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Secondary malignant neoplasms after intensive treatment of relapsed acute lymphoblastic leukaemia in childhood

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ABSTRACT

Purpose: To investigate the cumulative incidence of and the risk factors for developing second malignant neoplasms (SMN) in children and adolescents following treatment for relapse of acute lymphocytic leukaemia (ALL).

Methods: Patients (1376) up to 18 years of age with first relapse of non-B-cell ALL were treated and achieved a 2nd complete remission (CR). The treatment followed trial protocol in five consecutive multicentre trials of the ALL-REZ BFM Study Group between March 1983 and December 2001. The incidence of SMN was analysed, correlated with clinical and therapeutic parameters, and compared to the age-specific incidence rates of cancers as cited in German cancer registries.

Results: Out of the 1376 patients 21 were diagnosed with SMN including non-lymphoblastic leukaemia/myelodysplastic syndrome ($n = 6$), osteo-/Ewing's-/fibroblastic sarcoma ($n = 4$), B-cell ALL/lymphoma ($n = 2$), thyroid carcinoma ($n = 2$), basal cell carcinoma, adeno carcinoma, squamous cell carcinoma, meningioma, malignant histiocytosis, glioblastoma and anaplastic astrocytoma ($n = 1$ each). The overall cumulative risk of SMN at 15 years (median follow-up of 13.1 years) was 1.26% ± 0.38% (SE). SMN was found to be significantly associated with stem cell transplantation (SCT), and high cumulative doses of cranial irradiation, etoposide and cyclophosphamide. In multivariate analysis etoposide (VP16) and cyclophosphamide (CY) were found to be independently associated with SMN ($p = 0.047$ and 0.002). Compared to the incidence of neoplasm in the age-matched population, there was a 10-fold increase of neoplasia.

Conclusions: Despite repeated exposure to intense frontline and relapse treatment (including multiagent chemotherapy, cranial irradiation and stem cell transplantation in some patients) the cumulative incidence of SMN was unexpectedly low, though significantly higher than in the general age-matched population. The association of SMN to SCT seemed to be a secondary effect at least partially mediated by exposure to high doses of VP16 and CY given for conditioning therapy.

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1. Introduction

In about 20% of patients with childhood acute lymphoblastic leukaemia (ALL) the disease recurs after frontline therapy.[1] In large studies published, the event-free survival (EFS) rates after second line therapy for patients with first relapse was 35% at 5 years.[2,3] For patients at high risk of disease recurrence, stem cell transplantation (SCT) is a treatment option in second complete remission (CR). EFS rates of about 50% have been reported.[4,5] Lower relapse rates following SCT are purchased at the expense of higher therapy related acute and late toxicity, when compared to chemoradiotherapy alone.

As current treatment strategies enable a longer survival, patients are at higher risk of second malignant and non-malignant neoplasms (SMN).[6,7] SMN is a known complication of conventional anticancer therapy. In particular, irradiation, anthracyclines, etoposide and cyclophosphamide have been reported to be associated with SMN. Furthermore, younger patients were at higher risk of SMN arising in the CNS after cranial irradiation.[8,9]

Cytotoxic effects leading to DNA damage and impaired DNA repair mechanisms are thought to be the major causes of SMN after chemotherapy and radiationtherapy. Additionally, suppression of T-cell function caused by T-cell depletion of the recipient and/or the graft, as well as antigenic stimulation caused by HLA and non-HLA differences, are blamed for the enhanced risk of SMN in the context of allogeneic SCT.[6,9]

Most reports on SMN in childhood leukaemia refer to events after primary diagnosis. Since children with relapse receive additional multiagent chemotherapy at an even higher intensity, followed by cranial irradiation or allogeneic SCT in most patients, we hypothesised that this more aggressive treatment with higher cumulative doses of cytotoxic agents would lead to higher rates of SMN.

Here, we present data on cumulative incidence, risk factors for, type and outcome of SMN from a large cohort of patients after relapse therapy registered within five consecutive acute lymphoblastic leukaemia – relapse Berlin/Frankfurt/Muenster (ALL-REZ BFM) trials since 1983.

