

Effect of two additional doses of intrathecal methotrexate during induction therapy on serious infectious toxicity in pediatric patients with acute lymphoblastic leukemia

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Table S1 Definition of CNS status in AIEOP-BFM ALL 2009

CNS 1:

no clinical or imaging findings of CNS disease and absence of blasts on cytospin preparation in CSF, regardless of the number of white blood cells (WBCs) and regardless of red blood cells (RBCs) or bloody contamination.

CNS 2:

no clinical or imaging findings of CNS disease **and**

- o **CNS2a:** <10/μl RBCs and no macroscopical contamination with blood; ≤5/μl WBCs and cytospin positive for blasts
- o **CNS 2b:** Macroscopical contamination with blood and/or ≥10/μl RBCs; ≤5/μl WBCs and cytospin positive for blasts
- o **CNS 2c:** Macroscopical contamination with blood and/or ≥10/μl RBCs; >5/μl WBCs and cytospin positive for blasts but negative by algorithm as specified below (i.e. WBC/RBC in CSF < 2x WBC/RBC in blood)

CNS 3:

- o **CNS 3a:** <10/μl RBCs and no macroscopical contamination with blood; >5/μl WBCs and cytospin positive for blasts
- o **CNS 3b:** ≥10/μl RBCs and/or macroscopical contamination with blood, >5/μl WBCs and positive by algorithm (i.e. WBC/RBC in CSF > 2x WBC/RBC in blood).
- o **CNS 3c:** Clinical or imaging findings of CNS disease: CNS masses or meningeal infiltration on Magnetic Resonance Imaging (MRI) and/or Computed Tomography (CT) Cranial nerve palsies if not caused by extracerebral manifestations

Table S2: Definition of life-threatening

An adverse event is considered life-threatening if it places the patient, in the view of the investigator, at *immediate* risk of death from the event as it occurred, i.e., it does not refer to an event which hypothetically might have caused death if it were more severe. Life-threatening events therefore usually require an emergency intervention into the immediately life-threatening condition such as:

- artificial ventilation with intubation due to respiratory failure
- urgent intervention (drug therapy, e.g. vasopressor support, or cardiac resuscitation) due to circulatory/cardiac failure
- emergency surgery or emergency endoscopic intervention due to other serious clinical situations, e.g.
 - gastrointestinal bleeding or perforation
 - cerebral abscess/bleeding

An event requiring an intervention for preventing a life-threatening situation (e.g. dialysis due to acute renal failure in a clinically stable patient) is not life-threatening *per definitionem*.

Table S3: Life-threatening and fatal infections in induction according to the number of intrathecal doses of IT MTX related to different patient characteristics

	all		life-threatening infection			fatal infection		
	additional IT MTX in induction		additional IT MTX in induction			additional IT MTX in induction		
	No N	Yes N	No N (%)	Yes N (%)	P	No N (%)	Yes N (%)	P
all patients	4706	1350	77 (1.6%)	59 (4.4%)	< 0.001	16 (0.3)	22 (1.6)	< 0.001
male	2651	840	37 (1.4)	30 (3.6)	< 0.001	9 (0.3)	12 (1.4)	0.001
female	2055	510	40 (1.9)	29 (5.7)	< 0.001	7 (0.3)	10 (2.0)	< 0.001
age < 10 years	3590	993	43 (1.2)	40 (4.0)	< 0.001	4 (0.1)	12 (1.2)	< 0.001
age ≥ 10 years	1116	357	34 (3.0)	19 (5.3)	0.050	12 (1.1)	10 (2.8)	0.04
WBC < 50*10⁹/l	3989	939	63 (1.6)	42 (4.5)	< 0.001	13 (0.3)	14 (1.5)	< 0.001
WBC ≥ 50*10⁹/l	715	411	14 (2.9)	17 (4.1)	0.037	3 (0.4)	8 (1.9)	0.022
B-lineage	4151	1034	54 (1.3)	50 (4.8)	< 0.001	11 (0.3)	19 (1.8)	< 0.001
T-lineage	538	312	22 (4.1)	9 (2.9)	0.45	5 (0.9)	3 (1.0)	1.0
ETV6-RUNX1 neg.	3538	1125	69 (2.0)	48 (4.3)	< 0.001	15 (0.4)	18 (1.6)	< 0.001
ETV6-RUNX1 pos.	1098	222	7 (0.6)	11 (5.0)	< 0.001	1 (0.1)	4 (1.8)	0.003
FCM d15 < 10%	4010	1085	62 (1.5)	44 (4.1)	< 0.001	11 (0.3)	16 (1.5)	< 0.001
FCM d15 ≥ 10%	570	199	8 (1.4)	10 (5)	0.004	2 (0.4)	4 (2.0)	0.42
Prot. IA-Dexa	373	174	18 (4.8)	6 (3.4)	0.654	3 (0.8)	1 (0.6)	1.0
Prot. IA/IA'	4151	1034	54 (1.3)	50 (4.8)	< 0.001	11 (0.3)	19 (1.8)	< 0.001
Prot. IA-CPM	165	138	4 (2.4)	3 (2.2)	1.0	2 (1.2)	2 (1.4)	1.0

Table S3 legend:

WBC indicates white blood count; FCM-MRD, minimal residual disease by flow cytometry; Dexa, dexamethasone; IA/IA', Prednisone/Prednisolone with 4 or 2 doses (IA') of DNR in protocol IA; CPM, cyclophosphamide; IT, intrathecal; MTX, methotrexate; Prot., protocol.

