Supplemental data: *Asparaginase Enzyme Activity Levels and Toxicity in Childhood Acute Lymphoblastic Leukemia: a NOPHO ALL2008 study*

## Patients and methods

*Risk stratification and PEG-asparaginase treatment in the NOPHO ALL2008 protocol.*

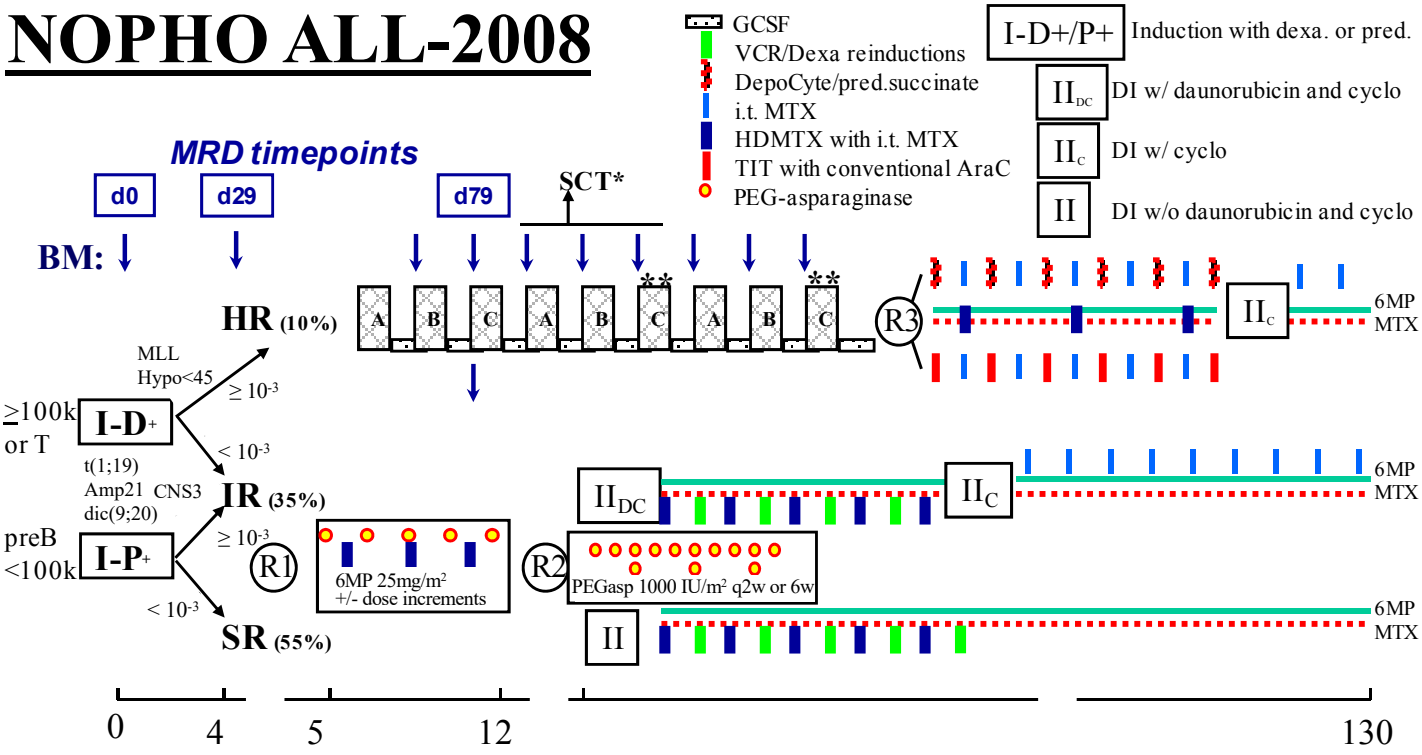
All Patients included in the NOPHO ALL2008 protocol were stratified into three risk groups: standard risk (SR), intermediate risk (IR), high risk (HR) at the end of induction therapy according to tumor burden at diagnosis, immunophenotype, cytogenetics, and minimal residual disease on days 15, 29, and 79. HR patients were furthermore stratified to receive hematopoietic stem cell transplantation or not.

