Prolonged *versus* standard native *E. coli* asparaginase therapy in childhood acute lymphoblastic leukemia and non-Hodgkin lymphoma: final results of the EORTC-CLG randomized phase III trial 58951

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 doi:10.3324/haematol.2017.165845

Received: February 6, 2017.

Accepted: July 21, 2017.

Pre-published: July 27, 2017.

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Definitions

DIAGNOSIS

Diagnosis of ALL was based on the presence of more than 25% lymphoblasts in at least one bone marrow aspirate; NHL was defined as any neoplastic proliferation within the lymphoid cell lineage with less than 25% lymphoblasts in the bone marrow. Cytomorphology, immunophenotyping and cytogenetics on blood and/or bone marrow (BM) samples as well as on samples of extramedullary tissue were reviewed by a central reference panel.

RISK GROUPS

Patients were assigned to different risk groups: very low risk (VLR), average risk (AR) and very high risk (VHR). The VLR group consisted of patients with progenitor B-cell ALL with white blood cell (WBC) counts below $10x10^9$/L, hyperdiploid karyotype (>50 chromosomes in at least 3 abnormal metaphases) and DNA index (DI) ≥ 1.16. The presence of central nerve system (CNS) or gonadal involvement or the presence of VHR criteria excluded patients from the VLR group. VHR criteria consisted of blast count in peripheral blood ≥ $1x10^9$/L at completion of the prephase (day 8), presence of t(9;22), t(4;11) or another MLL rearrangement, near-haploidy (< 34 chromosomes), hypodiploidy (35-40 chromosomes), acute undifferentiated leukemia (AUL), minimal residual disease (MRD) ≥ 10^{-2} at completion of induction (day 35) or failure to achieve complete remission (CR) or good partial response (GPR)[8,14]. The AR group was defined by the absence of VLR and VHR characteristics. AR patients were substratified in low average risk (AR1) and high average risk (AR2) groups. Patients belonged to the AR1 group if they had B-cell lineage ALL with WBC counts below $100x10^9$/L, initial CNS-2 or hemorrhagic cerebrospinal fluid (CSF), and becoming negative at day 4 of prephase. AR2 criteria included B-cell lineage ALL with WBC counts above $100x10^9$/L and T-cell lineage ALL. All patients with overt or non-equivocal CNS involvement at diagnosis or any CSF involvement at day 4, and all patients with gonadal involvement were allocated to the AR2 group.

CNS DISEASE

CNS status was defined as CNS-1 (no blast cells in a sample of cerebrospinal fluid), CNS-2 (< 5 WBC/mm^3 with blasts in a sample with ratio erythrocytes/leukocytes <10), CNS-3 (≥ 5 WBCs/mm^3 with blasts in a sample with ratio erythrocytes/leukocytes <10,

and/or any neurological abnormality attributed to leukemic involvement), or traumatic lumbar puncture with blast cells (TLP+) (> 5 WBC/mm3 with blasts in a sample with ratio erythrocytes/leukocytes ≥10). All patients with CNS-3 status or any CNS involvement at the lumbar puncture 3 days after the initial one, and without any VHR criteria, were included in the AR2 group.

REMISSION AND RELAPSE CRITERIA

Complete remission (CR) was defined as disappearance of all symptoms related to the leukemia or lymphoma in combination with less than 5% blasts in a hematologically recovered bone marrow and in absence of blasts in the CSF. Good partial response (GPR) was defined, for NHL and T-cell ALL, by the disappearance of all signs of lymphoma or leukemia, except for a residual mass on one tumor site with a diameter less than 30% of the original diameter

Statistical Analysis

DFS was computed from date of randomization to date of relapse at any site, or death, or until date of last follow-up for children in continuous complete remission (censored observations). OS was calculated from date of initial randomization to date of death or date of last follow-up for children alive (censored observations).

Patients eligible for the EORTC-CLG 58951 trial were prospectively registered at diagnosis. At the end of induction, patients eligible for the ASNase question were randomly assigned at the EORTC Headquarters, Brussels. Central randomization was stratified by the 1st randomized arm, EORTC risk group and treating site using a minimization technique.

Survival distributions were estimated according to the Kaplan-Meier technique; standard errors (SE) of the estimates were obtained via the Greenwood formula. The two-sided, log-rank test was used for comparisons of treatment outcome. All *P*-values were from two-sided tests. For exploratory purposes, we investigated predictive importance of several factors on the treatment differences regarding the efficacy outcomes. The forest plot technique was used to display treatment differences regarding the outcomes. The estimated HR and its 95% or 99% confidence interval (CI), were derived from log-rank test computations. Heterogeneities between the HRs were tested for significance using the Cochran's Q test, used in meta-analyses[16].

An intention-to-treat analysis was performed. The SAS 9.4 software (SAS Institute Inc, Cary, NC, USA) was used for data analysis.