2. Patients and methods

2.1. *Treatment protocols*

Between March 1983 and December 2001, 1731 children and adolescents up to 18 years of age with first relapse of non-B ALL were enrolled in five consecutive multicentre trials of the ALL-REZ BFM Study Group. One thousand three hundred and seventy-six of the enrolled patients were treated according to the main or pilot protocols; they achieved a complete 2nd remission (CR2) and were analysed in this study for the incidence of SMN as of September 2006. The median observation time was 13.1 years (minimum 4.3, interquartile range [IQR] 8.8–17.2, maximum 22.9 years).

Frontline therapy was given according to the protocols of the BFM and Cooperative ALL (COALL) study groups in most patients. In the ALL-REZ BFM trials, therapy was stratified according to several risk factors such as time point of relapse site of relapse and immunophenotype of leukaemia. By definition, late relapses occurred more than 6 months after cessation of frontline therapy, early relapses at least 18 months after primary diagnosis but less than 6 months after cessation of therapy, and very early relapses within 18 months after primary diagnosis. Factors associated with an unfavourable prognosis are early or very early time point of relapse, isolated bone marrow relapse or T-cell immunophenotype.[47]

According to the ALL-REZ BFM standard protocols, treatment of relapse consisted of up to 10 alternating courses of multi-drug chemotherapy,[10,3,11,12] followed by local irradiation to involved compartments, preventive CNS irradiation if indicated, and conventional maintenance therapy for one or two years. Table 1 outlines cumulative doses of those chemotherapeutic drugs, included within the BFM relapse protocols, with a potency to induce secondary malignancies.

Local radiotherapy was given at age adapted dosages. Children with haematological relapse without CNS involvement received cranial irradiation at a dose of 12 or 18 Gray 1989–1990 and at a dose of 12 Gray since 1990. Cranial irradiation was substituted by intensified intrathecal therapy when the previously applied irradiation doses exceeded 24 Gray or 18 Gray for children less than 2 years of age. In children with CNS involvement, cranial radiotherapy was administered in relapse trials ALL-REZ BFM 1983–1990 at doses of 12–24 Gray, 1990–1996 at doses of 12–18 Gray and since 1996 at doses of 15 or 18 Gray. In boys with unilateral testicular relapse, the involved testis was surgically removed and a biopsy was taken from the clinically not involved testis. If a leukaemic infiltration of a testis could be excluded, local radiotherapy was administered at doses of 15 or 18 Gray. In patients with bilateral testicular relapse either both testicles were removed or irradiated at a dose of 24 Gray.

Since 1983, patients with bone marrow relapse were eligible for allogeneic stem cell transplantation (SCT). Since the early 1990s when HLA compatible unrelated stem cell donors (MUD) became increasingly available, SCT from unrelated donors has been performed as alternative option in high risk patients.

The conditioning regimes used prior to SCT were not uniform: 89% of the patients received fractionated total body irradiation (FTBI, 12 Gray) and etoposide (VP16, 1×40–60 mg/kg) and/or cyclophosphamide (CY, 2×60 mg/kg). High-dose chemotherapy for myeloablative conditioning was applied to 11% of the patients including different drug-combinations with VP16, CY, busulfan (BU), or thiotepa but without FTBI.

3. Patient characteristics

Data of 1376 patients treated according to the ALL-REZ BFM protocols or pilot studies were analysed. Patient characteristics are shown in Table 2. The median age at relapse was 8.3 years (IQR 5.7–12.0 years). The male to female ratio was 1.8. Most of the relapses occurred at a late time point, predominantly with an isolated BM involvement. The median peripheral blast cell count at relapse diagnosis was 0.17/nl (IQR 0–2.1). In 10% of the patients a T-lineage immunophenotype of the leukaemic clone was observed, whereas all other patients suffered from B-cell precursor ALL relapse.

