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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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Abstract

Although initial central nervous system (CNS) involvement is rarely detected in childhood acute lymphoblastic leukemia (ALL), risk-adapted CNS-directed therapy is essential for all patients. Treatment intensity depends on the initial CNS status. In the AIEOP-BFM ALL 2009 trial, patients with cytomorphic detection of leukemic blasts in initial cerebrospinal fluid were classified as CNS2 or CNS3 and received five intrathecal doses of methotrexate (MTX) in induction therapy compared to patients with CNS1 status (no blasts detected) who received three doses. The impact of additional intrathecal (IT) MTX on systemic toxicity in induction therapy is unknown. Between June 1st 2010 and February 28th 2017, a total of 6,136 ALL patients aged 1-17 years were enrolled onto the AIEOP-BFM ALL 2009 trial. The effect of three versus five doses of IT MTX during induction therapy on the incidence of severe infectious complications was analyzed. Among 4,706 patients treated with three IT MTX doses, 77 (1.6%) had a life-threatening infection during induction as compared to 59 of 1,350 (4.4%) patients treated with five doses ($P < 0.001$; Odds Ratio 2.86 [95% Confidence Interval 1.99-4.13]). In a multivariate regression model, treatment with additional IT MTX proved to be the strongest risk factor for life-threatening infections (Odds Ratio 2.85 [1.96-4.14]). Fatal infections occurred in 16 (0.3%) and 38 (1.6%) patients treated with three or five IT MTX doses, respectively ($P < 0.001$). As the relevance of additional intrathecal MTX in induction for relapse prevention in CNS2 patients is unclear, doses of intrathecal therapy have been reduced for these patients. (*Clinicaltrials.gov identifiers: NCT01117441 and NCT00613457*).

Introduction

Involvement of the central nervous system (CNS) can be detected in about 3-5% of patients at initial diagnosis of acute lymphoblastic leukemia (ALL) and in 30-40% of pa-

tients at ALL relapse.^{1,2} Without CNS-directed therapy, relapses originating from the CNS can be expected in up to 75%.³ For a sustainable therapy for children with ALL a stratified prophylactic and therapeutic CNS-directed therapy is, therefore, indispensable. Introduction of cranial

radiotherapy (CRT) and intrathecal drugs have improved the outcome of CNS disease.⁴ Due to the long-term toxicity of CRT and steadily improving cure rates in ALL, CRT has mainly been replaced by systemic and intrathecal CNS-directed chemotherapy.⁵⁻⁹

In the AIEOP-BFM ALL 2009 trial, CNS status was determined by the number of nucleated cells and the presence of blasts in initial CSF before start of chemotherapy, and the presence of clinical and imaging findings of CNS disease. Patients with cytomorphologic detection of blasts in the initial CSF cytopsin were classified as CNS2 status if the number of nucleated cells was $\leq 5/\mu\text{L}$, whereas patients with higher cell counts and detection of blasts in the initial CSF cytopsin were classified as CNS3.

Due to the observation of a higher risk of relapse in patients with CNS2 status at the time of initial diagnostic workup, the study group from St. Jude Children's Research Hospital postulated the need for a more intensive intrathecal therapy for these patients.^{10,11} Based on the data of Mahmoud *et al.*, intensified therapy with two additional intrathecal doses of methotrexate (MTX) in the induction phase was introduced for patients with CNS2 status in the ALL-BFM 95 study of the Berlin-Frankfurt-Münster ALL group compared to patients with CNS1 status who received three doses. Analysis of the ALL-BFM 95 trial demonstrated that patients with CNS2 status had the same event-free survival (EFS) as patients with CNS1 status.¹² Thus, patients with CNS2 as well as those with CNS3 status continued to receive two additional intrathecal doses of IT MTX in induction therapy in subsequent AIEOP-BFM ALL trials.

There are few data on the effect of additional intrathecal treatment in induction chemotherapy on adverse events,

especially on severe infectious complication,¹³ and, to our knowledge, no data have been published on the effect of two additional IT MTX doses in induction on adverse infectious events. Therefore, we performed a retrospective analysis to investigate the toxic effects of two additional IT MTX doses in induction chemotherapy in the AIEOP-BFM ALL 2009 trial.

