

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Pui C-H, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. *N Engl J Med* 2009;360:2730-41.

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Supplementary Appendix

Study Monitoring and Statistical Analysis

Interim analyses for safety monitoring

The major adverse events monitored for safety included deaths, seizures, grade 4 infections, and grade 3 or 4 mucositis during remission induction, the delay in the initiation of consolidation therapy, and disseminated fungal infection until the end of second reinduction. Threshold rates were pre-specified for each type of event based on historical data. Stopping thresholds, statistical significance and power were determined by the sequential conditional probability ratio test (Xiong 1995). For example, for the infections in induction, we monitored against a baseline rate of 10% and would suggest modifying the study if there was statistical evidence that the infection rate exceeded 15%. The overall significance level would be 0.0636 with a power of 0.9713 to detect the unacceptable higher rate.

Interim Analysis	Sample Size (Patients)	Modify if # Patients with Grade 4 Infections \geq
1	30	9
2	60	14
3	120	23
4	180	31
5	240	37
6	300	45
7	360	53
8	420	58
9	480	63

For the efficacy, we monitored the rate of molecular remission (i.e., $<0.01\%$ blasts in bone marrow) after remission induction, with a rule determined by sequential conditional probability ratio tests. No interim analysis for any outcome was planned and no such analysis was performed.

The protocol progress was reviewed and monitored for safety, efficacy and publication of results by an independent Data Safety Monitoring Board (DSMB) every 6 months from the time that all patients enrolled in the first 6 months have completed remission induction in April 2001 until October 2007 when the DSMB determined that its monitoring role was completed.

Statistical design and analysis of the primary therapeutic aim

All patients in TOTAL XIII A and XIII B studies who had received prophylactic cranial irradiation served as historical controls. Since the irradiation was given when the patients remained in continuous complete remission at week 56 of continuation therapy, the comparison was conditioned on the subset of the same group of patients who remained in continuous complete remission at the same time point on Total XV study. The statistical inference consists of testing the null hypothesis that the distribution of subsequent remission duration is the same between the two cohorts against the alternative that the duration is superior when cranial irradiation is given, based on an unstratified Mantel-Haenszel test ($\alpha=\beta=0.10$). A secondary endpoint of proportion of isolated CNS relapse was also analyzed primarily for safety monitoring.

Xiong X. A class of sequential conditional probability ratio tests. J Am Statist Assoc 1995; 90:1463-1473.

Table 1. Remission Induction, Consolidation, and Early Continuation/Reinduction Therapy**A Remission induction**

Agent	Dosage	Schedule
Methotrexate	1 g/m ² IV over 4 or 24 hours	Day 1
Prednisone	40 mg/m ² /day	Days 5-32
Vincristine	1.5 mg/m ² per week	Days 5, 12, 19, 26
Daunorubicin	25 mg/m ² per week	Days 5, 12
L-asparaginase (Elspar)	10,000 U/m ² per dose IM (thrice weekly)	Days 6, 8, 10, 12, 14, 16, (19, 21, 23)*
Cyclophosphamide	1000 mg/m ² IV	Day 26
Cytarabine	75 mg/m ² per day IV	Days 27-30, 34-37
Mercaptopurine	60 mg/m ² per night	Days 26-39
Intrathecal cytarabine	Age-dependent	Day 1
Triple intrathecal	Age-dependent	Day 19 (8, 26)**

Intrathecal cytarabine (40, 50 or 60 mg for ages 1 to 1.99, 2 to 2.99 and ≥ 3 years, respectively).

Triple intrathecal treatments (methotrexate 8, 10 or 12 mg; hydrocortisone 16, 20 or 24 mg; and cytarabine 24, 30 or 36 mg for ages 1 to 1.99, 2 to 2.99 and ≥ 3 years, respectively); **Extra triple intrathecal treatment on days 8 and 26 for patients with high-risk features of CNS relapse (CNS-2, CNS-3, traumatic lumbar puncture with blasts, T-cell ALL with leukocyte count $> 50 \times 10^9/L$, B-cell precursor ALL with leukocyte count $> 100 \times 10^9/L$, or the presence of t(9;22)[*BCR-ABL1*], *MLL* rearrangement, or hypodiploidy < 45 chromosomes).