The treatment comprised four main components: (1) induction, (2) consolidation (standard risk and intermediate risk) or block therapy (high risk), (3) delayed intensifications, and (4) maintenance therapy. Consolidation treatment for SR/IR patients included intramuscular injections of PEG-asp (1000 IU/m2) every 2 weeks from treatment day 30 (total of five doses). Patients were randomized to receive 10 additional doses of PEG-asparaginase administered at 2-week intervals (standard arm) or 3 additional doses administered at 6-week intervals (experimental arm) from treatment weeks 14 to 33. Patients were included in randomization from January 1, 2009 in Sweden and Denmark; February 11, 2009, in Norway; June 1, 2009, in Finland; and January 7, 2010, in Iceland. Patients not invited to randomization because of being diagnosed before trial start, treated in non-participating countries (Lithuania and Estonia), or parents/patients rejecting randomization were all treated according to the standard arm with 15 scheduled doses PEG-asparaginase. The randomization was closed on March 1, 2016 because interim analysis showed no difference in disease-free survival between the 2 arms, but significantly reduced incidence of asparaginase-associated toxicities in the experimental arm. Afterwards, all children received PEG-asparaginase according to the experimental arm. Results of the randomization were published in 2019[1].

Non-high-risk with hypersensitivity to PEG-asparaginase were switched to *Erwinia chrysanthemi*-derived asparaginase 20,000 IU/m$^2$/dose 3 times per week for 2 weeks during delayed intensification.

## The NOPHO ALL2008 protocol

Protocol overview for treatment according to risk group. The number of weeks since diagnosis indicated at the bottom of the figure. PEG-asparaginase administrations for standard risk (SR) and intermediate risk (IR) are marked with red circles with red filling. R2 indicates the asparaginase randomization in the protocol.




R1 6MP dose increments (25-50-75 mg/m$^2$ if HDM w/o ANC<0.5 and T<50), N=900-1000

R2 PEG-asparaginase 1.000 IU/m$^2$ at 2 vs 6 weeks intervals weeks 13-33, N=900-1000

R3 Standard TIT vs DepoCyte/pred.succinate at 12w intervals x6, N=100-130

*SCT if d29 M2/3 or d79/post-B >10-3. ** blocks C2 and C3 are not given if post-A1 MRD <0.1%