Table 1 – Cumulative doses of drugs with potential to induce SMN given in subsequent protocols of the ALL-REZ BFM Study Group

ALL-REZ BFM	RG	DNR (mg/m^2)	DOX (mg/m^2)	VP16 (mg/m^2)	VM26 (mg/m^2)	CY (mg/m^2)	IFO (g/m^2)	MTX IT (12 mg)[a]	MTX IV (g/m^2)[b]
83	A		140 (4)	450 (3)	660 (4)		8 (20)	8/16	4 (8)
	B		140 (4)		660 (4)		8 (20)	8/16	4 (8)
	C		70 (2)		330 (2)		4 (10)	4/8	2 (4)
85/87	A	200 (4)			660 (4)		8 (20)	8/16	9 (9)
	B	200 (4)			660 (4)		8 (20)	8/16	8 (8)
	C	150 (3)			495 (3)		6 (15)	6/12	6 (6)
90	A/B	150 (3)		1350 (9)			6 (15)	9/12	30/6 (6)
	C	100 (2)		900 (6)			4 (10)	6/9	20/4 (4)
96	S1	105 (3)					6 (15)	8/12	7 (7)
	S2	140 (4)					8 (20)	10/15	9 (9)
	S3	35 (1)					2 (5)	4/6	3 (3)
	S4			900 (9)				8/12	
Conditioning regimens									
TBI/VP16				1800 (1)					
TBI/VP16/CY				1200 (1)		3600 (2)			
BU/VP16/CY				1200 (1)		3600 (2)			

In brackets the number of single doses is given. *Abbreviations*: ADR, adriamycin; BU, busulfan; DNR, doxorubicin; IFO, ifosfamide; IT, intrathecally; IV, intravenously; MTX, methotrexate; RG, risk group; TBI, total body irradiation; VP16, etoposide; VM 26, teniposide.
a After slash number of doses in CNS relapse is given.
b After slash the cumulative dose of randomized alternative is given.

In Table 3, therapeutic parameters and cumulative doses of radiation therapy and chemotherapeutic agents are displayed. Most patients (71%) received frontline therapy according to protocols of the BFM Study Group (*ALL-BFM* 76, $n = 7$; 79, $n = 14$; 81, $n = 92$; 83, $n = 152$; 86, $n = 177$; 90, $n = 315$; 95, $n = 190$; 2000, $n = 7$;[13–15]) 16% were treated according to COALL protocols (*COALL* 01-80, $n = 9$; 02-82, $n = 30$; 03-85, $n = 56$; 04-89, $n = 17$; 05-92, $n = 69$; 06-97, $n = 26$;[16,17]) and 13% have been treated according to other protocols. Fifty-four percent ($n = 706$) of patients have received cranial irradiation during frontline treatment, one received craniospinal irradiation, and no data were available for 75 (6%) patients.

At relapse, 1180 patients (86%) received second line therapy according to ALL-REZ BFM standard protocols (*ALL-REZ BFM 83*, $n = 77$; 85, $n = 118$; 87, $n = 163$; 90, $n = 243$; 95/96, $n = 481$), 196 patients (14%) according to pilot protocols. During relapse therapy, 438 patients (35%) did not receive irradiation, 420 patients (33%) only received cranial irradiation, 109 patients (9%) received craniospinal irradiation, 306 patients (21%) received total body irradiation, for 102 patients (7%) data are lacking. A total of 362 patients (27%) received stem cell transplantation in 2nd CR (matched family donor, $n = 180$; syngeneic donor, $n = 6$; mismatched family donor, $n = 21$; unrelated donor, $n = 106$; autologous, $n = 49$; no data in 16 patients).

With subsequent relapse ($n = 734$), no standardised protocol was available. Most patients received chemotherapy regimens adapted to ALL-REZ BFM protocols. Three hundred and twenty-eight patients have been registered again and treated with curative intention. Cranial irradiation was given in 5 patients, craniospinal in 10 patients, and FTBI in 92 patients in CR3 (no data in 44 patients). A total of 136 patients received stem cell transplantation in CR3 (matched family donor, $n = 40$; mismatched family donor, $n = 19$; unrelated donor, $n = 53$; autologous, $n = 24$; no data in 7 patients). From these, 8 had second transplantations (5 after allogeneic SCT, 3 after autologous SCT in CR2).

After completion of therapy, patients were examined regularly by their treatment institution to monitor remission status and late effects of therapy. Adverse events such as SMN were reported to the German Childhood Cancer Registry (GCCR) and to the study registry ALL-REZ BFM. For each reported SMN, additional information was obtained including standard clinical data and histopathological report. SMN in this context includes as well non-malignant neoplasms such as meningeoma and basalioma.