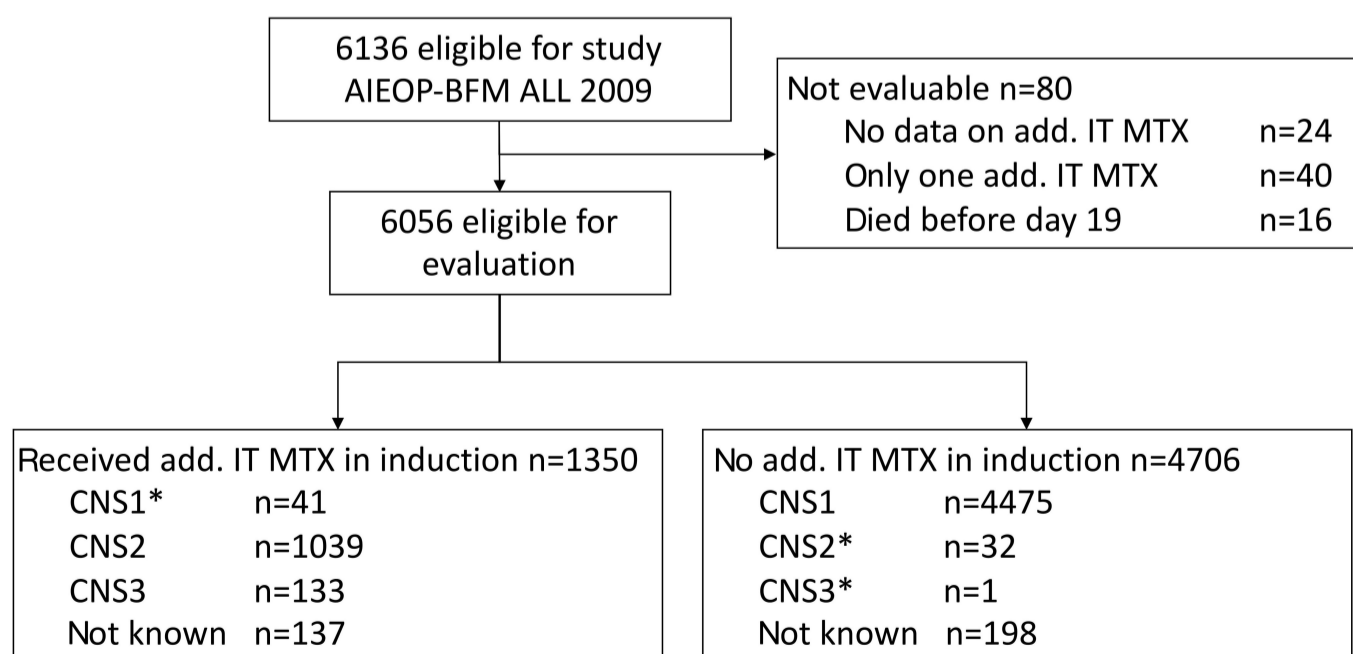
Methods

Patients and study design

Between June 1st 2010 and February 28th 2017, a total of 6,136 ALL patients under 18 years of age were enrolled onto the AIEOP-BFM ALL 2009 trial (registered at EudraCT n. 2007-004270-43). Patients were treated in participating study centers in Austria, Australia, the Czech Republic, Germany, Israel, Italy and Switzerland. Informed consent was obtained from the guardians of all patients. Eighty of the patients enrolled onto the study were not evaluable for analysis for the following reasons: only one additional dose of IT MTX in induction was administered (n=40), no information about additional IT MTX was available (n=24), or patients died before day 19, the day on which the first additional dose of IT MTX was scheduled per protocol (n=16) (Figure 1).

Cytomorphologic evaluation of CSF cytopsin preparations was carried out at the local treatment center; an additional central review of the cytopsin preparation was performed in 56% of the cases.