Suppl. table 1a: EORTC-CLG treatment protocol for very low risk patients

Drug	Dose	Days of administration
Induction: protocol IA		
Methotrexate (IT)		1 (prephase)
According to randomization		
PRED (PO)	60 mg/m²	1-7 (prephase)
DEX (PO)	6 mg/m²	1-7 (prephase)
According to randomization		
PRED (PO)	60 mg/m²	8-28, tapered over 9 days
DEX (PO)	6 mg/m²	8-28, tapered over 9 days
Vincristine (IV)	1.5 mg/m² (max 2 mg)	8,15,22,29
Daunorubicin (IV)	30 mg/m²	8,15
Methotrexate (IT)		12,25
Native *E.coli* ASNase	10,000 IU/m²	12,15,18,22,25,29,32,35
Consolidation: protocol IB		
Cytarabine (IV)	75 mg/m²	38-41,45-48,52-55,59-62
MTX chemotherapy (IT)		38,52
6-mercaptopurine (PO)	60 mg/m²	36-63
According to randomisation		
Short native *E.coli* ASNase	No ASNase	
Long native *E.coli* ASNase	5,000 IU/m²	38,41,45,48,52,55,59,62
Interval therapy		
6-mercaptopurine (PO)	25 mg/m²	1-56
Methotrexate (24 h) †	5,000 mg/m²	8,22,36,50
MTX chemotherapy (IT)		9,23,37,51
Late intensif.: protocol II		
DEX (PO)	6 mg/m²	1-21, taper over 9 days
Vincristine (IV)	1.5 mg/m² (max 2 mg)	8,15,22,29
Doxorubicin (IV)	30 mg/m²	8,15
Methotrexate (IT)		38
Cytarabine (IV)	75 mg/m²	38-41,45-48
6-thioguanine (PO)	60 mg/m²	36-49
According to randomisation		
Short native *E.coli* ASNase	10,000 IU/m²	8,11,15,18
Long native *E.coli* ASNase	10,000 IU/m²	8,11,15,18
and	5,000 IU/m²	22,25,29,32
Maintenance (74 weeks)		
6-mercaptopurine (PO)	50 mg/m²	Daily
Methotrexate (PO)	20 mg/m²	Weekly

IT indicates intrathecally; PO, orally; PRED, prednisolone; DEX, Dexamethasone; IV, intravenously;.
Methotrexate: For age less than 1 year: 6 mg, 1 year 8 mg, 2 years 10 mg, 3 years and more: 12 mg

† Leucovorin rescue 12 mg/m²/6h starts at H36.

Suppl. table 1b: EORTC-CLG treatment protocol for average risk (AR1 and AR2) patients

Drug	Dose	Days of administration
Induction: protocol IA		
Methotrexate (IT)		1
According to randomization		
PRED (PO)	60 mg/m²	1-7 (prephase)
DEX (PO)	6 mg/m²	1-7 (prephase)
According to randomization		
PRED (PO)	60 mg/m²	8-28, tapered over 9 days
DEX (PO)	6 mg/m²	8-28, tapered over 9 days
AR1		
Vincristine (IV)	1.5 mg/m² (max 2 mg)	8,15,22,29
Daunorubicin (IV)	30 mg/m²	8,15,22,29
Triple chemotherapy (IT)		12,25
Native *E.coli* ASNase	10,000 IU/m²	12,15,18,22,25,29,32,35
AR2		
Cyclophosphamide	1,000 mg/m²	9
Methotrexate (24 h) †	5 g/m²	8
Triple chemotherapy (IT)		9,25
Vincristine (IV)	1.5 mg/m² (max 2 mg)	8,15,22,29
Daunorubicin (IV)	40 mg/m²	15,22,29
Native *E.coli* ASNase	10,000 IU/m²	12,15,18,22,25,29,32,35
Consolidation: protocol IB		
Cyclophosphamide (IV)	1,000 mg/m²	36,63
Cytarabine (IV)	75 mg/m²	38-41,45-48,52-55,59-62
Triple chemotherapy (IT)		38,52
6-mercaptopurine (PO)	60 mg/m²	36-63
According to randomisation		
Short native *E.coli* ASNase	No ASNase	
Long native *E.coli* ASNase	5,000 IU/m²	38,41,45,48,52,55,59,62
Interval therapy		
6-mercaptopurine (PO)	25 mg/m²	1-56
Methotrexate (24 h) †	5,000 mg/m²	8,22,36,50
Triple chemotherapy (IT)		9,23,37,51
Late intensification: protocol II		
DEX (PO)	6 mg/m²	1-21, taper over 9 days
Vincristine (IV)	1.5 mg/m² (max 2 mg)	8,15,22,29
Doxorubicin (IV)	30 mg/m²	8,15,22,29
Triple chemotherapy (IT)		38
Cyclophosphamide (IV)	1,000 mg/m²	36
Cytarabine (IV)	75 mg/m²	38-41,45-48
6-thioguanine (PO)	60 mg/m²	36-49

According to randomization		
Short native *E.coli* ASNase	10,000 IU/m²	8,11,15,18
Long native *E.coli* ASNase	10,000 IU/m²	8,11,15,18
and	5,000 IU/m²	22,25,29,32
Maintenance (74 weeks)		
AR1		
6-mercaptopurine (PO)	50 mg/m²	Daily
Methotrexate (PO)	20 mg/m²	Weekly
Triple chemotherapy (IT)		Every 70 days, starting D22, 6 times
According to randomisation		
No pulses		
PRED (PO) ‡	60 mg/m²	Every 70 days, for 7 days, starting D57-63, 6 times
DEX (PO) ‡	6 mg/m²	Every 70 days, for 7 days, starting D57-63, 6 times
Vincristine	1.5 mg/m² (max 2 mg)	Every 70 days, on D57 and D63, 6 times
AR2		
6-mercaptopurine (PO)	50 mg/m²	Daily
Methotrexate (PO)	20 mg/m²	Weekly
Methotrexate (24 h) †	5,000 mg/m²	Every 70 days, starting D22, 6 times
Triple chemotherapy (IT)		Every 70 days, starting D23, 6 times
Native *E.coli* ASNase	25,000 IU/m²	Every 70 days, starting D23, 6 times
According to randomization		
No pulses		
PRED (PO) ‡	60 mg/m²	Every 70 days, for 7 days, starting D57-63, 6 times
DEX (PO) ‡	6 mg/m²	Every 70 days, for 7 days, starting D57-63, 6 times
Vincristine (IV)	1.5 mg/m² (max 2 mg)	Every 70 days, on D57 and D63, 6 times

