Comparison of *Escherichia coli*–asparaginase with *Erwinia*-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer—Children’s Leukemia Group phase 3 trial


Asparaginase is an enzyme used in the treatment of acute lymphoblastic leukemia and lymphoblastic lymphoma in children. It has minimal bone marrow toxicity. Its major side effects are anaphylaxis, pancreatitis, diabetes, coagulation abnormalities, and thrombosis, especially intracranial. It is derived from 2 different sources: *Escherichia coli* and *Erwinia chrysanthemi*. Nonrandomized clinical studies have suggested a similar efficacy of these 2 types of asparaginases and a lower toxicity for *Erwinia*-asparaginase. The European Organisation for Research and Treatment of Cancer—Children’s Leukemia Group (EORTC-CLG) 58881 trial randomized 700 children with acute lymphoblastic leukemia or lymphoblastic lymphoma to either *E coli*–or *Erwinia*-asparaginase at the same dosage of 10 000 IU/m² twice weekly to compare toxicity and efficacy. Coagulation abnormalities were more frequent in the *E coli*–asparaginase than in the *Erwinia*-asparaginase arm of the study (30.2% versus 11.9%, *P < .0001*). The incidence of other toxicity was not significantly different. In the *Erwinia*-asparaginase arm, more patients failed to achieve complete remission (4.9% versus 2.0%; *P = .038*) and the relapse rate was higher, leading to shorter event-free survival (hazard ratio, 1.59; 95% CI, 1.23-2.06; *P = .0004*).

The estimate of event-free survival rate (SE) at 6 years was 59.8% (2.6%) versus 73.4% (2.4%). Overall survival rate at 6 years was also lower in the *Erwinia*-asparaginase arm at 75.1% (2.3%) versus 83.9% (2.0%), *P = .002*. With the dose scheduling used in this protocol, *E coli*-asparaginase induced more coagulation abnormalities but was superior to *Erwinia*-asparaginase for the treatment of childhood lymphoid malignancies. (Blood. 2002;99:2734-2739)

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Introduction

The enzyme L-asparaginase has been used in the treatment of lymphoblastic malignancies in children since 1970. Its antileukemic effect is believed to result from the depletion of circulating asparagine, which is not essential for normal cells but essential for most malignant lymphoblastic cells. Asparaginase has minimal bone marrow toxicity. Its main side effects are anaphylaxis, pancreatitis, diabetes, and coagulation abnormalities that may lead to intracranial thrombosis or hemorrhage.

Clinically available asparaginase is derived from 2 sources: *Escherichia coli* and *Erwinia chrysanthemi*. In many countries, asparaginase from only one of these sources is available for front-line therapy of lymphoblastic malignancies. In 1990, when the study reported here was started, the 2 types of asparaginase were used as if they were one and the same drug. Doses and schedule, although variable from one protocol to another, were defined without consideration for the source of the enzyme. One clinical study using a historical comparison had in fact suggested that *Erwinia*-asparaginase was as effective as *E coli*-asparaginase but was less toxic. To get a clearer view on the relative efficacy and toxicities of the 2 drugs, we conducted the first randomized trial to compare them in front-line chemotherapy in children with newly diagnosed acute lymphoblastic leukemia (ALL) and lymphoblastic non-Hodgkin lymphoma.

Patients and methods

Patients

Patients were enrolled in 28 pediatric centers in Belgium, France, and Portugal in trial 58881 of the European Organisation for Research and Treatment of Cancer—Children’s Leukemia Group (EORTC-CLG). To be eligible for the trial, patients less than 18 years of age had to be
diagnosed with ALL according to French-American-British L1 or L2 cytology or lymphocytic non-Hodgkin lymphoma. Patients previously treated with corticosteroids for more than 7 days were excluded.