*Extra asparaginase on days 19, 21, and 23 for patients with $\geq 1\%$ residual leukemia cells in the bone marrow on day 19.

B Consolidation therapy

Agent	Dosage	Schedule
High-dose methotrexate*	Targeted to 33 μ M (low-risk) or 65 μ M (standard-/or high-risk)	Days 1, 15, 29 and 43
Mercaptopurine	50 mg/m ² per night	Days 1 to 56
Triple intrathecal	Age-dependent	Day 1, 15, 29 and 43

Methotrexate dosage was adjusted according to prior patient-specific pharmacokinetic data to achieve a steady-state concentration of 65 μ M (corresponding to an average dose of approximately 5 g/m²) in standard-risk cases, and 33 μ M (average 2.5 g/m²) in low-risk cases.

C Early continuation/reinduction therapy

Week	Low-risk Patients	Standard- or high-risk Patients
1	Mercaptopurine + dexamethasone + vincristine	Asparaginase + mercaptopurine + dexamethasone + vincristine + doxorubicin
2	Mercaptopurine + methotrexate	Asparaginase + mercaptopurine
3	Mercaptopurine + methotrexate	Asparaginase + mercaptopurine
4	Mercaptopurine + dexamethasone + vincristine	Asparaginase + mercaptopurine + dexamethasone + vincristine + doxorubicin
5	Mercaptopurine + methotrexate	Asparaginase + mercaptopurine
6	Mercaptopurine + methotrexate	Asparaginase + mercaptopurine
7	Dexamethasone + vincristine + asparaginase + doxorubicin	Asparaginase + dexamethasone + vincristine + doxorubicin
8	Vincristine + asparaginase	Asparaginase + vincristine + doxorubicin
9	Dexamethasone + vincristine + asparaginase	Asparaginase + dexamethasone + vincristine
10	Mercaptopurine + methotrexate	Asparaginase + mercaptopurine
11	Mercaptopurine + methotrexate	Asparaginase + mercaptopurine + vincristine + doxorubicin
12	Mercaptopurine + methotrexate	Asparaginase + mercaptopurine
13	Mercaptopurine + methotrexate	Asparaginase + mercaptopurine
14	Mercaptopurine + dexamethasone + vincristine	Asparaginase + mercaptopurine + dexamethasone + vincristine + doxorubicin

15	Mercaptopurine + methotrexate	Asparaginase + mercaptopurine
16	Mercaptopurine + methotrexate	Asparaginase + mercaptopurine
17	Dexamethasone + vincristine + asparaginase + doxorubicin	Asparaginase + dexamethasone + vincristine
18	Vincristine + asparaginase	Asparaginase + vincristine
19	Dexamethasone + vincristine + asparaginase	Asparaginase + vincristine + dexamethasone + high-dose cytarabine
20	Mercaptopurine + methotrexate	-----
21	Mercaptopurine + methotrexate	Mercaptopurine + methotrexate
22	Mercaptopurine + methotrexate	Mercaptopurine + methotrexate
23	Mercaptopurine + methotrexate	Cyclophosphamide + cytarabine
24	Mercaptopurine + dexamethasone + vincristine	dexamethasone + vincristine

Mercaptopurine – 75 mg/m² PO every evening for 7 days for low-risk group; 50 mg/m² in the first 16 weeks and 75 mg/m² thereafter for the standard- and high-risk groups. The starting dose for patients with heterozygous deficiency of thiopurine methyltransferase was 60 mg/m² instead of 75 mg/m².

Dexamethasone – 8 mg/m² PO per day in 3 divided doses for 5 days for low-risk group and 12 mg/m² for standard-risk group; 8 mg/m² on days 1 to 8 and 15 to 21 during reinduction I (weeks 7 to 9) and reinduction II (weeks 17 to 19) for both groups.

Asparaginase – 10,000 U/m² IM thrice weekly for 9 doses during each reinduction for low-risk group; and 25,000 units /m² IM weekly for 19 doses for the standard- and high-risk groups; in patients with allergic reactions to *E coli* asparaginase, *Erwinia* asparaginase 20,000 units /m² thrice weekly during reinduction treatment for the low-risk group, and 25,000 units /m² twice weekly in standard-risk group; in patients with allergic reactions to both *E coli* and *Erwinia* asparaginase, or in those for whom *Erwinia* asparaginase was not available, pegaspargase (Oncaspar) 2500 units /m² per week.