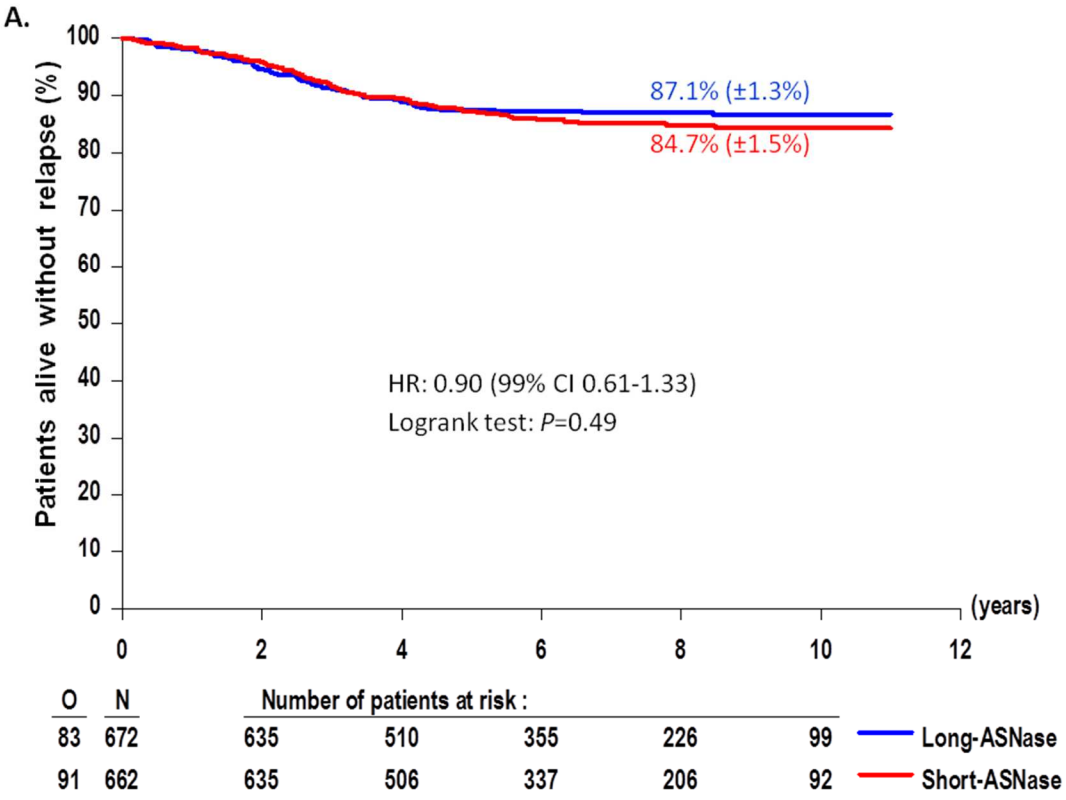
IT indicates intrathecally; PO, orally; PRED, prednisolone; DEX, Dexamethasone; IV, intravenously; D X, day X.

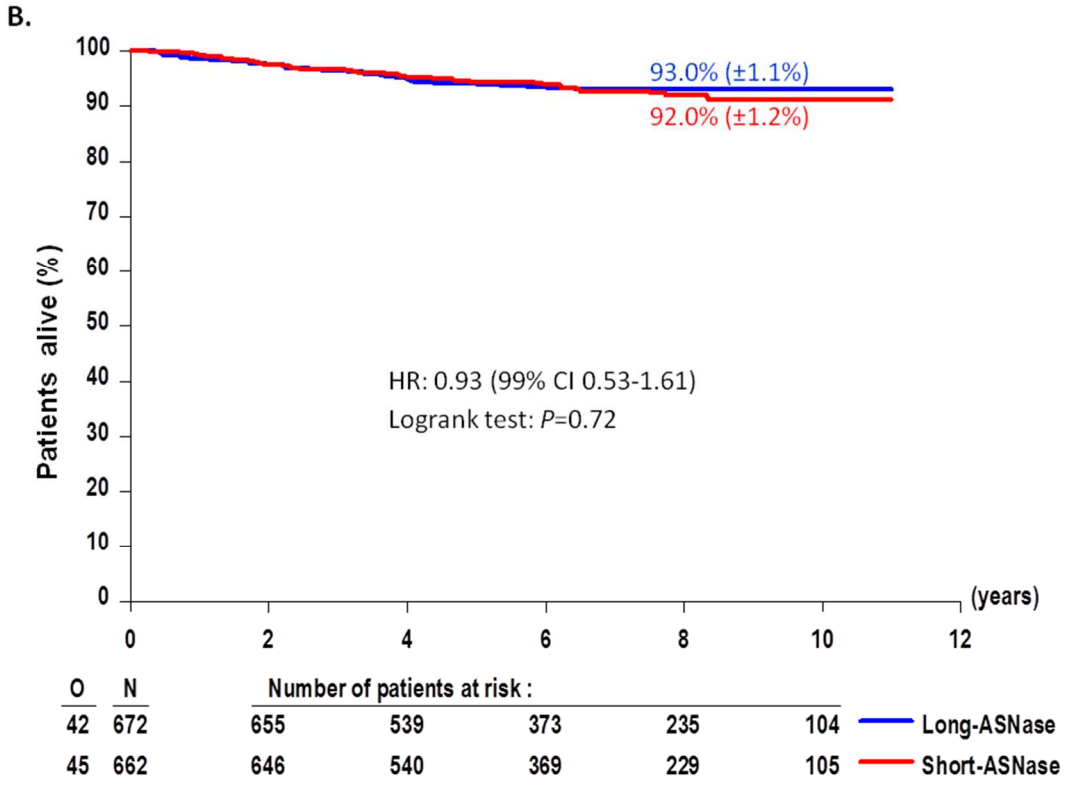
Methotrexate: Less than 1 year: 6 mg, 1 year 8 mg, 2 years 10 mg, 3 years and more: 12 mg
Cytarabine: Less than 1 year: 15 mg, 1 year 20 mg, 2 years 25 mg, 3 years and more: 30 mg
Hydrocortisone: Less than 1 year: 7.5 mg, 1 year 10 mg, 2 years 12.5 mg, 3 years and more: 15 mg

† Leucovorin rescue 12 mg/m²/6h starts at H36.