Patients without blasts in CSF were classified as having CNS1 status independent of the CSF cell count. Patients with non-traumatic lumbar puncture and detection of blasts on the diagnostic CSF cytopsin were classified as



* Patients were treated in deviation from the protocol plan

Figure 1. Consolidated standards for reporting of trials (CONSORT) diagram. add. : additional; IT: intrathecal; MTX: methotrexate; n: number.

either CNS2a if the number of nucleated cells was $\leq 5/\mu\text{L}$ or CNS3a if the number of nucleated cells was $>5/\mu\text{L}$. Patients with traumatic lumbar puncture and detection of blasts were classified as having CNS2b status if the nucleated cell count was $\leq 5/\mu\text{L}$ or as either CNS2c or CNS3b (depending on the estimated level of blood contamination) if the nucleated cell count was $>5/\mu\text{L}$. (For details see the *Online Supplementary Appendix*).

The study protocol was approved by the competent ethics committees of the national co-ordinating centers and by local ethics committees where required.

Treatment

Patients were treated according to the AIEOP-BFM ALL 2009 protocol. The treatment plan of the induction phase Protocol IA is shown in Table 1.

According to the protocol, IT MTX was administered on days 1, 12 and 33 for patients classified as CNS1, and on days 1, 12, 19, 26 and 33 for patients classified as CNS2 or CNS3 during induction phase Protocol IA.

Documentation of life-threatening infections

Data on serious adverse events were regularly collected in the AIEOP-BFM ALL 2009 study according to the regulatory requirements and were classified as infectious events based on the information provided by the investigators. The assessment of life-threatening status was made by the investigator and centrally reviewed by qualified persons in the national study co-ordinating centers of the participating groups according to the ICH Harmonised Tripartite Guideline. An adverse event was considered as life-threatening if its occurrence placed the patient at immediate risk of death. A severe adverse event that might have caused death

if it had occurred in a more severe form, was not considered as life-threatening, as long as the patient was not at immediate risk of death. Details of the definition of life-threatening are provided in *Online Supplementary Table S2*.

Statistical analysis

All analyses were performed on the basis of the number of IT MTX doses the patient had actually received. Differences in the distribution of individual parameters among patient subsets were analyzed using the χ^2 or Fisher's exact test for categorized variables and the Mann-Whitney U test for continuous variables. The association between rates of adverse events and prognostic factors was examined by univariate and multivariate logistic regression analysis to calculate Odds Ratios (OR) and their 95% Confidence Intervals (CI). To describe the impact of CNS status on relapse risk, Cox proportional hazard model was used for uni- and multivariate analysis. Differences in the distribution of categorical variables were analyzed using the Fisher's exact test. Analyses were carried out using SAS version 9.4.

Results

Patients' characteristics

A total of 6,056 patients were eligible for this analysis. Of these patients, CNS status was not evaluable in 335, mostly due to lack of sufficient diagnostic material or because the diagnostic spinal tap was delayed >72 hours after start of prednisone according to protocol. Among evaluable patients, 4,516 had CNS1 (74.5%), 1,071 CNS2 (17.6%), and 134 CNS3 (2.2%) status. IT MTX treatment was administered in deviation from the protocol in 41 patients

Table 1. Induction treatment in the AIEOP-BFM ALL 2009 study.

Treatment phase/drug	Single or daily dose	Days of application per phase ^a
Prephase: Prednisone (PO/IV) Methotrexate (IT)	60 mg/m ² /d Age-adjusted ^b	1-7 1
Induction: Protocol IA Prednisone/prednisolone (PO/IV) or Dexamethasone (PO/IV) ^c Vincristine (IV) Daunorubicin (PI over 1 h) Cyclophosphamide (PI over 1 h) ^f PEG-L-asparaginase (PI over 2 h) Methotrexate (IT)	60 mg/m ² /d 10 mg/m ² /d 1.5 mg/m ² /dose (max. 2 mg) 30 mg/m ² /dose 1000 mg/m ² /dose 2500 IU/m ² /dose (max. 3750 IU) Age-adjusted ^b	8-28 ^d 8-28 ^d 8, 15, 22, 29 8, 15, 22, 29 ^e 10 12, 26 12, 33 ^g