**Figure S1: Individual asparaginase enzyme activity measurements combined for each patient and stratified by toxicity**

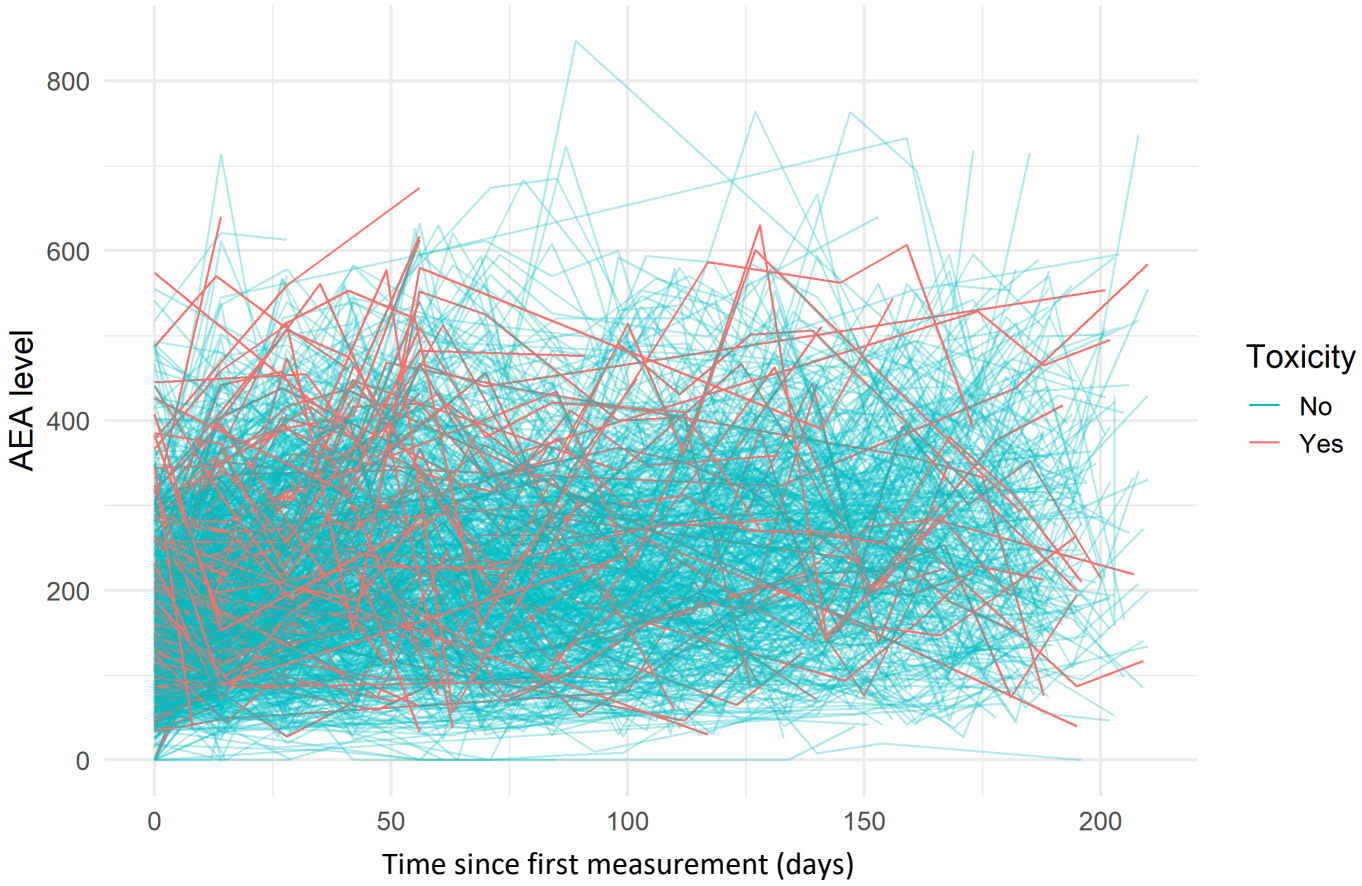



Table S1: Adjusted hazard ratios for each outcome by median asparaginase enzyme activity level stratified by number of scheduled doses of PEG-asparaginase

| | Number of scheduled doses | | | | Association in the groups |
|---|---|---|---|---|---|
| | 8 doses$ | | 15 doses§ | | |
| Patients | 269 | | 686 | | |
| Samples, median (IQR) | 3 (IQR, 2-4) | | 7 (IQR, 5-10) | | |
| **Outcome, HR* (95% CI)** | | | | | |
| Overall toxicity | 0.61 (0.24–1.50) | p = 0.296 | 1.23 (1.02–1.49) | p = 0.034 | p = 0.995 |
| Pancreatitis | – & | – | 1.42 (1.13–1.78) | p = 0.003 | – |
| Thromboembolism | 0.72 (0.23–2.22) | p = 0.566 | 1.00 (0.70–1.48) | p = 0.947 | p = 0.580 |
| Osteonecrosis | 0.83 (0.47–1.46) | p = 0.520 | 1.66 (1.22–2.26) | p = 0.001 | p = 0.034 |
| Relapse | 0.70 (0.42–1.22) | p = 0.195 | 0.98 (0.70–1.36) | p = 0.894 | p = 0.300 |

*$ Patients randomized to 8 doses*
*§ Patients randomized to 15 doses or treated with 15 doses as standard of care*
**Hazard ratio per 100 IU/L increase in median asparaginase enzyme activity*
*& Estimation of hazard ratio not possible due to only one first-event in this subgroup*

**Figure S2: Adjusted hazard ratio of pancreatitis by median asparaginase enzyme activity level:** Hazard ratio of 1.40 per 100 IU/L increase in AEA (95% CI, 1.12—1.75), p = .002.

