/dose twice a week for 4 doses, starting on day 1), protocol II (10,000 IU/m²/dose twice a week for 4 doses starting on day 8), and HR blocks (25,000 IU/m²/dose either on day 6 [before July 2000] or on days 6 and 11 [after amendment on July 1, 2000] of each block). In the case of an HSR to the native *E. coli* ASNase product, the administration of PEG-ASNase (Oncaspar, 1000 IU/m²/dose IV, 1 dose replacing a 2-week phase of native *E. coli* ASNase) or Erwinase (10,000 U/m²/dose IM, every 2 d) was recommended for the remaining time scheduled for ASNase therapy. In case of further HSR, it was recommended to switch to the respective other substitute product. The study included 4 randomized questions. (1) All patients: either prednisone at 60 mg/m²/day or dexamethasone at 10 mg/m²/day for 3 weeks plus 9-day tapering phase (randomization R1) during induction protocol IA after a 7-d prednisone prephase.[13] (2) SR patients: either reinduction protocol III (branch SR-1) or protocol II (branch SR-2) (randomization R2).[14] (3) MR patients: either reinduction protocol III twice (branch MR-1) or protocol II (branch MR-2) (randomization R3).[15] (4) HR patients of BFM countries were randomized to receive either 3 HR blocks (HR-3', HR-2', and HR-1') plus 3 times protocol III (branch HR-1) or 6 HR blocks (HR-1', HR-2', HR-3', HR-1', HR-2', HR-3') plus protocol II (branch HR-2) during postconsolidation phases (randomization R4).[16] ASNase doses are indicated as red arrows. Percentages represent the cumulative incidence of HSR per ASNase-containing treatment phase, calculated for all patients treated in the respective treatment arm and including randomized and nonrandomized patients. The number of patients under observation at the start of the respective treatment phases can be found in Suppl. Figure S3. alloHSCT = allogeneic hematopoietic stem cell transplantation; *Escherichia coli* = *E. coli*; HR = high-risk; IA-P/D = protocol IA with either prednisone or dexamethasone; IB = protocol IB; II = protocol II; III = protocol III; IM = interim maintenance therapy; M = protocol M; MR = medium risk; MT = maintenance therapy; SR = standard risk.

those with treatment changes due to ASNase-related HSR. Due to the retrospective nature of the analysis, specific criteria to define HSR or its severity could not be applied. The diagnosis of HSR was, therefore, based on the clinical judgement of the treating physician. Patients who switched to PEG-ASNase or Erwinase for unknown reasons or for reasons other than HSR were retained in the analysis.

Of the 1207 patients who met the inclusion criteria (Suppl. Table S1), 492 patients were identified with ASNase product change or discontinuation in the consequence of HSR to first-line native *E. coli* ASNase (Suppl. Figure S1, Suppl. Table S2) with a 1-year cumulative incidence (CI) of HSR of 42.3% (standard error [SE] 1.5%) (Suppl. Figure S2A). It is likely that these reactions diagnosed as allergy included some allergic-like reactions because ASNase activity level measurements, which would allow differentiation from allergy,[17] were not systematically performed in ALL-BFM 2000. The HSR incidence was particularly

high in the HR group, reaching a CI of 65.2% (SE 3.8%). CI in non-HR patients was 40.6% (SE 2.5%) in SR and 37.0% (SE 1.9%) in MR (Suppl. Figure S2B) with the vast majority of reactions occurring on re-exposure in reinduction (Figure 1; Suppl. Figure S3). Although our data suggest that an ASNase schedule with repetitive ASNase doses (as in the HR intensified consolidation regimen) and a long ASNase-free interval[3] (as in the non-HR branch) produce high incidence of HSR, it is remarkable that only very few HSR to the first-line product occurred in the late phases of treatment. This was evident in the MR-1 arm with only 3 HSR in 135 patients in the second protocol III, and – although in a small number of patients – it can also be suspected in the HR arm (Figure 1; Suppl. Figure S3). A similar effect was observed in the DCOG ALL-10 study[18] and also in our AIEOP-BFM ALL 2009 trial with a 20-week phase with IV PEG-ASNase during reinduction/maintenance and the vast majority of clinical HSR presenting to the first 2 doses in

reinduction.[19] These observations suggest that either sensitization or peripheral immune tolerance to ASNase is determined already early after initial antigen exposure. Patients sensitized to ASNase would, therefore, usually develop HSR within a certain period of time while those who were able to achieve peripheral immune tolerance would usually tolerate repeated doses.