^aAdjustments of time schedule were allowed if clinical condition and bone marrow recovery were inadequate. ^bAge-adjusted dose: 1 to <2 years, 8 mg; 2 to <3 years, 10 mg; ≥ 3 years, 12 mg. ^cDexamethasone only for patients with T-cell acute lymphoblastic leukemia (T-ALL) and prednisone good response (PGR). ^dSteroids were tapered over 9 additional days. ^eRandomization: only two doses on days (d) 8 and 15 were given to patients randomized into the experimental arm in randomization R1. ^fOnly for T-ALL / prednisone-poor response patients. ^gAdditional intrathecal (IT) methotrexate (MTX) therapy on day 19 and 26 was administered to patients with CN2 and CNS3 status. PO: oral administration; IV: intravenous; PI: infusion; d: day; h: hour.

with CNS1 status and 33 patients with CNS2 or CNS3 status; therefore, 4,706 patients were treated with three and 1,350 with five doses of IT MTX in induction (Figure 1). Table 2 shows the initial patients' characteristics and the type of Protocol IA according to the intrathecal doses given. There was a significantly higher proportion of patients who received additional IT MTX among male patients, older patients (>10 years), T-lineage, *ETV6-RUNX1* negativity, with high initial white blood cell (WBC) count ($\geq 50 \times 10^9/L$), and patients with $\geq 10\%$ blasts in FCM-MRD at day (d) 15. Accordingly, it was higher in patients who received one of the two T-ALL protocol variants, i.e., "IA Dexa" (dexamethasone instead of prednisolone) or protocol variant "IA-CPM" (with additional cyclophosphamide for high-risk T-ALL patients).

Incidences of life-threatening and fatal infections according to number of intrathecal methotrexate doses

Incidences of life-threatening and fatal infections in induction phase are shown in Table 3 with reference to patients' characteristics and the number of IT MTX doses in induction. Females showed a higher incidence of life-threatening infections (2.7%) compared to males (1.9%), although this did not reach statistical significance ($P=0.053$). A statistically significantly higher incidence of

life-threatening infections was seen in older patients (3.6% in patients aged ≥ 10 years vs. 1.8% in patients <10 years; $P<0.001$), in patients with T-lineage ALL (3.6% vs. 2.0% in B-lineage ALL; $P=0.005$), *ETV6-RUNX1* negativity (2.5% vs. 1.4% in patients with *ETV6-RUNX1* positivity; $P=0.012$) and in patients assigned to the treatment with Protocol IA-Dexa (4.4% vs. 2.0% or 2.2% in patients assigned to Protocol IA/IA' or IA-CPM, respectively; $P=0.004$). Patients aged ≥ 10 years of age in addition had a significantly higher incidence of fatal infections (1.5%) compared to younger patients (0.3%; $P<0.001$).

Patients who received five doses of IT MTX had a statistically significantly higher incidence of life-threatening infections (4.4%) compared to patients with three doses of MTX (1.6%; $P<0.001$). Fatal infections appeared in 1.6% in patients with additional IT MTX versus 0.3% without additional IT MTX ($P<0.001$). Patients with CNS2 and CNS3 status had a statistically significantly higher incidence of life-threatening infections in induction (4.4% and 3.0%, respectively) compared to patients with CNS1 status (1.6%; $P<0.001$). There was no significant difference in the incidence of life-threatening infections between patients with CNS2 and those with CNS3 status ($P=0.45$).

In addition, we analyzed the incidence of life-threatening infections in the consolidation phase which followed in-

Table 2. Proportion of patients with additional intrathecal methotrexate in induction related to different initial patients' characteristics and (as assigned) type of Protocol IA.

	No additional IT MTX* N (%)	Additional IT MTX* N (%)	P
Gender			
Male	2,651 (75.9)	840 (24.1)	<0.001
Female	2,055 (80.1)	510 (19.9)	
Age in years			
< 10	3,590 (78.3)	993 (21.7)	0.04
≥ 10	1,116 (75.8)	357 (24.2)	
Initial WBC, $\times 10^9/L$			
< 50	3,989 (80.9)	939 (19.1)	<0.001
≥ 50	715 (63.5)	411 (36.5)	
Immunophenotype			
B-lineage	4,151 (80.1)	1,034 (19.9)	<0.001
T-lineage	538 (63.3)	312 (36.7)	
<i>ETV6-RUNX1</i>			
Negative	3,538 (75.9)	1,125 (24.1)	<0.001
Positive	1,098 (83.2)	222 (16.8)	
FCM-MRD d15			
<10% blasts	4,010 (78.7)	1,085 (21.3)	0.005
$\geq 10\%$ blasts	570 (74.1)	199 (25.9)	
Type of Protocol IA			
IA-Dexa	373 (68.2)	174 (31.8)	<0.001
IA/IA'	4,151 (80.1)	1,034 (19.9)	
IA-CPM	165 (54.5)	138 (45.5)	