Patients were considered to have central nervous system (CNS) involvement if they had cranial nerve palsy or at least 5 leukocytes/μL cerebrospinal fluid with leukemic cells seen on cytocentrifuged preparations. Immunophenotype was determined using standard techniques, and positivity for each marker was defined as more than 20% of leukemic cells expressing that marker. Chromosome analysis used standard techniques. Bone marrow smears, immunophenotypes, and cytogenetics were reviewed centrally.

**Treatment**

Patients were randomized to *Erwinia*-asparaginase (Erwinia, Ipsen, Maidenhead, United Kingdom) or *E coli*-asparaginase (Paronal, Medac, Hamburg, Germany for the Belgian centers, or Kidrolase, Bellon, Montrouge, France for the French and Portuguese centers, both produced by Kyowa Hakko, Tokyo, Japan). Physicians had to switch to the other asparaginase in case of allergy grade 1 or higher. In case of pancreatitis or thrombosis, asparaginase was eliminated from the treatment. Informed consent was required before entry, in accordance with the Helsinki protocol. The trial also randomized patients to receive additional monthly intravenous mercaptopurine during maintenance therapy, and high-risk patients (see below) to receive high-dose cytarabine during interval therapy.

Protocol design was similar to that of the BFM-90 protocol. Patients were stratified into low- and high-risk categories according to their risk factor calculated as a function of blood blast count, hepatomegaly, and positivity for each marker was defined as more than 20% of leukemic cells expressing that marker. Chromosome analysis used standard techniques. Bone marrow smears, immunophenotypes, and cytogenetics were reviewed centrally.

Protocol design was similar to that of the BFM-90 protocol.15 Patients were stratified into low- and high-risk categories according to their risk factor calculated as a function of blood blast count, hepatomegaly, and splenomegaly.15,16 A very-high-risk group was defined by the presence of at least one of the following criteria regardless of risk factor: more than 1000 blasts/μL in the blood after 7 days of prednisolone and intrathecal methotrexate on day 1, translocation t(9;22) or t(4;11), near-haploidy, undifferentiated immunophenotype, or complete remission (CR) not achieved after protocol IA for leukemia patients or after protocol IB for lymphoma patients.

Strategy for low- and high-risk patients was based on induction-consolidation, CNS-directed therapy with high-dose methotrexate, and then reinduction, followed by maintenance therapy for a total treatment duration of 2 years (Table 1). CNS-directed therapy consisted of high-dose intravenous methotrexate and 10 intrathecal injections of methotrexate; cranial irradiation was not given even for patients with CNS involvement at diagnosis. The latter received an additional 10 intrathecal injections of methotrexate and during maintenance 5 high-dose methotrexate infusions. Asparaginase was administered intravenously twice weekly. A total of 12 doses of 10 000 IU each was planned, 8 during protocol I and 4 during protocol II. No study for asparaginase pharmacokinetics was planned because no laboratory of our group had that expertise at the time the trial started.

Treatment of very-high-risk patients called for rotating chemotherapy courses and also for allogeneic bone marrow transplantation for patients with an HLA-identical sibling. After completion of protocol IA, very-high-risk patients received consolidation therapy of 6 weeks' duration consisting of cyclophosphamide, asparaginase, oral mercaptopurine, and high-dose methotrexate and cytarabine (IB protocol). They then received a combination of oral dexamethasone, high-dose cytarabine, mitoxantrone, etoposide, and asparaginase ("VANDA" block).18 VANDA was followed by interval therapy with only 3 administrations of high-dose methotrexate, combined with high-dose cytarabine. Then 2 sequences of 3 R-blocks were administered according to the BFM relapse protocol,17 followed by maintenance therapy. Cranial radiotherapy was not given. Total duration of treatment was also 2 years.