ALL-BFM 2000 included 4 randomized questions (Figure 1). Randomization R1 compared dexamethasone and prednisone in induction protocol IA.[13] Incidence of HSR was significantly higher in dexamethasone-treated versus prednisone-treated patients in SR and MR (CI of HSR: SR/Pred 32.3% [SE 3.7%], SR/Dexa 48.5% [SE 4.3%], $P = 0.007$; MR/Pred 31.5% [SE 2.9%], MR/Dexa 45.9% [SE 3.2%], $P < 0.001$) (Figure 2), whereas no difference was observed in HR (HR/Pred 64.0 % [SE 6.2%], HR/Dexa 64.0% [SE 6.2%], $P = 0.59$) (Figure 2). This observation provides interesting insights into possible immunological processes following ASNase exposure during glucocorticoid therapy. Glucocorticoids have various effects on regulatory T cells (Treg), which play a key role in immune tolerance (reviewed in Cari et al[20]). In the phase of first exposure to an antigen, glucocorticoids lead to a decrease of Treg cells and may consequently be able to prevent immune tolerance and promote allergic sensitization.[20] This was shown in various animal models of allergic asthma and rhinitis, but might be transferable to our study in which the patients were exposed to glucocorticoids during the phase of potential sensitization to ASNase. We hypothesize that this immunomodulatory effect varies depending on the type of glucocorticoid leading to the different incidence of HSR observed in dexamethasone-treated or prednisone-treated non-HR patients. In HR, where we found only a shift toward earlier presentation of HSR in the dexamethasone arm, other specific effects of HR therapy may override the differential effects of the induction glucocorticoids.

No difference in the incidence of HSR was seen between the R2 and R3 randomization arms in non-HR patients[14,15] (Figure 1; Suppl. Figure S4A) demonstrating that the dexamethasone prephase in the standard-of-care reinduction protocol II had no protective effect on HSR. In the R4 randomization in the HR group, we saw a borderline significant higher incidence of HSR in the standard-of-care arm HR-2 (71.6% versus 56.0%, $P = 0.08$; Suppl. Figure S4B). This was exclusively due to a twice as high HSR incidence in the second HR block (Figure 1; Suppl. Figure S3D), which was identical (HR-2') in both arms.

The therapy up to this point differed only in the type of the preceding HR block, which was HR-1' in the standard-of-care and HR-3' in the experimental arm,[16] suggesting that the difference may be due to the different chemotherapy of these previous blocks. Interestingly, also in our succeeding AIEOP-BFM ALL 2009 study (HR blocks administered in the order HR-1', HR-2', and HR-3'), the incidence of HSR was almost twice as high in HR-2' compared with HR-1'.[19] An effect of cytotoxic therapy on immunoregulatory T cells is quite conceivable and has been experimentally demonstrated by others.[21]

Overall, 6.3% of our patient cohort did not receive all prescribed ASNase doses (details in Suppl. Figure S1). Our data did not indicate a disadvantage of incomplete ASNase therapy in terms of event-free survival (EFS) or cumulative incidence of relapse (CIR), neither in the non-HR nor in the HR patients (ASNase discontinued versus complete: 5-year pEFS, non-HR: 87% [SE 6%] versus 89% [SE 1%], $P = 0.89$; HR: 63% [SE 8%] versus 66% [SE 4%], $P = 0.83$; 5-year CIR, non-HR: 12.5% [SE 6.0%] versus 9.2% [SE 0.9%], $P = 0.76$; HR: 22.2% [SE 6.6%] versus 23.7% [SE 3.8%], $P = 0.69$) (Suppl. Figure S5A-D). This is in contrast to the results of others who have demonstrated that early discontinuation of ASNase adversely effects leukemia outcome.[22–24] However, these apparent discrepancies may, at least in part, reflect varying importance of ASNase in different chemotherapy regimens. Following this, the particularly intensive BFM HR regimen may be able to compensate for omitted ASNase doses. In the NOPHO ALL 2008 study, the authors were only able to demonstrate a significant adverse effect of suboptimal ASNase therapy after including patients with silent inactivation.[22] As no systematic measurements of ASNase enzyme activity were performed in our study, we were not able to evaluate the contribution of silent inactivation to suboptimal ASNase therapy.