4. Statistical methods

SMN was defined as malignant (or non-malignant for selected entities) neoplasm which occurred after attaining a 2nd or a 3rd complete remission (CR2 or 3) of ALL. Follow-up was calculated from the time of diagnosis of ALL relapse until death, the occurrence of a SMN, or until the last report of survival. In case of the inclusion of a subsequent relapse as a competing event, relapse-free survival was calculated from the time of relapse diagnosis until the occurrence of the 2nd relapse.

Cumulative incidences were calculated according to Kalbfleisch and Prentice.[19] Death and SMN were included as competing events; survival was counted as censored event. Cumulative incidences of SMN depending on cumulative drug doses were calculated including subsequent relapse as competing event additional to death in CR2 and SMN,

Table 2 – Frequencies of clinical parameters by occurrence and cumulative incidence of secondary malignant neoplasms in patients with 1st relapse of ALL, treated according to protocols ALL-REZ BFM 83–96

	Total	Secondary malignant neoplasm					CumInc% ± SE% (15y)	p
		No	%	Yes	%	p		
Total	1376	1355	100	21	100		1.26 ± 0.38	
Prognostic parameters								
Relapse status						0.002		0.001
1st relapse	642	625	46	17	81		2.31 ± 0.61	
1st + 2nd relapse	734	730	54	4	19		0.68 ± 0.35	
Gender						1.0		0.821
Male	886	872	64	14	67		1.62 ± 0.45	
Female	490	483	36	7	33		1.01 ± 0.48	
Age at relapse						0.828		0.790
<5 years	231	228	17	3	14		1.33 ± 0.77	
⩾5 and <10 years	630	619	46	11	52		1.63 ± 0.55	
⩾10 years	515	508	37	7	33		1.24 ± 0.50	
Time point of relapse						0.807		0.736
Very early	271	268	20	3	14		1.16 ± 0.67	
Early	407	400	30	7	33		1.79 ± 0.74	
Late	698	687	50	11	52		1.37 ± 0.45	
Site						0.873		0.705
Bone marrow isolated	804	791	58	13	62		1.65 ± 0.48	
Bone marrow combined	315	310	23	5	24		0.98 ± 0.56	
Extramedullary isolated	257	254	19	3	14		1.37 ± 0.80	
PBC						0.330[a]		0.243
<1/μl	475	471	36	4	20		1.00 ± 0.51	
⩾1 and <10,000/μl	731	718	54	13	65		1.61 ± 0.48	
⩾10,000/μl	138	135	10	3	15		2.27 ± 1.30	
No data	32	31	(2)	1	(5)		–	
Immunophenotype[b]						0.552[a]		0.466
Pro-B ALL	79	78	1	1	6		1.42 ± 0.35	
Common ALL	847	834	66	13	59	BCP		
Pre-B ALL	224	221	17	3	18			
Pre-T ALL	31	31	2	–				
Cortical T-ALL	28	27	12	1	6	T	2.30 ± 0.42	
Mature T-ALL	75	73	6	2	12			
Biphenotypic	7	7	1	–			–	
No data	85	84	(6)	1	(5)		–	

Abbreviations: CumInc, cumulative incidence; PBC, peripheral blast-cell count.
a Missing values excluded.
b Immunophenotype according to EGIL classification.

since information on drug doses after 2nd relapse was incomplete in most patients. The cumulative doses of drugs and radiation therapy were calculated based on treatment schedules of the respecting frontline and relapse protocols for the individual risk groups and the individual conditioning regimens, if applicable. Patients with incomplete information on cumulative drug doses have been excluded from the analysis. For patients with 2nd relapse, data on cumulative drug doses were widely not reliable. Therefore, in regard to the calculation of the cumulative incidence of SMN depending on cumulative drug doses, information on treatment and events after subsequent relapse have been excluded considering 2nd relapse as competing event. Differences of cumulative incidences between subgroups were tested according to Gray[20] using the *R*-software for statistical computing. A p value of <0.05 was regarded as significant. To test the independence of factors predictive for survival without SMN, multivariate Cox-Regression analysis and the Forward Wald tests have been applied. The probability of event-free and overall survival has been calculated according to Kaplan–Meier statistic.[18]

The independence of categorical parameters was calculated using the (Pearson) χ^2, or Fisher's exact test. The distribution of continuous variables was calculated using the Mann–Whitney *U* test.