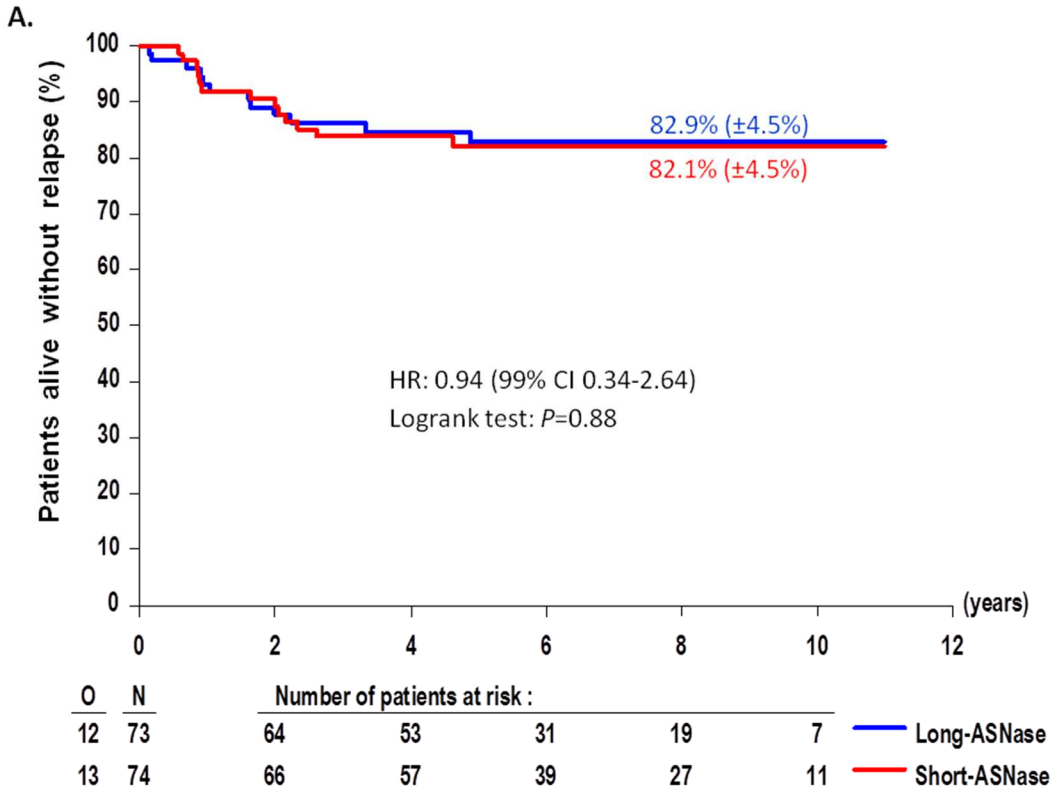
‡ Same corticosteroid as initial randomisation (protocol IA).

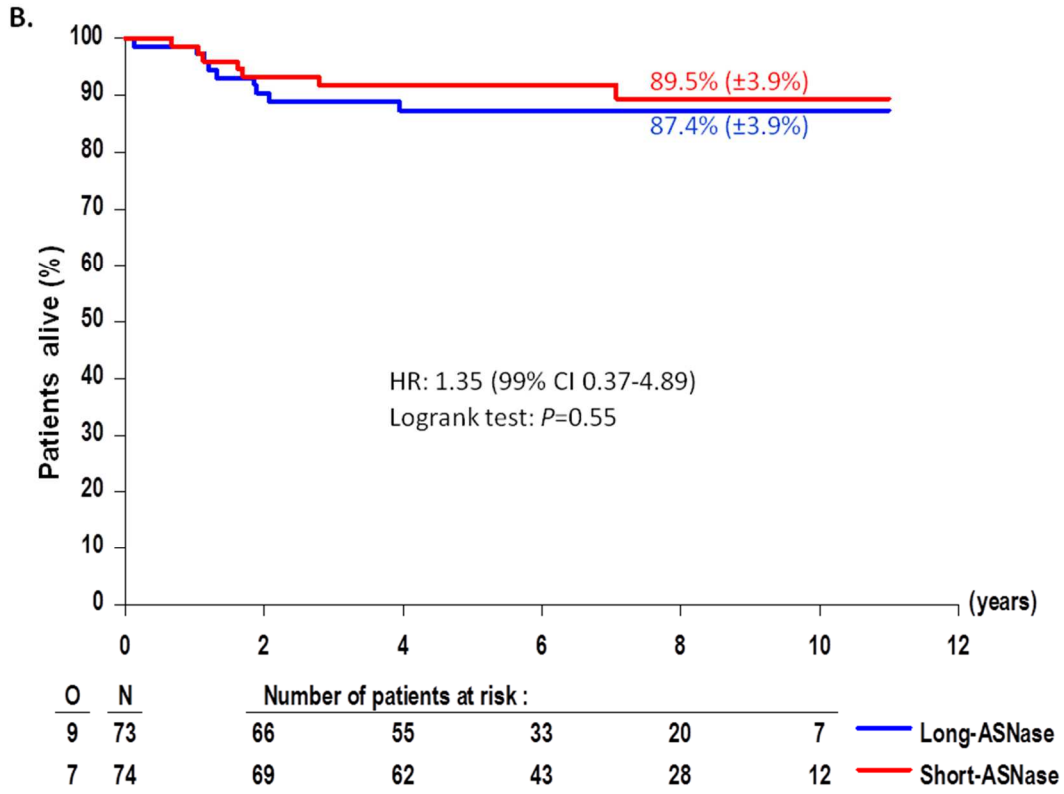
Suppl. figure 1: Treatment comparison for disease free survival (A) and overall survival (B) in B-cell ALL of the EORTC-CLG 58951 trial
O, observed number of events; N, number of patients randomly assigned.