*Data refer to patients with successful investigation of the respective criteria. IT: intrathecal; MTX: methotrexate; N: number; WBC: white blood count; FCM-MRD: minimal residual disease by flow cytometry; d: day; Dexa: dexamethasone; IA/IA' : prednisone/prednisolone with 4 or 2 doses (IA') of daunorubicin in Protocol IA; CPM: cyclophosphamide.

duction Protocol IA. Treatment in this phase was independent of the CNS status. Incidences of life-threatening infection in consolidation phase were 0.78% (35/4516), 0.84% (9/1071), and 0.75% (1/134) in patients with CNS1, CNS2 and CNS3 status, respectively.

In a multivariate logistic regression model including gender, age, WBC, *ETV6-RUNX1* status, minimal residual disease by flow cytometry (FCM-MRD) d15, type of Protocol IA and the intrathecal MTX doses in induction as co-variables, female gender, age ≥ 10 years, Protocol IA-Dexa, and the treatment with additional IT MTX showed independent significance on the risk of life-threatening infection in induction, with the highest effect for the treatment with additional IT MTX doses among the parameters analyzed (OR 2.85 [95% CI 1.96-4.14]; $P < 0.001$) (Table 4).

Detailed data of subgroups with life-threatening and fatal

infections in induction therapy according to the number of intrathecal doses of IT MTX related to different patients' characteristics are provided in *Online Supplementary Table S3*.

To analyze the impact of CNS status on relapse risk, multivariate cause-specific Cox regression analyses on relapse incidence including gender, age ($</\geq 10$ years), WBC ($</\geq 50 \times 10^9/L$), risk group (high-risk [HR] / non-HR), *ETV6-RUNX1* rearrangement (for precursor B-cell ALL [pB-ALL] only), and CNS status (CNS1 [reference] / CNS2/CNS3) as co-variables were performed separately for pB-ALL and T-cell ALL (T-ALL). Hazard ratios for CNS2 status were 1.13 (95% CI: 0.92-1.39; $P=0.25$; $n=4,919$) or 1.10 (0.66-1.84; $P=0.71$; $n=738$) in patients with pB-ALL or T-ALL, respectively. The hazard ratios for CNS3 status were 1.59 (95% CI: 0.85-3.0; $P=0.15$) in B-ALL and 2.65 (95% CI: 1.56-4.51; $P < 0.001$) in T-ALL.

Table 3. Life-threatening and fatal infections in induction therapy related to patients' characteristics, type of Protocol IA (as assigned), and number of intrathecal methotrexate administrations.