Standardised incidence ratios (SIR), defined as the number of observed divided by the number of expected malignancies, were used to calculate the risk of SMN compared to the general population.[21] The age- and sex-specific number of expected malignancies in the normal population was registered by the following population-based German cancer registries: German Childhood Cancer Registry (children up to 15 years of age, whole Germany),[22] Krebsregister Saarland (persons aged 15 years or more in the German State Saarland),[23] Gemeinsames Krebsregister der Länder Berlin, Brandenburg, Mecklenburg-Vorpommern, Sachsen-Anhalt und der Freistaaten Sachsen und Thüringen (persons aged 15 years or more in the federal states of the former German Democratic Republic).[23]

Table 3 – Frequencies of therapeutic parameters by occurrence and cumulative incidence of secondary malignant neoplasms in patients with 1st relapse of ALL, treated according to protocols ALL-REZ BFM 83–96

	Total	Secondary malignant neoplasm					CumInc% ± SE% (15y)	p[a]
		No	%	Yes	%	p[a]		
Total	1376	1355	100	21	100		1.26 ± 0.38	
Therapeutic parameters								
Frontline therapy						0.845		0.812
ALL-BFM	981	967	71	15	71		1.49 ± 0.42	
COALL	217	213	16	4	19		1.95 ± 0.97	
Others	178	176	13	2	10		0.57 ± 0.57	
Relapse therapy						0.553		0.237
ALL-REZ BFM 83/85/87	438	432	32	6	29		0.70 ± 0.40	
ALL-REZ BFM 90	441	432	32	9	43		2.06 ± 0.68	
ALL-REZ BFM 95/96	497	491	36	6	29		1.31 ± 0.54	
Cranial irradiation (cum.dose)						0.014		.019
0–18 Gy	631	625	56	6	29		1.06 ± 0.44	
>18 Gy	499	484	44	15	71		2.52 ± 0.72	
No data	246	246	(18)	–	–		–	
Stem cell transplantation						0.025		0.021
None	870	861	64	9	43		0.81 ± 0.32	
MRD	217	214	16	3	14		0.92 ± 0.87	
MUD, MMF/UD[b]	194	189	14	5	24		2.06 ± 1.06	
Autologous	79	75	6	4	19		3.80 ± 2.26	
No data	16	16	(1)	–	–		–	
Cumulative dose of single drugs[c]								
Daunorubicin (mg/m^2)						0.258		0.302
<260	393	382	39	11	52		2.05 ± 0.78	
⩾260	614	604	61	10	48		1.15 ± 0.44	
No data	369	369	(27)	–	–		–	
Doxorubicin (mg/m^2)						0.774		0.879
<180	177	173	17	4	19		1.80 ± .1.04	
⩾180	839	822	83	17	81		1.39 ± 0.42	
No data	360	360	(27)	–	–		–	
Etoposide (mg/m^2)						0.024		0.006
0	526	520	55	6	29		0.58 ± 0.33	
>0	438	423	45	15	71		2.61 ± 0.78	
No data	412	412	(30)	–	–		–	
Teniposide (mg/m^2)						1.0		0.253
0	574	562	57	12	57		1.89 ± 0.60	
>0	433	424	43	9	43		.96 ± 0.48	
No data	369	369	(27)	–	–		–	
Cyclophosphamide (mg/m^2)						0.002		0.0005
⩽3000	823	812	82	11	52		.88 ± 0.33	
>3000	184	174	18	10	48		4.12 ± 1.54	
No data	369	369	(27)	–	–		–	
Ifosphamide (mg/m^2)						0.076		0.335
⩽6000	509	494	50	15	71		1.86 ± 0.62	
>6000	498	492	50	6	29		1.04 ± 0.47	
No data	369	369	(27)	–	–		–	
Methotrexate IV (mg/m^2)						0.075		0.350
⩽8000	493	480	42	13	62		1.71 ± 0.60	
>8000	681	673	58	8	38		0.92 ± 0.37	
No data	202	202	(15)	–	–		–	
Methotrexate IT (mg/m^2)						0.267		0.712
⩽192	510	498	43	12	57		1.43 ± 0.54	
>192	664	655	57	9	43		1.10 ± 0.41	
No data	202	202	(15)	–	–		–	

Abbreviations: Gy, Gray; IV, intravenously; IT, intrathecally; MRD, matched related donor; MUD, matched unrelated donor; MMF/UD, mismatch family/unrelated donor.

a Missing values excluded.

b MUD n = 156, MMF/UD n = 38.

c Subsequent relapse is included as competing event for the calculation of cumulative incidence of SMN by drug doses.