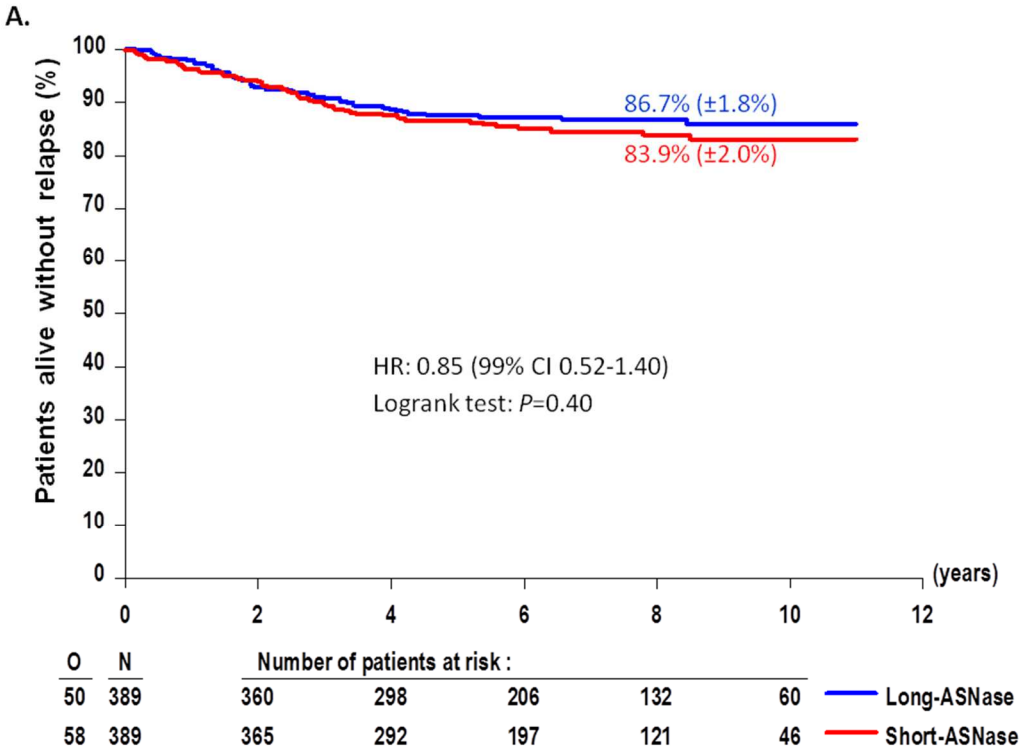


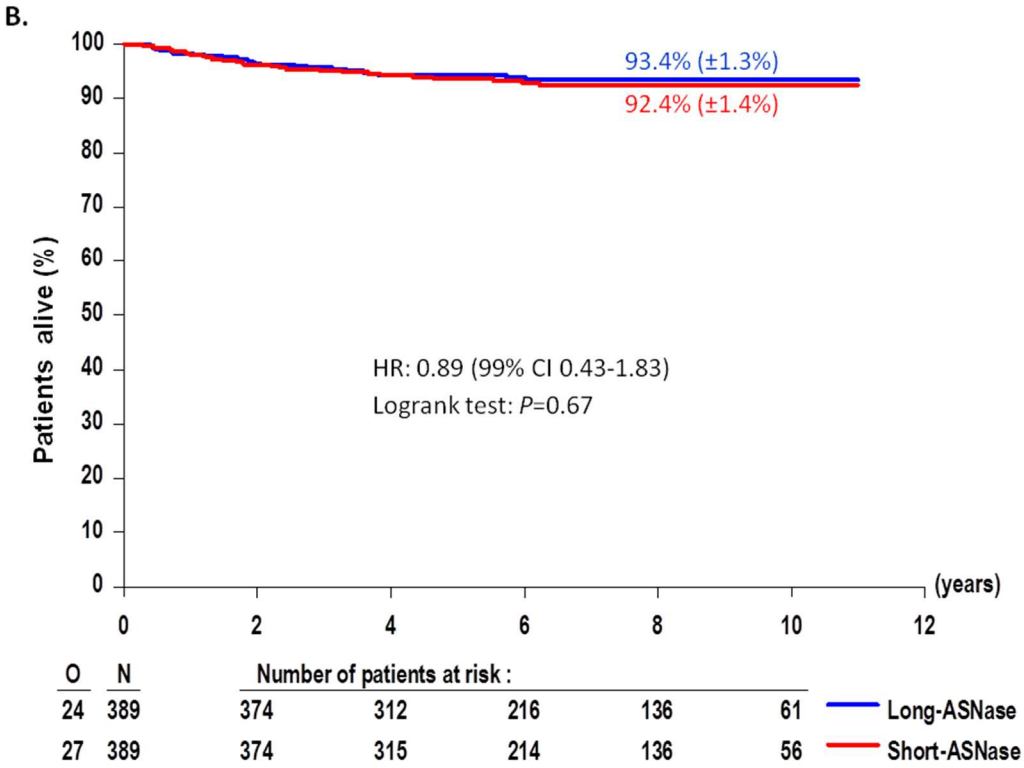
Suppl. figure 2: Treatment comparison for disease free survival (A) and overall survival (B) in T-cell ALL of the EORTC-CLG 58951 trial
O, observed number of events; N, number of patients randomly assigned.