	Life-threatening infections in induction							Fatal infections in induction				
	Total		No		Yes		P	No		Yes		P
	N	%	N	%	N	%		N	%	N	%	
Total	6,056	100	5,920	97.8	136	2.2	-	6,018	99.4	38	0.6	-
Gender												
Male	3,491	100	3,424	98.1	67	1.9	0.053	3,470	99.4	21	0.6	0.87
Female	2,565	100	2,469	97.3	69	2.7		2,548	99.3	17	0.7	
Age in years												
<10	4,583	100	4,500	98.2	83	1.8	<0.001	4,567	99.7	16	0.3	<0.001
≥ 10	1,473	100	1,420	96.4	53	3.6		1,451	99.5	22	1.5	
Initial WBC, $\times 10^9/L$												
<50	4,928	100	4,823	98.1	105	2.1	0.22	4,901	99.5	27	0.5	0.14
≥ 50	1,126	100	1,095	97.2	31	2.8		1,115	99.0	11	1.0	
CNS status												
CNS1	4,516	100	4,442	98.4	74	1.6	<0.001	4,500	99.6	16	0.4	<0.001
CNS2	1,071	100	1,024	95.6	47	4.4		1,055	98.5	16	1.5	
CNS3	134	100	130	97.0	4	3.0		132	98.5	2	1.5	
Immunophenotype												
B-lineage	5,185	100	5,081	98.0	104	2.0	0.005	5,155	99.4	30	0.6	0.24
T-lineage	850	100	819	96.4	31	3.6		842	99.1	8	0.9	
<i>ETV6-RUNX1</i>												
Negative	4,663	100	4,546	97.5	117	2.5	0.012	4,630	99.3	33	0.7	0.24
Positive	1,320	100	1,302	98.6	18	1.4		1,315	99.6	5	0.4	
FCM-MRD d15												
<10% blasts	5,095	100	4,989	97.9	106	2.1	0.58	5,068	99.5	27	0.5	0.42
$\geq 10\%$ blasts	769	100	751	97.7	18	2.3		763	99.2	6	0.8	
Type of Protocol IA												
IA-Dexa	547	100	523	95.6	24	4.4	0.004	543	99.3	4	0.7	0.18
IA/IA'	5,185	100	5,081	98.0	104	2.0		5,155	99.4	30	0.6	
IA-CPM	303	100	269	97.7	7	2.3		299	98.7	4	1.3	
Add. IT MTX in P IA												
No	4,706	100	4,629	98.4	77	1.6	<0.001	4,690	99.7	16	0.3	<0.001
Yes	1,350	100	1,291	95.6	59	4.4		1,328	98.4	22	1.6	

N: number; WBC: white blood count; CNS: central nervous system; FCM-MRD: minimal residual disease by flow cytometry; d: day; Dexa: dexamethasone; IA/IA': prednisone/prednisolone with 4 or 2 doses (IA') of daunorubicin in Protocol CPM: cyclophosphamide; IT: intrathecal; MTX: methotrexate; Add.: additional.

Discussion

Our data show a highly significant impact of two additional IT MTX doses in induction therapy on the incidence of life-threatening and fatal infections. These additional doses were indicated to be given to patients with CNS2 or CNS3 status at initial diagnosis and were omitted in patients with CNS1 status. Almost all patients had eventually received the IT therapy in induction in accordance with the protocol. This high correlation between the CNS status and IT therapy made it difficult to discriminate between whether the higher risk of severe infection is caused by the intensified IT therapy or is a specific feature of the CNS involvement itself. The multivariate logistic regression analysis did not allow inclusion of the status due to the high correlation, but it showed that the adverse effect of additional IT MTX was largely independent of the other relevant patient-, leukemia-, and therapy-related parameters included in the model. We conclude from the data that it is most likely that the additional IT therapy is the decisive risk factor rather than the feature “leukemia with CNS involvement”. This conclusion was supported by the finding of similar incidences of life-threatening infections in patients with CNS1, CNS2 and CNS3 status in the subsequent consolidation phase.

There were no significant differences in interim analyses on relapse incidence between CNS1 and CNS2 status. However, the vast majority of patients with CNS2 status had received two additional doses of IT MTX. The erstwhile introduction of intensified IT MTX in induction for CNS2 in ALL-BFM therapy was based on observations in protocols other than the ALL-BFM protocol and, like other therapy modifications, was continued in subsequent ALL-BFM protocols. Given the data now available on adverse events, we have to question the impact of the additional IT MTX on ALL-BFM therapy.

Severe infectious complications are the main cause for early death, mortality, and treatment delay in induction therapy in childhood ALL.^{14,15} Risk factors for infectious complications have been described¹⁴⁻¹⁷ and can be divided into factors relating to patients' characteristics (e.g., age), to disease (e.g., high initial WBC), or to treatment (e.g., dexamethasone therapy). So far, single therapeutic interventions like two additional IT MTX applications have not been included in risk factor analysis. Published data on the impact of 2-4 additional intrathecal treatments (MTX/cytarabine/hydrocortisone) in induction by the St. Jude Study group did not show any significant difference in the rates of grade 4 or 5 infections or grade 2-4 seiz-

Table 4. Uni- and multivariate logistic regression analyses on life-threatening infections in Protocol IA.