Table 4 – Individual clinical characteristics of patients with SMN, course of treatment and outcome

Patent no.	Relapse status	Age at relapse (years)	Sex	Time[a] to relapse (years)	Site	Immuno-pheno-type	Frontline protocoll, risk group	Relapse protocoll ALL-REZ BFM, SG	SCT, conditioning regimen	Radiatio (Gy)	Time[b] to SMN (years)	Type of SMN	Outcome[c], Survival after SMN (years)
1	1st Rel	6.35	M	2.5	Testis	Common	ALL-BFM 81, MR	83, SG2	–	Cranial 18, testis 18	9.1	MDS/AML	Death, 0.2
	2nd Rel	8.9		2.5	BM		–	85, SG2	MRD, TBI/CY	TBI 12	6.5		
2		7.8	F	4.0	BM	Common	PinkelVII+ADR	83, SG2	MRD, TBI	TBI 12	20.3	Basal Cell Ca	CR2, 0.8
3		6.4	F	1.8	BM+CNS	BCP	ALL-BFM 81, MR	83, SG2	Syngen, TBI/CY	Cranial 24, TBI 12	18.7	Thyroid carcinoma	CR2, 4.2
4		10.0	M	2.5	Testis	Common	ALL-BFM 81, SR	85, SG1	–	Cranial 18, Testis 16	2.3	CML	CR2, 17.5
5		10.8	M	4.4	BM+CNS	Common	ALL-BFM 81, SR	85, SG2	–	Cranial 24	18.3	Adeno Ca ileocaecal	CR2, 2.6
6		4.5	F	2.3	BM+Bone	Common	ALL-BFM 83, MR	87, SG2	–	Cranial 18+12	2.1	AML	Death, 0.8
7	1st Rel	4.9	M	2.3	BM	Common	ALL-BFM 86, MR	90, SG3	Autolog., TBI/VP16	Cranial 12, TBI 12	5.0	Squamous cell carcinoma	Death, 6.3
	2nd Rel	6.5		1.6	BM		–	MB91	MMRD, BU/CY	TNI 3.75	3.4		
8		7.8	F	4.8	BM	Common	COALL-03-85,LR	90, SG2	–	Cranial 12	5.0	Meningeoma	CR2, 6.0
9		13.3	M	2.5	BM	Common	ALL-BFM 86, MR	90, SG3	Syngen,TBI/VP16	Cranial 18, TBI 12	5.7	Osteosarcoma	Death, 5.7
10		9.1	M	1.7	BM	Pre-B	ALL-BFM 86, MR	90, SG3	MRD, TBI/CY	Cranial 15, TBI 15.75	7.3	Osteosarcoma	CR2, 7.1
11	1st Rel	8.3	M	2.7	BM	Common	COALL-04-89, HR	90, SG2	–	Cranial 18	8.1	Thyroid carcinoma	CR3, 6.1
	2nd Rel	10.4		2.1	CNS			90, SG2	–	Cranial 18	6.0		
12	1st Rel	6.9	M	4.7	BM	Pro-B	ALL-BFM 86, SR	90, SG2	–	Cranial 18	7.7	Ewing sarcoma	CR3, 14.3
	2nd Rel	11.9		5.1	Testis		–	96, SG1	–	Testis 15	2.7		
13		16.8	F	3.0	BM	Pre-B	ALL-BFM 90, MR	90, SG2	–	Cranial 12	3.7	MDS/AML	Death, 0.1
14		10.5	M	6.4	BM	Common	COALL-03-85,HR	90, SG2	–	Cranial 25+12	2.1	AML	Death, 0.2
15		2.7	M	0.4	BM	T	ALL-BFM 90, HR	90, SG4	MUD, TBI/CY	TBI 12	0.7	Malignant Histiocytosis	Death, 0.1
16		7.1	F	4.3	BM	Common	ALL-BFM 90, SR	96, SG2	–	Cranial 12	1.8	MDS	Death, 1.6
17		8.5	F	0.8	BM+CNS	T	ALL-BFM 95, HR	96, SG4	MUD, TBI/VP16/TEP	TBI 12, cranial 6+18	5.3	Glioblastoma	Death, 1.2
18		12.2	M	3.6	BM	Common	ALL-BFM 95, HR	96, SG2	MUD, TBI/VP16/TEP	Cranial 12, TBI 12	0.6	B-NHL	CR2, 5.2
19		15.0	M	3.8	BM	Common	COALL-05-92, HR	96, SG2	MUD, TBI/VP16/CY	TBI 12	5.7	Fibroblast Sarcoma	CR2, 1.3
20		8.8	M	1.3	BM+CNS	T	ALL-BFM 95, HR	96, SG4	MMUD, TBI/VP16/TEP	Cranial 12, TBI 12	0.4	B-ALL	Death, 0
21		10.1	M	1.3	CNS	Pre-B	ALL-BFM 95, MR	96, SG2	Autolog, TBI/VP16	TBI 12, cranial 6	4.4	Anaplastic Astrocytoma	CR2, 0.5