Suppl. figure 3: Treatment comparison for disease free survival (A) and overall survival (B) in prednisolone group versus disease free survival (C) and overall survival (D) in the dexamethasone group of the EORTC-CLG 58951 trial
O: observed number of events; N, number of patients randomly assigned.





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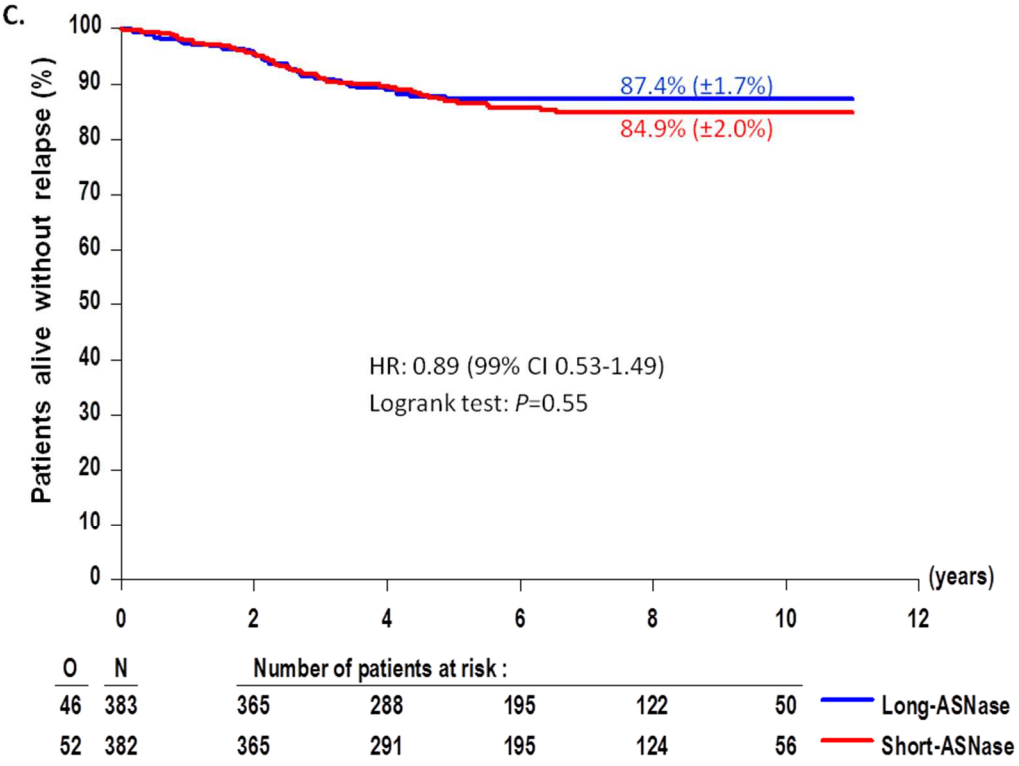
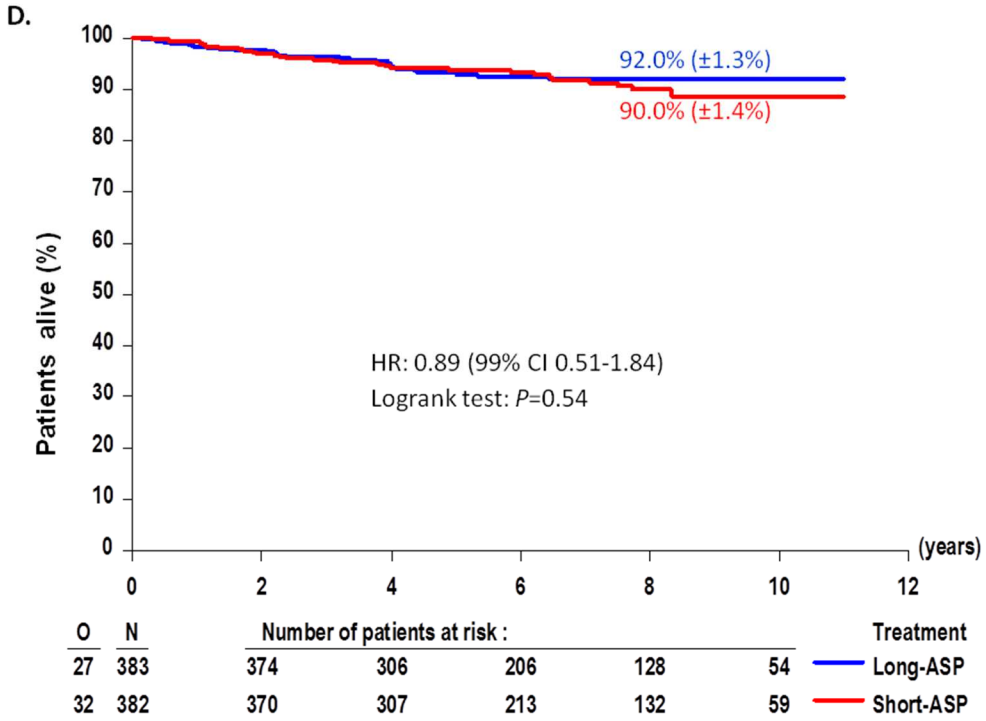