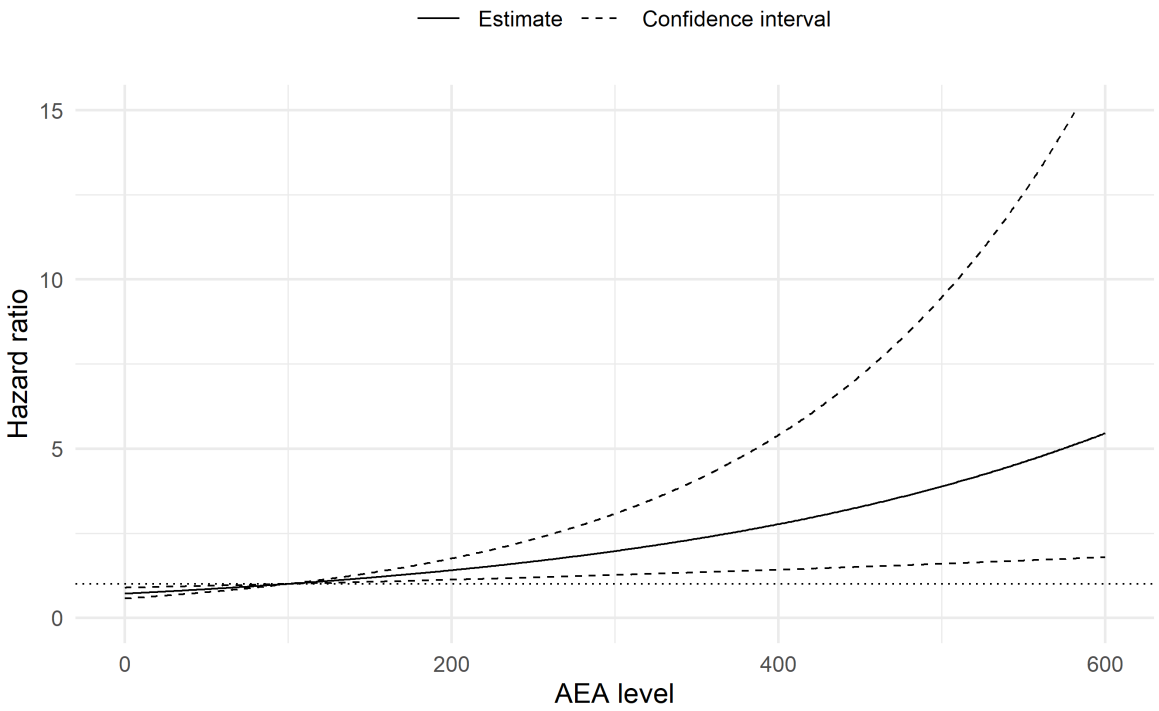



**Figure S3: Adjusted hazard ratio of thromboembolism by median asparaginase enzyme activity level:** hazard ratio 0.99 (95% CI, 0.70–1.40), p = 0.96