	Univariate analysis			Multivariate analysis		
	Odds Ratio	95% CI	P	Odds Ratio	95% CI	P
Gender						
Male	1			1		
Female	1.39	0.97-1.97	0.075	1.65	1.14-2.37	0.008
Age in years						
<10	1			1		
≥10	1.99	1.38-2.89	<0.001	1.80	1.21-2.66	0.003
Initial WBC, x10 ⁹ /L						
<50	1			1		
≥50	1.23	0.81-1.9	0.33	0.86	0.54-1.37	0.51
Immunophenotype						
B-lineage	1			-		-
T-lineage	1.63	1.08-2.63	0.019		Excluded from model*	
<i>ETV6-RUNX1</i>						
Negative	1			1		
Positive	0.53	0.31-0.89	0.018	0.69	0.40-1.21	0.201
FCM-MRD d15						
<10% blasts	1			1		
≥10% blasts	1.14	0.69-1.91	0.57	0.99	0.57-1.65	0.96
Protocol IA-Dexa						
No	1			1		
Yes	2.12	1.31-3.41	0.002	1.75	1.05-2.97	0.034
Add. IT MTX in P IA						
No	1			1		
Yes	2.86	1.99-4.13	<0.001	2.85	1.96-4.14	< 0.001

WBC: white blood count; FCM-MRD: minimal residual disease by flow cytometry; Dexa: dexamethasone; IT: intrathecal; MTX: methotrexate; CI: Confidence Interval; d: day; Add.: additional. *Immunophenotype was not included in multivariate model due to high correlation with type of Protocol IA (P IA).

ures.¹³ In contrast to treatment under the AIEOP-BFM ALL 2009 study protocol, patients in the St. Jude study received triple IT treatment (ITT) and leucovorin rescue (5 mg/m² per dose, max. 5 mg) which was administered orally at 24 and 30 hours after each ITT during induction. Besides the smaller size of the cohort and other differences in treatment and stratification compared to the AIEOP-BFM ALL 2009 protocol, the leucovorin rescue may have protected patients against an additional risk of infection. Moreover, the overall number of grade 4 and 5 infections was slightly higher in the St. Jude study compared to the incidence of infections defined as life-threatening and fatal infections in the AIEOP-BFM 2009 trial (8.6% vs. 5.9%, respectively).

Evidently there is a systemic therapeutic effect of IT MTX in childhood ALL. This was demonstrated for response to prednisone pre-phase plus IT-MTX, with a significantly higher rate of good response to prednisone in patients with pB-ALL who received IT MTX on d1 as compared to those who did not receive IT MTX before d7.^{18,19}

Only limited data are available on systemic side effects of IT MTX, but IT MTX administration results in greater systemic exposure compared to oral administration of the same dose.²⁰ The CSF, with its blood-brain barrier, seems to act as an MTX reservoir with the ability to prolong systemic MTX exposure in a bi-exponential manner.²¹⁻²³ In addition, IT MTX can cause acute tumor lysis syndrome after a single administration.²⁴⁻²⁶ Together, these observations lead to the widely accepted assumption that IT MTX has a systemic effect, which in turn may be associated with severe adverse events, as our study has now clearly demonstrated.

When discussing the consequences of the observation that two additional IT MTX doses in patients with CNS2 status severely increase the risk of severe and life-threatening infections, several aspects have to be taken into account. 1) The use of systemic leucovorin rescue in IT MTX overdose turned out to be beneficial, as described in case series.^{27,28} However, data are scarce on the impact of systemic leucovorin rescue in regular IT MTX administration, not only with respect to toxicity, but also on a plausible systemic antileukemic effect. There is a lack of systematic study data for leucovorin rescue in IV and IT MTX therapy that could answer this question. In general, the frequency of IV MTX-induced oral mucositis was described to decrease when the leucovorin dose was increased, and that response was related to the dose.²⁹ 2) Cytomorphology as the sole method of CSF assessment is still insufficient, as it is prone to preanalytical error and observer-derived vagueness.^{30,31} Interim analyses of data of the AIEOP-BFM ALL 2009 trial revealed relevant differences in the proportion of patients with CNS2 status between the participating groups (3.3-35.1%; *data not shown*). The reason for these differences is not