Abbreviations: BM, bone marrow; CML, chronic myeloid leukaemia; CY, cyclophosphamide; F, female; HR, high risk; M, male; MDS, myelodysplastic syndrome; MR, intermediate risk; NHL, non-hodgkin lymphoma; SG, strategy group; SR, standard risk; TBI, total body irradiation; TNI, total lymph node irradiation.

a Time from initial diagnosis of ALL to relapse diagnosis.

b Time from relapse diagnosis to diagnosis of SMN.

c Time from diagnosis of SMN to evaluation.

5. Results

5.1. *Treatment outcome after first relapse of ALL*

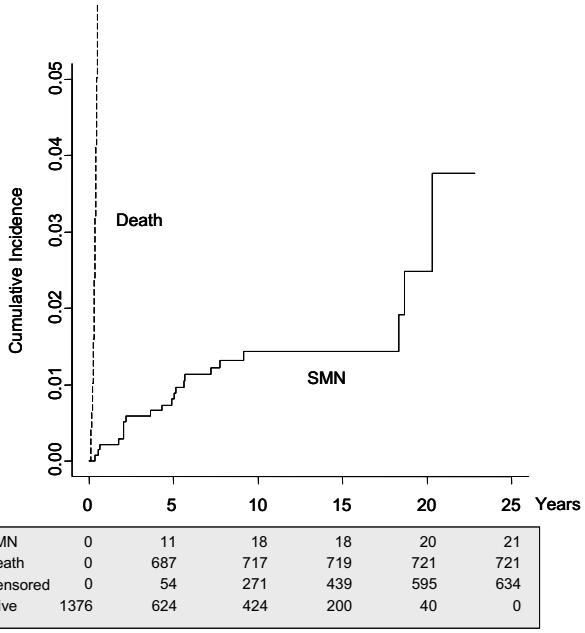
Among the 1376 patients at risk for SMN (treated according to the ALL-REZ BFM trial protocol between March 1983 and December 2001), 734 (53%) of treated patients suffered a subsequent relapse, 95 (7%) died during CR2, 626 (45%) died after 2nd relapse, 518 (38%) are alive in CR2, and 104 (8%) are alive after 2nd relapse. Seventeen (1.2%) patients suffered a SMN in CR2 4 in CR3. Twelve patients are lost to follow-up in CR2. Thus, a total of 21 SMN occurred in relation to 622 so far surviving patients (3.2%) at a median observation time of 13.1 years. The probability of event-free and overall survival at 10 years was .38 ± 0.01 and .47 ± 0.01, respectively.