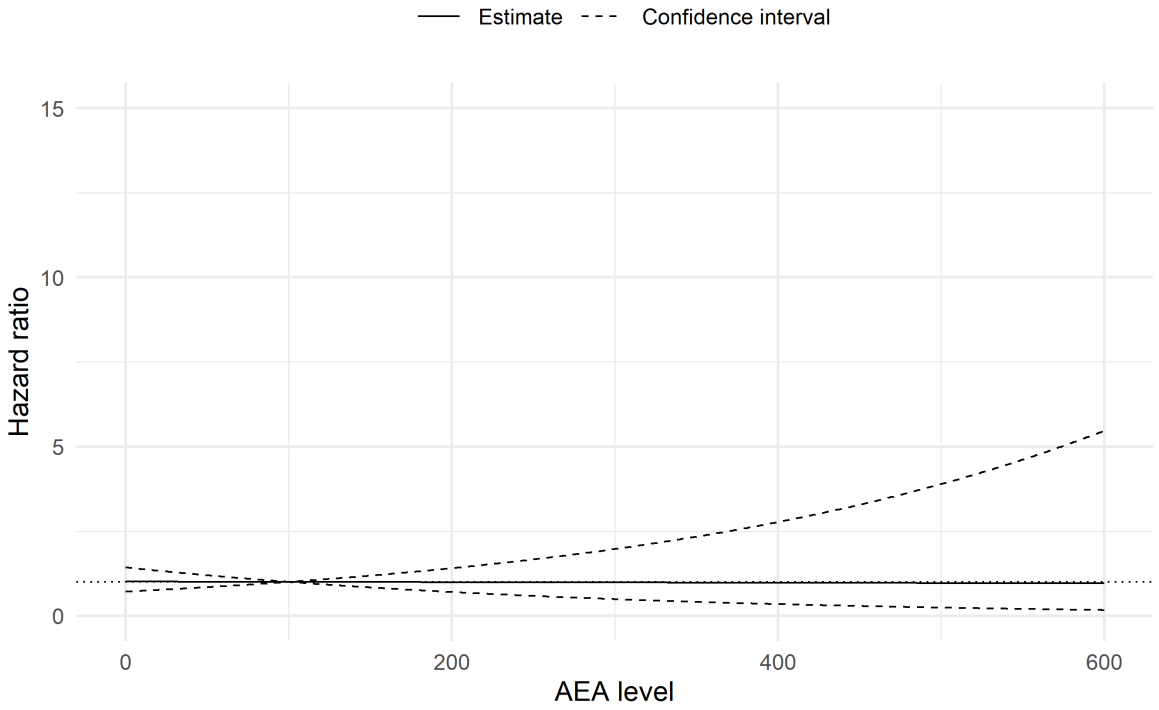


**Figure S4:** Adjusted hazard ratio of osteonecrosis by median asparaginase enzyme activity level: hazard ratio 1.36 per 100 IU/L increase in AEA (95% CI, 1.04–1.77), p = .02

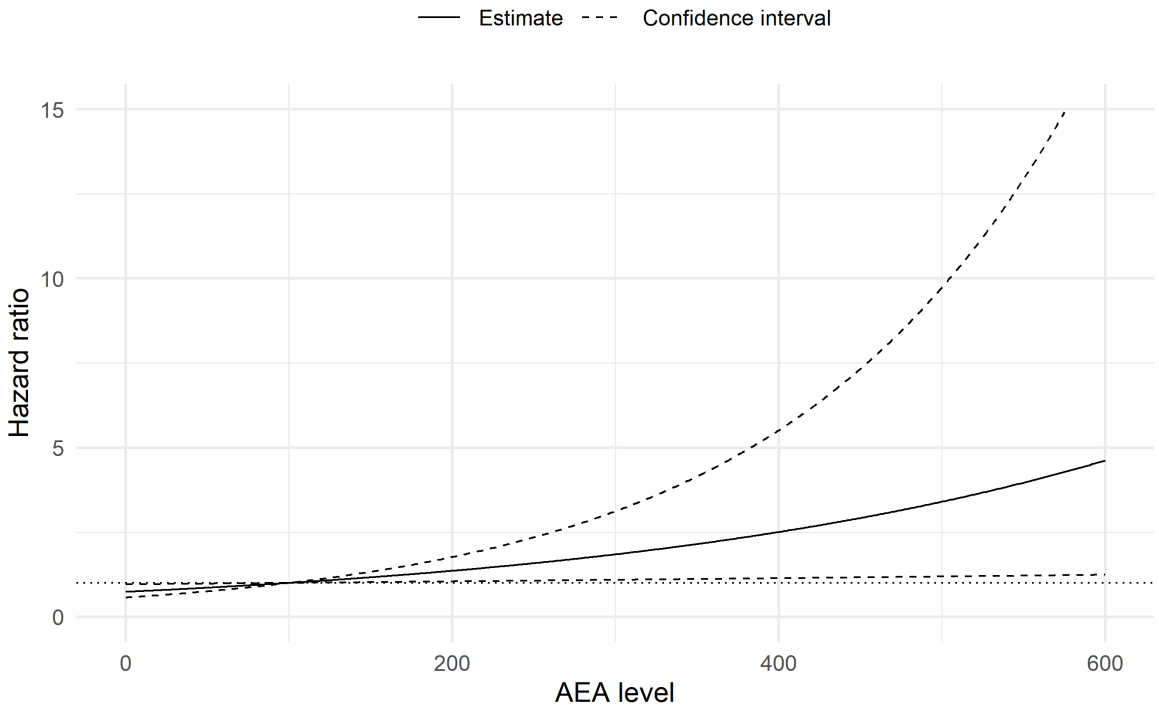



**Figure S5:** Adjusted hazard ratio of treatment discontinuation by median asparaginase enzyme activity level: hazard ratio 0.99 per 100 IU/L increase in AEA (95% CI, 0.81–1.19), p = 0.85