**Definitions**

Complete remission was defined as cellular bone marrow with fewer than 5% leukemic cells and no evidence of leukemia or lymphoma at any other site. Remission failure was defined as failure to reach CR at the completion of protocol I. Relapse was defined as the reappearance of more than 25% leukemic cells in the bone marrow or of any leukemic cell at another site. Coagulation abnormalities were defined as any clinical or biologic abnormality requiring a modification of chemotherapy or supportive care. Investigators were advised to consider such a modification for hypoamrinogenemia below 0.5 g/L. Allergy, neurotoxicity, liver toxicity, and infection were graded according to World Health Organization (WHO) criteria. To ensure comparability with other studies, National Cancer Institute (NCI) risk groups for leukemia patients were used according to consensus conference recommendations: NCI standard-risk group consisted of patients aged 1 to 9 years at diagnosis with an initial white blood cell (WBC) count less than 50 x 109/L. Other patients were considered as having NCI high-risk leukemia.

**Statistical methods**

Randomization was done centrally (EORTC Data Center, Brussels) and was stratified according to center, disease (leukemia versus lymphoma), risk...
factor (< 0.8, 0.8-1.19, ≥ 1.2), and immunophenotype (B versus T lineage) for leukemia patients, and by Murphy stage (stage I-II versus III-IV) for lymphoma patients. Randomization was not stratified by the presence of t(9;22). Subsequent randomizations were stratified according to treatment arm and initial risk factor or Murphy stage.

The primary end point was event-free survival calculated from the date of CR to the date of first relapse or death. For patients who failed to reach CR by the end of protocol I, the failure was considered as an event at time 0. The secondary end points were the rate of CR after induction and consolidation, disease-free survival (time from CR until relapse or death), and survival (time from randomization until death, whatever the cause). Actuarial curves were computed using the Kaplan-Meier technique, and the SEs of the estimates were obtained using the Greenwood formula. To summarize the overall treatment difference, the hazard ratio for the daily risk of event in the Cox proportional hazards model. This model was also used to adjust one in each arm, initially erroneously diagnosed and subsequently randomized to. Such a proportion did not allow comparison of the incidence of toxicity in protocol IIA.

The Fisher exact 2-tailed test was used (StatExact) to compare the rates of complete remission after induction and consolidation. The odds ratio estimates and their exact 95% CIs were used to express the results. The same methods were used for treatment comparisons of the incidence of grade 3 to 4 toxicity during the induction period.

### Results

#### Patient characteristics

Between November 1990 and October 1993, 702 patients were enrolled. Seven hundred were considered eligible for entry into the study, 354 in the E coli–asparaginase arm and 346 in the Erwinia-asparaginase arm. Two patients with Burkitt lymphoma, one in each arm, initially erroneously diagnosed and subsequently treated with another protocol were excluded from the analysis. Enrollment was stopped early because the treatment difference in terms of event-free survival yielded a P < .001.

Patient characteristics according to treatment arms are shown in Table 2. A total of 653 patients (93%) had ALL. The 2 arms were comparable for usual prognostic factors, except for a slight imbalance in the incidence of t(9;22). Forty-seven patients with lymphoblastic lymphoma were randomized and the 2 arms were also comparable at presentation.

#### Protocol compliance and toxicity

During protocol IA, 81% of the patients in the E coli–asparaginase arm and 88% of the patients in the Erwinia-asparaginase arm received 8 doses of the asparaginase they had been randomized to receive (Table 3). Coagulation abnormalities were more often observed in the Erwinia-asparaginase arm: 30.2% versus 11.8%; odds ratio, 3.20; P < .0001 (Table 4). The incidence of other grade 3 or 4 toxic effects observed during protocol IA was low and comparable in the 2 arms. Three patients died before reaching CR. Grade 3 or 4 allergy had a low incidence in the 2 groups: 2.5% versus 2.6%.

During protocol IIA a similar proportion of patients in the 2 arms received the planned asparaginase treatment: 66% versus 69% (Table 3). Twenty-nine percent of patients in each arm received at least one dose of the asparaginase they had not been randomized to. Such a proportion did not allow comparison of toxicity in protocol IIA.