Vincristine – 2 mg/m² IV, except for weeks 7-9 and 17-19 when given at 1.5 mg/m²; Methotrexate - 40 mg/m² IV or IM; Doxorubicin 30 mg/m² IV; High-dose cytarabine - 2 g/m² IV every 12 hours for 4 doses; Cyclophosphamide - 300 mg/m² IV; Cytarabine - 300 mg/m² IV.

Triple intrathecal therapy - low-risk cases with CNS-1 status: weeks 7, 12, 17, 24, 32, 40, and 48; low-risk cases with CNS-2, traumatic lumbar punctures with blasts or leukocyte count $\geq 100 \times 10^9/L$: weeks 7, 12, 17, 24, 28, 32, 36, 40, 44, and 48; standard-risk cases: weeks 7, 12, 17, 24, 28, 32, 36, 40, 44 and 48; other standard-risk cases with leukocyte count $\geq 100 \times 10^9/L$, T-cell ALL with WBC $\geq 50 \times 10^9/L$, presence of Philadelphia chromosome, *MLL* rearrangement, hypodiploidy <45, or CNS-3 status: weeks 3, 7, 12, 17, 24, 28, 32, 36, 40, 44, 48, 56, 64, 72, 80, 88 and 96.

Table 2. Continuation therapy (from week 21 to the end of therapy)*

Week	Low-risk Patients	Standard- or high-risk Patients
21	Mercaptopurine + methotrexate	Mercaptopurine + methotrexate
22	Mercaptopurine + methotrexate	Mercaptopurine + methotrexate
23	Mercaptopurine + methotrexate	Cyclophosphamide + cytarabine
24	Mercaptopurine + dexamethasone + vincristine	Dexamethasone + vincristine
25	Mercaptopurine + methotrexate	Mercaptopurine + methotrexate
26	Mercaptopurine + methotrexate	Mercaptopurine + methotrexate
27	Mercaptopurine + methotrexate	Cyclophosphamide + cytarabine
28	Mercaptopurine + dexamethasone + vincristine	Dexamethasone + vincristine

*See footnote of Table 1 in the original article for the details of intrathecal treatments

Mercaptopurine – 75 mg/m² per night for 7 days; Methotrexate - 40 mg/m² IV or IM per week; Cyclophosphamide - 300 mg/m² IV on day 1; Cytarabine - 300 mg/m² IV on day 1; Dexamethasone – 8 mg/m² PO per day in 3 divided doses for 5 days for low-risk group and 12 mg/m² for standard-risk group; Vincristine – 2.0 mg/m² IV (maximum 2 mg) on day 1.

Low-risk patients received daily mercaptopurine and weekly methotrexate, interrupted by pulses of dexamethasone, vincristine, and mercaptopurine every 4 weeks (up to week 100); after which only mercaptopurine and methotrexate will be given. Standard-risk patients received three drug pairs given in 4-week blocks: mercaptopurine plus methotrexate in the first and second weeks, cyclophosphamide plus cytarabine in the third week (replaced by mercaptopurine and methotrexate after week 67), and dexamethasone plus vincristine in the fourth week with added mercaptopurine between week 68 and week 100 (replaced by mercaptopurine and methotrexate after week 100). The total duration of continuation treatment was 120 weeks for girls and 146 weeks for boys.

The dosages of mercaptopurine and methotrexate were tailored to the limits of tolerance (leukocyte count between 1.5 and 3.0 x 10⁹/L and absolute neutrophil count above 0.5 x 10⁹/L); higher counts were permissible a week following dexamethasone treatment. Thiopurine methyltransferase phenotype and genotypes were determined prospectively in all patients, with doses of mercaptopurine lowered in those with heterozygous enzyme deficiency (e.g., 60 mg/m² instead of 75 mg/m²; no homozygous deficient patients were observed); subsequent doses of mercaptopurine (not methotrexate) were further reduced in patients with poor tolerance and enzyme deficiency.