Table S4: pB-ALL. Life-threatening and fatal infections in induction according to the number of intrathecal doses of IT MTX related to different patient characteristics

	all		life-threatening infection			fatal infection		
	additional IT MTX in induction		additional IT MTX in induction			additional IT MTX in induction		
	No N	Yes N	No N (%)	Yes N (%)	<i>P</i>	No N (%)	Yes N (%)	<i>P</i>
all patients (pB-ALL)	4151	1034	54 (1.3%)	50 (4.8%)	< 0.001	11 (0.3)	19 (1.8)	< 0.001
male	2243	592	22 (1.0)	27 (3.7)	< 0.001	5 (0.2)	9 (1.5)	0.001
female	1908	442	32 (1.7)	28 (6.3)	< 0.001	6 (0.3)	10 (2.3)	< 0.001
age < 10 years	3280	821	34 (1.0)	34 (4.1)	< 0.001	4 (0.1)	10 (1.2)	< 0.001
age ≥ 10 years	871	213	20 (2.3)	16 (7.5)	0.001	7 (0.8)	9 (4.2)	0.001
WBC < 50*10⁹/l	3698	829	45 (1.2)	38 (4.6)	< 0.001	10 (0.3)	13 (1.6)	< 0.001
WBC ≥ 50*10⁹/l	451	205	9 (2.0)	12 (5.9)	0.015	1 (0.2)	6 (2.9)	0.005
ETV6-RUNX1 neg.	2995	811	46 (1.5)	39 (4.8)	< 0.001	10 (0.3)	15 (1.8)	< 0.001
ETV6-RUNX1 pos.	1098	221	7 (0.6)	11 (5.0)	< 0.001	1 (0.1)	4 (1.8)	0.003
FCM d15 < 10%	3642	893	48 (1.3)	39 (4.4)	< 0.001	10 (0.3)	16 (1.8)	< 0.001
FCM d15 ≥ 10%	398	95	3 (0.8)	7 (7.4)	0.001	0 (0.0)	2 (2.1)	0.037

Table S4 legend:

WBC indicates white blood count; FCM-MRD, minimal residual disease by flow cytometry; IT, intrathecal; MTX, methotrexate.

Table S5: T-ALL. Life-threatening and fatal infections in induction according to the number of intrathecal doses of IT MTX related to different patient characteristics

	all		life-threatening infection			fatal infection		
	additional IT MTX in induction		additional IT MTX in induction			additional IT MTX in induction		
	No N	Yes N	No N (%)	Yes N (%)	<i>P</i>	No N (%)	Yes N (%)	<i>P</i>
all patients (T-ALL)	538	312	22 (4.2%)	9 (2.9%)	0.45	5 (0.9)	3 (1.0)	1.0
male	397	245	14 (3.5)	8 (3.3)	1.0	4 (1.0)	3 (1.2)	1.0
female	141	67	8 (5.7)	1 (1.5)	0.277	1 (0.7)	0 (0.0)	1.0
age < 10 years	298	169	8 (2.7)	6 (3.6)	0.585	0 (0.1)	2 (1.2)	0.13
age ≥ 10 years	240	143	14 (5.8)	3 (2.1)	0.123	5 (2.1)	1 (0.7)	0.42
WBC < 50*10⁹/l	276	107	18 (6.5)	4 (3.7)	0.340	3 (1.1)	1 (0.9)	1.0
WBC ≥ 50*10⁹/l	262	205	4 (1.5)	5 (2.4)	0.515	2 (0.8)	2 (1.0)	1.0
FCM d15 < 10%	356	191	13 (3.7)	5 (2.6)	0.621	1 (0.3)	0 (0.0)	1.0
FCM d15 ≥ 10%	168	102	5 (3.0)	3 (2.9)	1.0	2 (1.2)	2 (2.0)	0.63
Prot. IA-Dexa	373	174	18 (4.8)	6 (3.4)	0.654	3 (0.8)	1 (0.6)	1.0
Prot. IA-CPM	165	138	4 (2.4)	3 (2.2)	1.0	2 (1.2)	2 (1.4)	1.0

Table S5 legend:

WBC indicates white blood count; FCM-MRD, minimal residual disease by flow cytometry; Dexa, dexamethasone; CPM, cyclophosphamide; IT, intrathecal; MTX, methotrexate; Prot., protocol

Table S6: Multivariate logistic regression analysis on life-threatening infections in protocol IA, stratified by gender

	Male			Female		
	Odds ratio	95% CI	<i>p-value</i>	Odds ratio	95% CI	<i>p-value</i>
Age (years)						
< 10	1			1		
≥ 10	1.75	1.02-3.00	0.041	1.87	1.06-3.27	0.030
initial WBC (10⁹/L)						
< 50	1			1		
≥ 50	0.83	0.44-1.57	0.57	0.89	0.45-1.78	0.75
ETV6-RUNX1						
Negative	1			1		
Positive	0.67	0.29-1.52	0.34	0.73	0.35-1.52	0.40
FCM-MRD d15						
<10% blasts	1			1		
≥10% blasts	0.92	0.44-1.93	0.82	1.06	0.50-2.22	0.88
Protocol IA-Dexa						
no	1			1		
yes	1.68	0.87-3.22	0.12	1.93	0.81-4.57	0.14
add. IT MTX in P IA						
no	1			1		
yes	2.80	1.67-4.71	<0.001	2.93	1.72-4.99	< 0.001

Table S6 legend:

WBC indicates white blood count; FCM-MRD, minimal residual disease by flow cytometry; of DNR in protocol IA Dexa, dexamethasone; CPM, cyclophosphamide; IT, intrathecal; MTX, methotrexate; P/Prot., protocol.