#### Efficacy

After induction (protocol IA), 335 leukemia or lymphoma patients (94.5%) reached CR in the E coli–asparaginase arm and 315 (91.0%) in the Erwinia-asparaginase arm (Table 5). Four leukemia patients (1.2%) never achieved CR at the completion of protocol I in the E coli–asparaginase arm and 12 (3.8%) in the Erwinia-asparaginase arm: odds ratio, 3.23; P = .042 (Table 6). Three patients with lymphoblastic lymphoma in the E coli–asparaginase arm and 5 in the Erwinia-asparaginase arm did not achieve CR. For the whole group, the estimated odds ratio for remission failure was 2.56, P = .038 (Table 5).

Median follow-up was 6.9 years (range, 4.8-9.0 years). Relapse rate was approximately 1.5 times higher in the Erwinia-asparaginase arm, regardless of the site, in leukemia (Table 6) and in lymphoma patients (2 versus 5 relapses). The rate of death in CR was similar: 11 patients (3.2%) versus 8 (2.4%). Event-free survival was shorter in the Erwinia-asparaginase arm (P = .0004; Figure 1A). Its rate at 6 years (SE) was 59.8% (2.6%) versus 73.4%

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*Percentages were computed on successful cytogenetic examinations. NCI risk groups were as defined by the consensus conference.*
bordered by a 2.4% in the E. coli–asparaginase arm, and the estimated hazard ratio for remission failure, relapse, or death was 1.59 (95% CI, 1.23–2.06). For leukemic patients the estimated hazard ratio for remission failure, relapse, or death was 1.60 (95% CI, 1.22–2.09) after adjustment for NCI risk group, very-high-risk features, and sex, which appeared to be independent strong prognostic factors. When restricted to leukemia patients with a successful cytogenetic examination, the comparison adjusted for the presence of t(9;22) yielded similar results.

The effects of the other 2 randomizations on the difference between outcome for the 2 types of asparaginase were as follows. First, the addition of high-dose cytarabine during interval therapy had no effect on disease-free survival, and it did not interact with the difference in outcome between the asparaginase arms. Second, patients randomized to receive additional monthly intravenous mercaptopurine during maintenance had a shorter disease-free survival. Among the patients randomized for asparaginase, 638 remained in CR at the beginning of maintenance therapy. A total of 224 patients were randomized to receive additional monthly intravenous mercaptopurine, 229 were randomized not to receive it, and 185 were not randomized. In the 3 subgroups the hazard ratio for death or relapse according to the type of asparaginase was calculated. The three 95% CIs for these hazard ratios were, respectively, 1.32 to 3.37, 0.65 to 1.92, and 1.02 to 2.98. They all contained the overall estimate of 1.63 calculated in the 638 patients, so there is no proof so far that addition of monthly intravenous mercaptopurine interacts with the difference in outcome between the asparaginase arms.

Estimated overall survival rate at 6 years was 83.9% (2.0%) in the E. coli–asparaginase arm versus 75.1% (2.3%) in the Erwinia–asparaginase arm (P = .002; Figure 1B). Estimated hazard ratio for death was 1.66 (95% CI, 1.20–2.23). The comparison yielded a similar result when adjusted for NCI risk group and sex, and, for leukemia patients, for very-high-risk features or for t(9;22) in those with a successful cytogenetic examination.

**Discussion**

Seven hundred children with ALL or lymphoblastic lymphoma were randomized to receive either E. coli– or Erwinia-asparaginase. Median follow-up was 6.9 years. Two main conclusions can be drawn from the results of this randomized trial. First, E. coli–asparaginase is more toxic because it induces more coagulation abnormalities. Second, clinical efficacy of E. coli–asparaginase is superior to that of Erwinia-asparaginase at the dosage of 10 000 IU/m² twice weekly. The type of asparaginase not only affects early response to treatment but also the risk of relapse, event-free survival, and overall survival.

Overall toxicity of asparaginase was low. The most frequent side effects were coagulation abnormalities, which were more frequent in the E. coli–asparaginase arm, as previously reported. Our results confirm a trend toward more neurotoxicity and convulsions with E. coli–asparaginase. However, their frequency (2.5% grade 3 or 4 neurotoxicity, 1.7% convulsions) remained moderate compared to the rate of relapse and death.