The finding of the exceptionally high incidence of HSR to ASNase in the ALL-BFM 2000 study led to a change in the ASNase regimen in the subsequent trial AIEOP-BFM ALL 2009 using PEG-ASNase as first-line product.[19] The observations in this retrospective study have generated new ideas and hypotheses about the pathogenesis of HSR to ASNase. These may also apply to the therapy with the now more widely used PEG-ASNase, although the incidence of HSR has been shown to be lower with the pegylated product.[7,10,19,24,25] Our hypotheses still need supporting evidence, and future studies on the interaction of the cellular immune system during chemotherapy may contribute to understanding the complex interactions in order to optimize ASNase therapy for ALL.

AUTHOR CONTRIBUTIONS

AM, CR, AB, M Schrappe, VC, DS, JB, MV, and MZ participated in the study design and data analyses. AM, AA, RB, BB, NB, GC, CF, AK, FN, AS, M Stanulla, M Schrappe, and MZ enrolled patients to the study or participated in data collection. All authors participated in writing the manuscript.
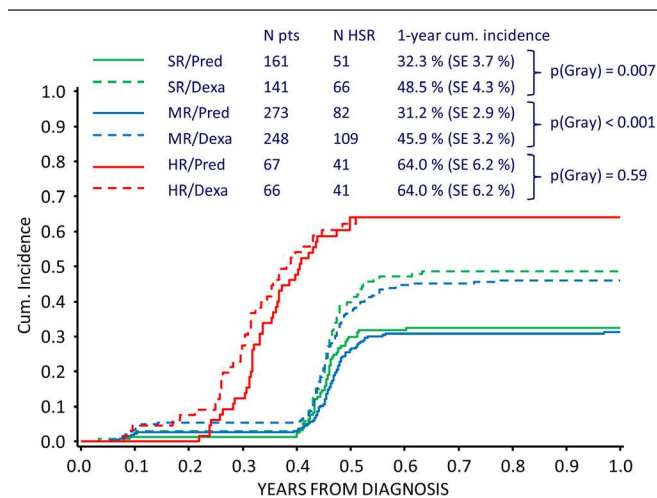
Figure 2. Cumulative incidence of hypersensitivity reaction to native *E. coli* asparaginase in patients included in the randomization R1 receiving either Pred or Dexa during the induction phase. Results are shown stratified by risk-adapted treatment branch. Dexa = dexamethasone; *Escherichia coli* = *E. coli*; HR = high risk; HSR = hypersensitivity reaction to native *E. coli* asparaginase; MR = medium risk; Pred = prednisone; SR = standard risk.

lectures, consultancy or advisory board participation from the following companies: Jazz Pharmaceuticals, Amgen, Novartis, MSD, Pfizer, Takeda and Gilead. Compensation for travel expenses from Jazz Pharmaceuticals. AB: CoImmune: Research Support (to Fondazione M.Tettamanti); Amgen, Novartis, CoImmune: Consultancy, Honoraria. JB: JAZZ and Servier: institutional grants for research and consulting. VC: Medac, Sigma-Tau and Shire: research funds; Sigma-Tau and Shire: consultancy honoraria. CL-K: Clinigen: Honoraria; Servier: Travel Grants. AS: consultancy honoraria – Amgen, Novartis, Pfizer, Jazz, Miltenyi, Clinigen. M Stanulla: Servier and Jazz: Travel grants. M. Schrappe: Amgen, Shire, Servier and Novartis: Research Funding; Servier and Jazz: Honoraria. All the other authors have no conflicts of interest to disclose.