Table 3. Reintensification Therapy

Agent	Dosage	Schedule
Dexamethasone	20 mg/m ² per day PO or IV	Days 1-5
Cytarabine	2 g/m ² (every 12 hours) for 4 doses	Days 1-2
Etoposide	100 mg/m ² (every 12 hours) for 5 doses	Days 3-5
Asparaginase	25,000 U/m ² IM	Day 6

Table 4. Clinical and biologic features of the 11 patients with isolated CNS relapse

Patient No.						Minimal residual disease				
	Age at diagnosis (Year)	Leukocyte count x 10 ⁹ L	Sex	Race	Subtype	Day 19 (%)	Day 46 (%)	CNS Status	Initial Remission Duration (Year)	Second remission duration(Year)
1	2.03	86.2	Male	African American	B-cell precursor	0.06	0.00	CNS-1	3.2	2.5
2	5.87	3.50	Male	White	Hyperdiploidy >50	0.02	0.00	CNS-1	2.97	0.43
3	4.30	34.8	Male	African American	t(1;19) [<i>TCF3-PBX1</i>]	1.12	0.00	CNS-2	1.9	5.52
4	5.20	7.2	Male	African American	t(1;19) / [<i>TCF3-PBX1</i>]	0.00	0.00	CNS-2	1.8	1.8
5	11.13	53.7	Male	African American	t(1;19) / [<i>TCF3-PBX1</i>]	0.27	0.00	CNS-2	3.1	2.5
6	1.18	656.2	Female	White	T Cell	0.88	0.03	CNS-3	1.9	2.3
7	1.86	567.0	Female	White	T Cell	0.02	0.00	CNS-2	0.4	3.6
8	5.39	657.0	Female	African American	T Cell	2.62	1.59	CNS-2	0.4	4.2
9	6.06	3.4	Male	White	T Cell	0.00	0.00	CNS-2	1.3	2.0
10	7.08	34.1	Male	White	T Cell	0.14	0.00	CNS-1	2.3	2.0
11	10.90	125.8	Male	White	T Cell	0.00	0.00	traumatic with blasts	0.9	4.2

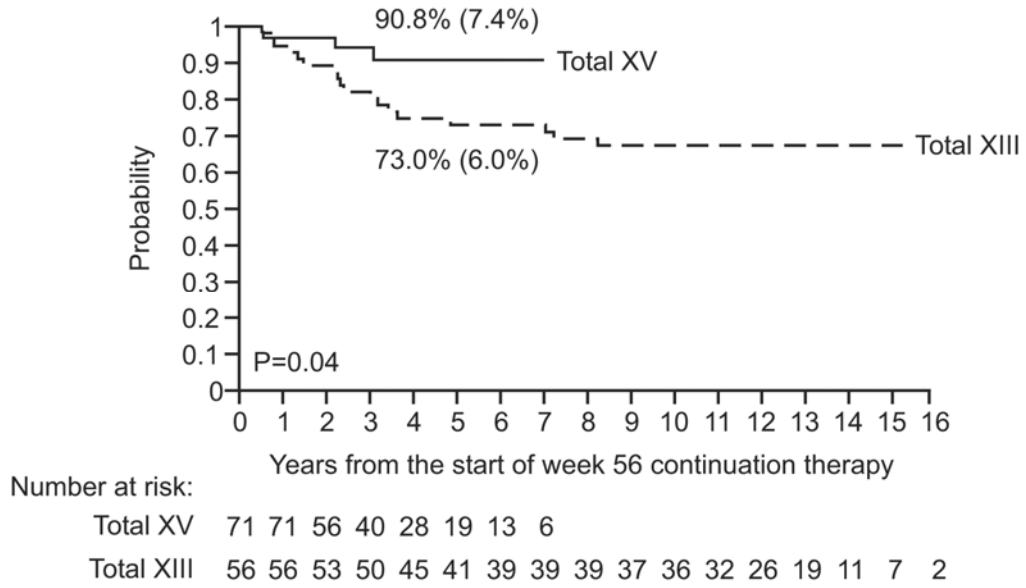


Figure 1. Comparison of continuous complete remission duration in 71 high-risk patients in Total XV study who received chemotherapy only versus 56 historical controls who received prophylactic cranial irradiation in Total XIII studies. The 5-year rates are means \pm standard error.