In accordance with previous reports, we found no difference between the 2 types of asparaginases in the rates of allergy, liver toxicity, or insulin-requiring diabetes. Frequency of pancreatitis and severe infections was similar, whereas other reports concerning these side effects are conflicting.

Three controlled studies have randomized patients to receive additional asparaginase during postremission therapy in childhood lymphoid malignancies. In 2 of them, additional E. coli–asparaginase improved outcome for patients with ALL and advanced stage lymphoblastic lymphoma. The largest study, which randomized 1085 patients, administered either E. coli– or Erwinia-asparaginase at equal dosage. It failed to show any impact on outcome of additional asparaginase during postremission therapy. Although our study closed in 1993, it is still, to our knowledge, the only comparative study of the 2 types of asparaginases during the remission-induction phase in such a large number of children, and certainly the only large study in which reliable 6-year survival figures are available. Its results suggest that asparaginase in the remission-induction phase may still have an impact on final outcome in this era of multi-agent therapies.

This difference in efficacy between asparaginases was not expected when the trial was begun, but is in keeping with recent...
The serum half-life of Erwinia-asparaginase activity is significantly shorter, 0.65 day versus 1.24 days for E coli–asparaginase. Asparagine depletion during reinduction in the BFM-90 trial was achieved in 26% of patients receiving Erwinia-asparaginase and in 60% to 90% of the patients receiving E coli–asparaginase. Time to recovery of serum asparagine level after administration was 4 days for Erwinia-asparaginase versus 11 days for E coli–asparaginase.

In all treatment protocols so far, the dosing schedule of asparaginase has been defined regardless of the type of asparaginase used, although the regimens have varied considerably from one protocol to another, from 6000 IU daily to 25,000 IU once a week. IU is defined by a chemical in vitro activity and not by a biologic in vivo effect. A recent study suggests that increasing the dose and decreasing the time interval between Erwinia-asparaginase administrations results in pharmacodynamics similar to that of lower and less frequent doses of E coli–asparaginase. However, it has not been demonstrated that this strategy leads to the same clinical outcome, and it may be more toxic. Whether other as yet unknown qualitative differences between the 2 sources of asparaginases could be responsible for their unequal efficacy cannot be demonstrated by these studies and remains undecided.

Thus, E coli–asparaginase can be recommended for first-line therapy, reserving Erwinia-asparaginase for allergic patients, because (1) most patients allergic to the former are not immediately allergic to the latter, (2) our results were analyzed according to the intention-to-treat principle and 29% of patients in the E coli–asparaginase arm were actually switched to Erwinia-asparaginase because of allergy, and (3) it has been demonstrated that this switch does not modify clinical outcome.

Table 6. ALL patients: outcome by arm

<table>
<thead>
<tr>
<th></th>
<th>E coli–asparaginase</th>
<th>Erwinia-asparaginase</th>
<th>Odds ratio (95% CI) (P*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR not reached after induction</td>
<td>N = 334 (100%)</td>
<td>N = 319 (100%)</td>
<td>1.89 (0.87, 4.29) (.107)</td>
</tr>
<tr>
<td>Remission failure</td>
<td>12 (3.6)</td>
<td>21 (6.6)</td>
<td>3.23 (0.96, 13.84) (.042)</td>
</tr>
<tr>
<td>CR reached</td>
<td>330 (98.8)</td>
<td>307 (96.2)</td>
<td></td>
</tr>
<tr>
<td>Continuous CR</td>
<td>242 (73)</td>
<td>190 (62)</td>
<td></td>
</tr>
<tr>
<td>Relapses</td>
<td>77 [23]</td>
<td>110 [36]</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>45 [14]</td>
<td>64 [21]</td>
<td></td>
</tr>
<tr>
<td>Other isolated</td>
<td>3 [1]</td>
<td>5 [2]</td>
<td></td>
</tr>
<tr>
<td>Other combinations</td>
<td>4 [1]</td>
<td>8 [3]</td>
<td></td>
</tr>
</tbody>
</table>

Remission failure means patient never achieved CR at the end of induction-consolidation. Parentheses for columns 2 and 3: percentages were computed on all patients included. Brackets for columns 2 and 3: percentages were computed on patients having reached CR.