SOURCES OF FUNDING

REFERENCES

1. Rizzari C, Conter V, Stary J, et al. Optimizing asparaginase therapy for acute lymphoblastic leukemia. *Curr Opin Oncol*. 2013;25(Suppl 1):S1–S9.
2. Pieters R, Hunger SP, Boos J, et al. L-asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. *Cancer*. 2011;117:238–249.
3. Liu C, Kawedia JD, Cheng C, et al. Clinical utility and implications of asparaginase antibodies in acute lymphoblastic leukemia. *Leukemia*. 2012;26:2303–2309.
4. Müller HJ, Beier R, Löning L, et al. Pharmacokinetics of native Escherichia coli asparaginase (Asparaginase medac) and hypersensitivity reactions in ALL-BFM 95 reinduction treatment. *Br J Haematol*. 2001;114:794–799.
5. Battistel AP, Rocha BSD, Santos MTD, et al. Allergic reactions to asparaginase: retrospective cohort study in pediatric patients with acute lymphoid leukemia. *Hematol Transfus Cell Ther*. 2021;43:9–14.
6. Petersen WC Jr, Clark D, Senn SL, et al. Comparison of allergic reactions to intravenous and intramuscular asparaginase in children with acute lymphoblastic leukemia. *Pediatr Hematol Oncol*. 2014;31:311–317.
7. Henriksen LT, Harila-Saari A, Ruud E, et al. PEG-asparaginase allergy in children with acute lymphoblastic leukemia in the NOPHO ALL2008 protocol. *Pediatr Blood Cancer*. 2015;62:427–433.
8. Avramis VI, Sencer S, Periclou AP, et al. A randomized comparison of native Escherichia coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. *Blood*. 2002;99:1986–1994.
9. Woo MH, Hak LJ, Storm MC, et al. Hypersensitivity or development of antibodies to asparaginase does not impact treatment outcome of childhood acute lymphoblastic leukemia. *J Clin Oncol*. 2000;18:1525–1532.
10. Brigitha LJ, Fiocco M, Pieters R, et al. Hypersensitivity to Pegylated E. coli asparaginase as first-line treatment in contemporary paediatric acute lymphoblastic leukaemia protocols: a meta-analysis of the Ponte di Legno Toxicity working group. *Eur J Cancer*. 2022;162:65–75.
11. Conter V, Bartram CR, Valsecchi MG, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. *Blood*. 2010;115:3206–3214.
12. Schrappe M, Valsecchi MG, Bartram CR, et al. Late MRD response determines relapse risk overall and in subsets of childhood T-cell ALL: results of the AIEOP-BFM-ALL 2000 study. *Blood*. 2011;118:2077–2084.
13. Möricke A, Zimmermann M, Valsecchi MG, et al. Dexamethasone vs prednisone in induction treatment of pediatric ALL: results of the randomized trial AIEOP-BFM ALL 2000. *Blood*. 2016;127:2101–2112.
14. Schrappe M, Bleckmann K, Zimmermann M, et al. Reduced-intensity delayed intensification in standard-risk pediatric acute lymphoblastic leukemia defined by undetectable minimal residual disease: results of an international randomized trial (AIEOP-BFM ALL 2000). *J Clin Oncol*. 2018;36:244–253.
15. Locatelli F, Valsecchi MG, Möricke A, et al. Protocol II vs protocol III given twice during reinduction therapy in children with medium-risk ALL. *Blood*. 2017;130:2146–2149.
16. Attarbaschi A, Mann G, Zimmermann M, et al. Randomized post-induction and delayed intensification therapy in high-risk pediatric acute lymphoblastic leukemia: long-term results of the international AIEOP-BFM ALL 2000 trial. *Leukemia*. 2020;34:1694–1700.
17. Kloos RQ, Pieters R, Escherich G, et al. Allergic-like reactions to asparaginase: Atypical allergies without asparaginase inactivation. *Pediatr Blood Cancer*. 2016;63:1928–1934.
18. Tong WH, Pieters R, de Groot-Kruseman HA, et al. The toxicity of very prolonged courses of PEGasparaginase or Erwinia asparaginase in relation to asparaginase activity, with a special focus on dyslipidemia. *Haematologica*. 2014;99:1716–1721.
19. Rizzari C, Möricke A, Valsecchi MG, et al. Incidence and characteristics of hypersensitivity reactions to PEG-asparaginase observed in 6136 children with acute lymphoblastic leukemia enrolled in the AIEOP-BFM ALL 2009 study protocol. *HemaSphere*. 2023;7:e893.
20. Cari L, De Rosa F, Nocentini G, et al. Context-dependent effect of glucocorticoids on the proliferation, differentiation, and apoptosis of regulatory T cells: a review of the empirical evidence and clinical applications. *Int J Mol Sci* . 2019;20:1142.
21. Winzler C, Fantinato M, Giordan M, et al. CD4(+) T regulatory cells are more resistant to DNA damage compared to CD4(+) T effector cells as revealed by flow cytometric analysis. *Cytometry A*. 2011;79:903–911.
22. Gottschalk Hojfeldt S, Grell K, Abrahamsson J, et al. Relapse risk following truncation of pegylated asparaginase in childhood acute lymphoblastic leukemia. *Blood*. 2021;137:2373–2382.
23. Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. *Blood*. 2001;97:1211–1218.
24. Gupta S, Wang C, Raetz EA, et al. Impact of asparaginase discontinuation on outcome in childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group. *J Clin Oncol*. 2020;38:1897–1905.
25. Place AE, Stevenson KE, Vrooman LM, et al. Intravenous pegylated asparaginase versus intramuscular native Escherichia coli L-asparaginase in newly diagnosed childhood acute lymphoblastic leukaemia (DFCI 05-001): a randomised, open-label phase 3 trial. *Lancet Oncol*. 2015;16:1677–1690.