entirely clear. Different approaches regarding centralized or local cytopsin assessment along with various technical reasons may contribute to the variation observed. It is, therefore, questionable whether CNS2 status with its assumed heterogeneity is a good parameter on which to justify treatment modification. A more precise leukemia detection in the CSF at diagnosis and during treatment is needed, e.g., by applying highly sensitive molecular genetic or flow cytometric detection methods. 3) In order to describe the prognostic potential of these new methods, treatment outcome should not be biased by treatment modification such as the application of two additional IT MTX doses. 4) In most patients, lumbar puncture is carried out in some kind of analog-sedation or anesthesia. This procedure in itself harbors the risk of various minor and major adverse events,³² including infection and cardio-respiratory incidents, entailing the need for further medical interventions with their own rates of adverse events, including infections. 5) The acute and late neurotoxicity and cognitive impairment associated with and caused by IT MTX has to be mentioned³³⁻³⁵ as a further reason to scrutinize the number of IT MTX treatments. Nevertheless, the ultimate goal of contemporary protocols should be the avoidance of CRT wherever possible.

Therefore, intrathecal therapy in induction was de-escalated in the subsequent currently ongoing trial AIEOP-BFM ALL 2017: patients with CNS2 status are no longer treated with additional IT MTX in induction, whereas the approach was not changed for patients with CNS3. An increased risk of relapse in CNS2 patients cannot be completely ruled out after omitting the two intrathecal administrations in induction. This potential risk may also vary depending on the underlying treatment protocol, particularly, but not exclusively, with respect to CNS-directed treatment elements such as the total number of intrathecal administrations, high-dose MTX and cranial radiotherapy. Ultimately, we have to weigh an unknown benefit of the two additional IT MTX administrations in terms of relapse incidence against the strong evidence of a significantly increased risk of serious and potentially fatal infectious complications. Another potential risk might be an increase in the incidence of non-remission due to resistant CNS disease at the end of induction as a consequence of reduced IT induction therapy. In the AIEOP-BFM ALL 2009 study, this was an extremely rare event concerning less than 1 out of 5,000 patients. Additional IT doses, given individually to patients who still have evidence of leukemic blasts in the CSF at the time of the second therapeutic lumbar puncture on d12 may be considered in order to minimize the risk of higher incidence of resistant CNS disease.

The uniform CNS-directed treatment of patients with CNS1 and CNS2 status forms a basis for prospective studies evaluating the prognostic relevance of the CNS2 status and of additional methods that are more reliable

than the cytomorphological CSF assessment. The ongoing AIEOP-BFM ALL 2017 trial addresses these questions.

Disclosures

AM has provided consultancy services for Clinigen and has received consultancy honoraria from BTG. AA has received honoraria for lectures, consultancy or advisory board participation from JazzPharma, Amgen, Novartis, MSD, Pfizer and Gilead, and compensation for travel expenses from JazzPharma and Sehas, has provided consultancy services for Amgen, and has received honoraria from Novartis and Medison Pharma. CR has received honoraria for lectures, presentations, or educational events from Amgen, Jazz, Servier, Clinigen and Serb. GC and/or study group have received research support from Amgen, JazzPharma, Novartis and Servier. GC has received travel support or speaker's honoraria from Amgen, JazzPharma, Novartis and Servier. MS and/or study group have received research support from SHIRE, JazzPharma, Servier, Sigma-Tau and Novartis. MS has received honoraria from Servier, Novartis, and JazzPharma. All of the other authors have no conflicts of interest to disclose.

Contributions

MS, VC, MZ, MGV, AB, GM and FN were involved in designing and planning the study. AM, JH, SV, GC and MS wrote the manuscript. JH, SV, AA, DB, NB, AC, LD, SE, SI, GM, FN, DS, JS, EZ and MS helped in collecting the data and provided patients for the study. MGV and MZ were the study statis-

ticians. MGV, MZ, AM, JH, SV and DS oversaw data checking and reporting during the study period, and analyzed the data. All authors contributed to the data interpretation, drafting, and revision of the manuscript, and gave final approval to submit the manuscript for publication.

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Data-sharing statement

Queries regarding data sharing should be addressed to the corresponding author.

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