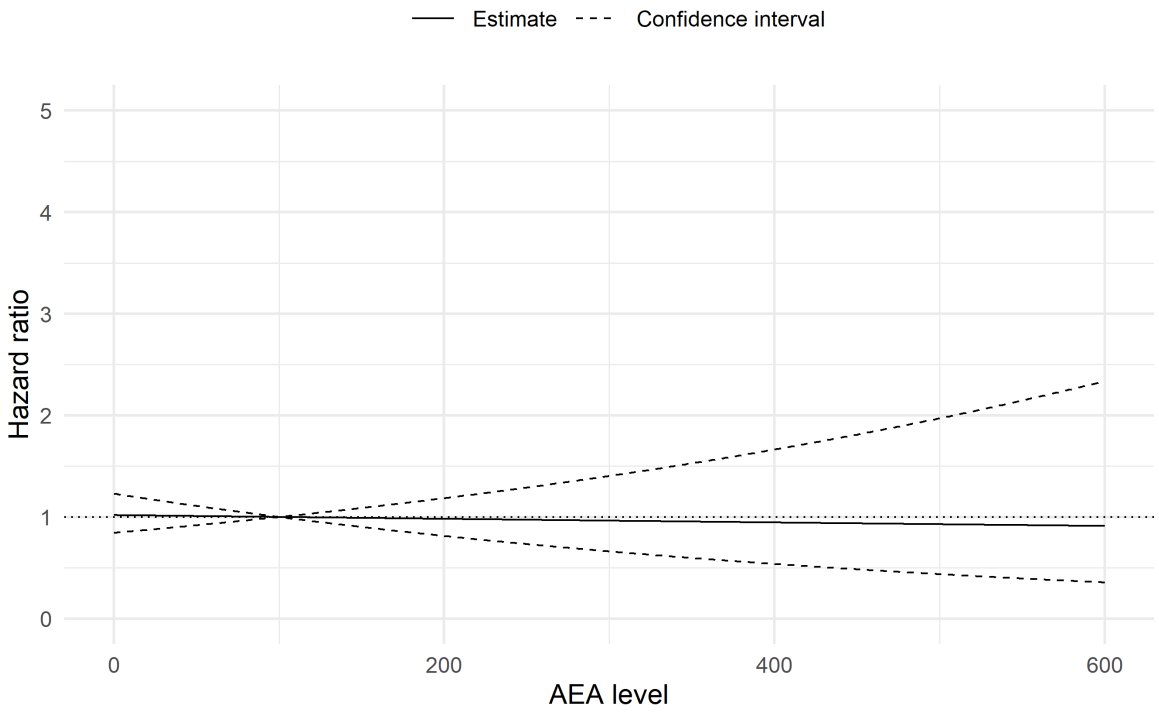


Table S2 Characteristics of patients with truncation of PEG-asparaginase treatment

| | | Asparaginase enzyme activity levels | | | |
|---|---|---|---|---|---|
| | | Inactivation | Measurable AEA | | |
| | | | Low levels | Therapeutic levels | High levels |
| | Cohort N = 1155 | (0 IU/L) n = 200 | (0–99 IU/L) n = 82 | (100–250 IU/L) n = 543 | (>250 IU/L) n = 330 |
| Truncation of Peg-asparaginase | | | | | |
| Yes | 268 (23.2%) | 149 (55.8%) | 7 (2.6%) | 73 (27.1%) | 39 (24.1%) |
| No | 872(75.5%) | 51 (58.5%) | 74 (8.5%) | 460 (52.8%) | 287 (32.9%) |
| Unknown | 15 (1.3%) | 0 | 1 (6.7%) | 10 (66.7%) | 4 (26.7%) |
| Reason for truncation | | | | | |
| Allergy | 161 (60.1%) | 149 | 3 | 7 | 2 |
| Pancreatitis | 57 (21.3%) | 0 | 3 | 33 | 21 |
| Thromboembolism | 15 (5.6%) | 0 | 0 | 11 | 4 |
| Osteonecrosis | 0 (0.0%) | 0 | 0 | 0 | 0 |
| Others* | 35 (13.1%) | 1 | 1 | 21 | 12 |

** E.g. hyperlipidemia, hepatoxicity, sepsis, seizure, abdominal pain, and parental refusal.*

**Reference list, supplemental data:**


1. Albertsen BK, Grell K, Abrahamsson J, et al. Intermittent Versus Continuous PEG-Asparaginase to Reduce Asparaginase-Associated Toxicities: A NOPHO ALL2008 Randomized Study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2019:Jco1801877.