*Fisher exact test.

Figure 1. Event-free survival and survival for the patient cohort. (A) Event-free survival for patients randomized to E coli–asparaginase (solid line) or Erwinia-asparaginase (broken line). O indicates observed number of events (remission failure, relapse, or death in CR); N, total number of patients randomized. (B) Survival for patients randomized to E coli–asparaginase (solid line) or Erwinia-asparaginase (broken line). O indicates observed number of deaths; N, total number of patients randomized.

In conclusion, our trial demonstrates the superiority of E coli–asparaginase compared to Erwinia-asparaginase in lymphoid malignancies of childhood, when used at the dose of 10,000 IU/m² twice a week. Our findings underscore the importance of asparaginase in induction therapy of childhood lymphoid malignancies. In modern multianti therapy, minor differences in treatment regimens may lead to substantial differences in outcome, suggesting the need for caution when modifying current therapeutic protocols.

Acknowledgment

A complete list of the participating institutions and investigators appears in the Appendix at the end of this article.
References


Appendix

Participating institutions and investigators of EORTC-CLG: EORTC Data Center, Gabriel Solbu, Stefan Suci, Christine Waterkeyn; HU des Enfants, Bruxelles, Dr Azzi, Dr Finster, Dr Sariban; CHR Grenoble, Dr Bachelot, Dr Plantaz; AZK VUB Brussels, Dr Maurus, Dr Otten; Hôpital Américain Reims, Dr Behar, Dr Munzer; UZ Gent, Dr Benoît, Dr Dhooge, Dr Laureys; Hôpital Debrousse Lyon, Dr Bertrand, Dr Manel, Dr Philippe, Dr Souillet; CHU Angers, Dr Blanchet, Dr Daulet, Dr Gamelin, Dr Le Moine, Dr Pein, Dr Pelliier, Dr Rialland; CHU Strasbourg, Dr Babin-Boileiot, Dr Falkenrot, Dr Lutz; CHU Caen, Dr Bourd, Dr Minkelos; UZ Gasthuisberg Leuven, Dr Brock, Dr Uyttebroeck; CHR Citadelle Liège, Dr Chantaine, Dr Dresse, Dr Hooyx; Hôpital Edouard-Herriot Lyon, Dr Charrin, Dr Magaud; CHU Toulouse, Dr Daugast, Dr Robert, Dr Rubie; Hôpital Saint Antoine Lille, Dr Demory; Fondation Lenval Nice, Dr Deville, Dr Soled; Institut Curie, Paris, Dr Fagnou, Dr Michon, Dr Pacqueulent; CHU Lille, Dr Dournier, Dr Mazinge, Dr Nelken; CH St-Joseph-l’Espérance, Montagne, Dr Francotte, Dr Hainaut, Dr Philippot; Hôpital Robert-Debré, Paris, Dr Duval, Dr Fenneteau, Dr Grandchamp, Dr Lescoeur, Dr Rohrich, Dr Vilmer; AK Antwerpen, Dr Gyseleculck; CHU Nantes, Dr Harousseau, Dr Méchinaud; CHU Montpellier, Dr Margueritte; CHU La Timone, Marseille, Dr Michel; CHU Poitiers, Dr Millot; Hôpital Cimiez, Nice, Dr Monpoux, Hospital S. Hugo, Porto, Dr Nortzon, CH Verviers, Dr Bitar, Dr Paulus; Clinique de l’Esperance, Montagne, Dr Philippot; CHU Reims, Dr Pignon; CHU Besançon, Dr Plouvier; Centre Lacassagne, Nice, Dr Thys.
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