Patients alive without relapse (%)
100
90
80
70
60
50
40
30
20
10
0
87.4% (±1.7%)
84.9% (±2.0%)
HR: 0.89 (99% CI 0.53-1.49)
Logrank test: P=0.55
(years)
0
2
4
6
8
10
12
O N
Number of patients at risk :
46 383 365 288 195 122 50 Long-ASNase
52 382 365 291 195 124 56 Short-ASNase

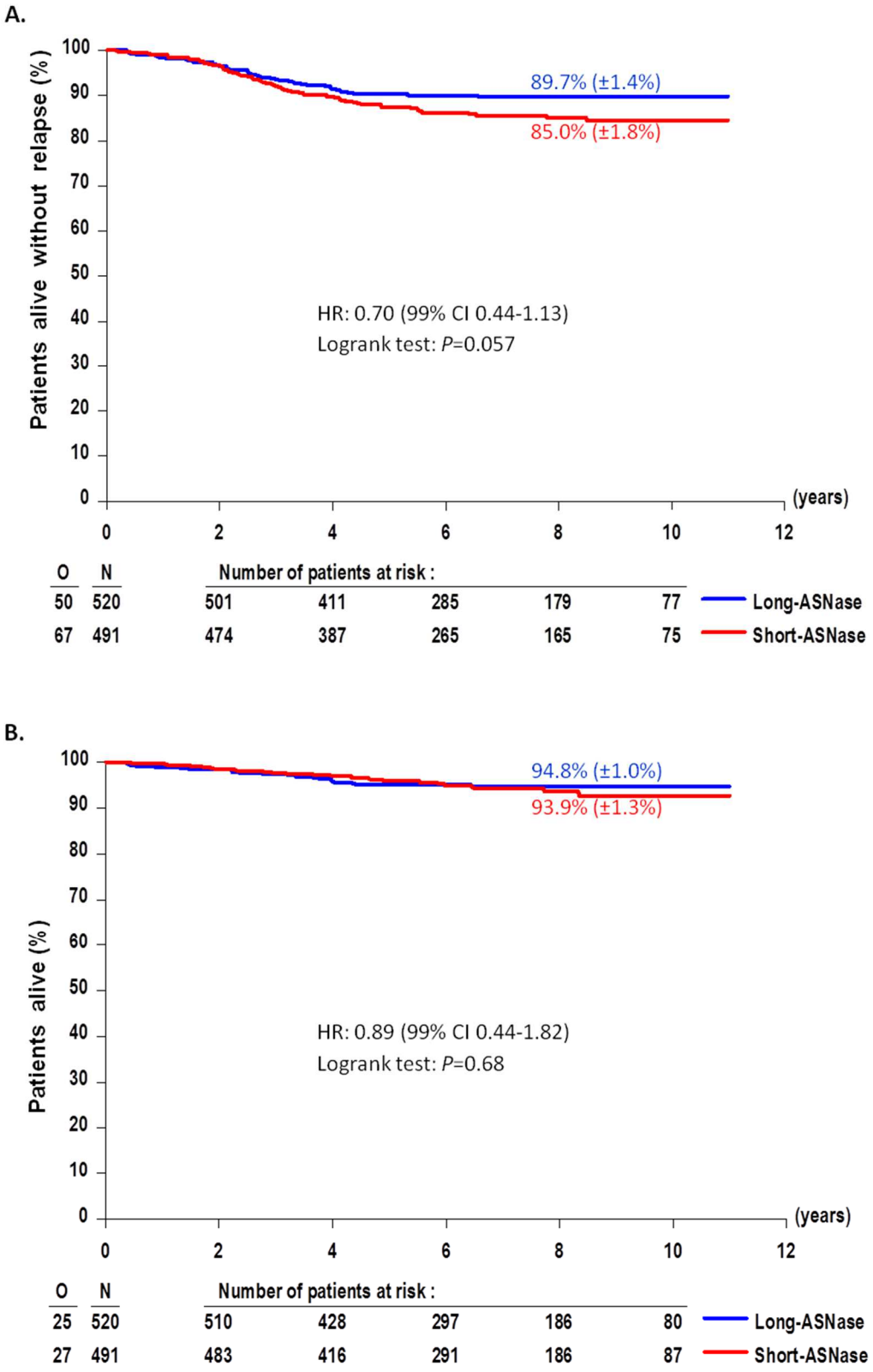
D.


Patients alive (%)
100
90
80
70
60
50
40
30
20
10
0
92.0% (±1.3%)
90.0% (±1.4%)
HR: 0.89 (99% CI 0.51-1.84)
Logrank test: P=0.54
(years)
0
2
4
6
8
10
12
O N
Number of patients at risk :
Treatment
27 383 374 306 206 128 54 Long-ASP
32 382 370 307 213 132 59 Short-ASP

Suppl. figure 4: Treatment comparison for disease free survival (A) and overall survival (B) in ALL with NCI Standard Risk and disease free survival (C) and overall survival (D) in ALL with NCI High Risk

NCI Standard Risk indicates ALL with WBC<50x10^9/l and age 1 - <10 years, NCI High Risk: ALL with WBC≥50x10^9/l or age < 1 or ≥10 years.

O, observed number of events; N, number of patients randomly assigned.



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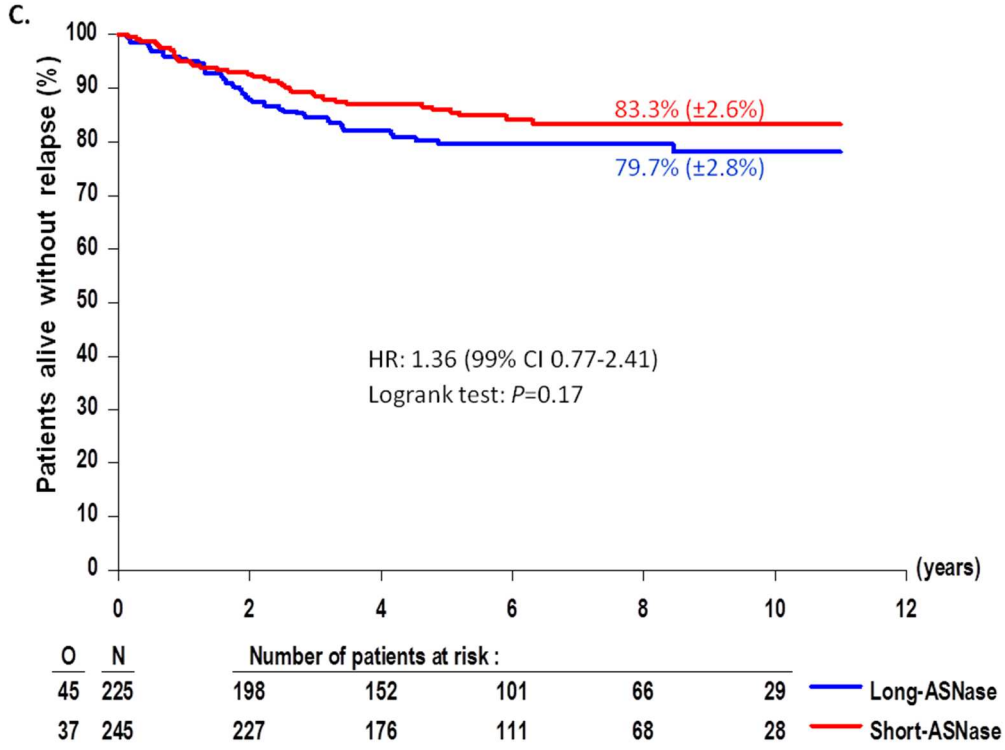
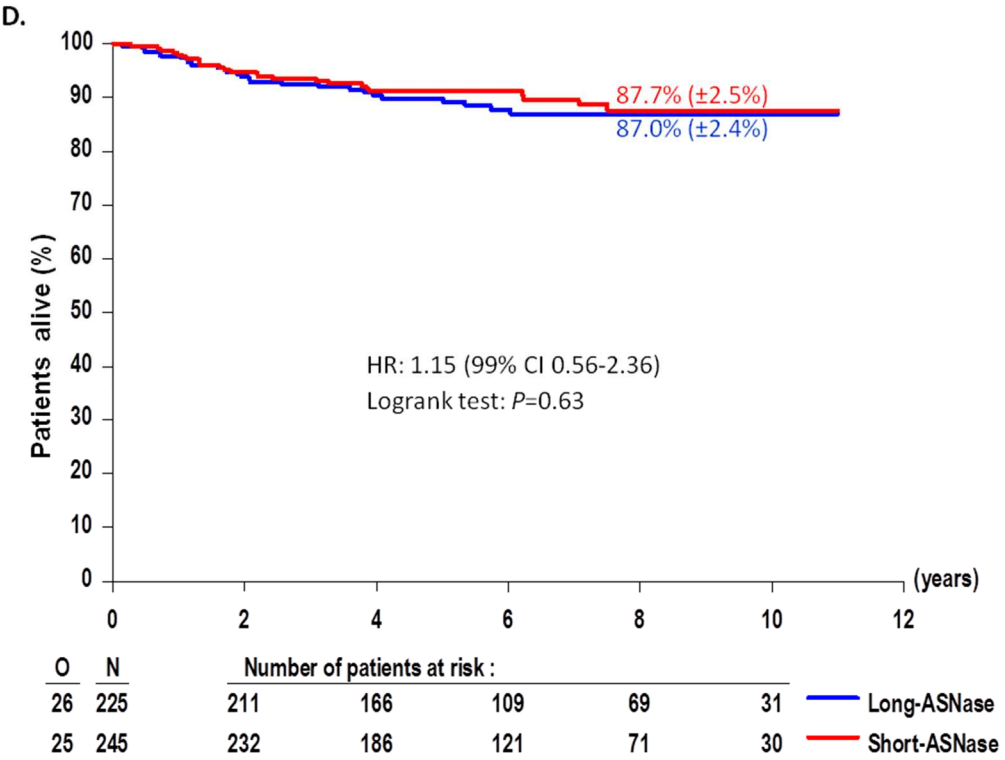

Patients alive without relapse (%)
100
90
80
70
60
50
40
30
20
10
0
83.3% (±2.6%)
79.7% (±2.8%)
HR: 1.36 (99% CI 0.77-2.41)
Logrank test: P=0.17
(years)
0
2
4
6
8
10
12
O N
Number of patients at risk :
45 225 198 152 101 66 29 Long-ASNase
37 245 227 176 111 68 28 Short-ASNase

D.


Patients alive (%)
100
90
80
70
60
50
40
30
20
10
0
87.7% (±2.5%)
87.0% (±2.4%)
HR: 1.15 (99% CI 0.56-2.36)
Logrank test: P=0.63
(years)
0
2
4
6
8
10
12
O N
Number of patients at risk :
26 225 211 166 109 69 31 Long-ASNase
25 245 232 186 121 71 30 Short-ASNase