5.2. *Types and cumulative incidences of SMN*

Twenty-one SMN have been observed after a median interval from relapse diagnosis of 4.9 years (IQR 2.0–7.5 years). Remarkably, all except 3 SMN occurred within 10 years of initial diagnosis of the 1st relapse. Individual characteristics are displayed in Table 4. Frequencies of clinical and therapeutic parameters are displayed in Tables 2 and 3. Of the 21 SMN, six were haematological myeloid malignancies, including two episodes of acute myeloid leukaemia (AML), one episode of uncomplicated myelodysplastic syndrome (MDS), two with MDS transforming into a myeloid leukaemia (MDS/AML, 1 SMN in CR3) and one with chronic myeloid leukaemia (CML). Furthermore two patients with thyroid carcinoma (one of them in CR3) have been registered, one patient with a B-cell non-Hodgkin lymphoma (NHL), one patient with a B-cell ALL after initial T-cell ALL, two patients with osteosarcoma, 1 patient with Ewing's sarcoma (SMN in CR3), one patient with fibroblastic sarcoma, one patient with basal cell carcinoma, one patient with adenocarcinoma, one patient with squamous-cell carcinoma (SMN in CR3), one patient with meningioma, one patient with a malignant histiocytosis, one patient with glioblastoma and one patient with anaplastic astrocytoma. Except for basal cell carcinoma and meningioma all neoplasms were malignant. The exact classification of malignant histiocytosis remains controversial, a secondary haemophagocytic lymphohistiocytosis after allogeneic SCT may be considered as a differential diagnosis. The overall cumulative risk for developing a SMN was 1.26% (±0.38% SE) at 15 years (Fig. 1).

None of the patients had a family history suggesting a predisposition for cancer. One of the two patients with osteosarcoma (No. 10, Table 4) had received growth hormone therapy after allogeneic SCT. The other patient with osteosarcoma (No. 9, Table 4) developed a further SMN of MDS, five years after the first SMN of osteosarcoma.

The development of SMN was not significantly associated with clinical parameters, as listed in Table 2. Although the frequency and the cumulative incidence of SMN is significantly lower in patients with 2nd relapse due to a larger reference cohort for the comparably early SMN after 2nd relapse, the rate in relation to surviving patients was not different (2.6 in 1st relapse versus 2.9 in 2nd relapse) and the parameter is not predictive in multivariate analysis. Furthermore, there



SMN	0	11	18	18	20	21
Death	0	687	717	719	721	721
Censored	0	54	271	439	595	634
Alive	1376	624	424	200	40	0

Fig. 1 – Cumulative incidence (CI) of secondary malignant neoplasm (SMN) in patients with 1st relapse of ALL, treated according to protocols ALL-REZ BFM 83–96. N = 1376, SMN = 21, CI (15 years) = 1.255% (±0.38% SE). Grey box: cumulative numbers of SMN, death, censored observations and remaining numbers of surviving patients at risk.

was no correlation to the type of frontline protocol or the relapse trial. The rate and cumulative incidence of SMN, however, was significantly higher in patients with higher cumulative doses of cranial radiation therapy (Fig. 3) and

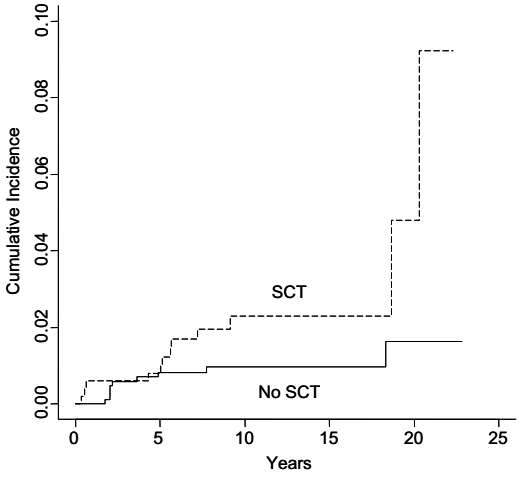


Fig. 2 – Cumulative incidence (CI) of secondary malignant neoplasm (SMN) in patients with 1st relapse of ALL, treated according to protocols ALL-REZ BFM 83–96 by post-remission therapy with SCT versus chemo/radiotherapy. SCT: N = 490, alive = 202, dead = 272, SMN = 12, CI (15 years) = 2.30% (±0.73% SE). No SCT: N = 870, alive = 412, dead = 449, SMN = 9, CI (15 years) = 1.00% (±0.41% SE). P (Gray) = 0.030.

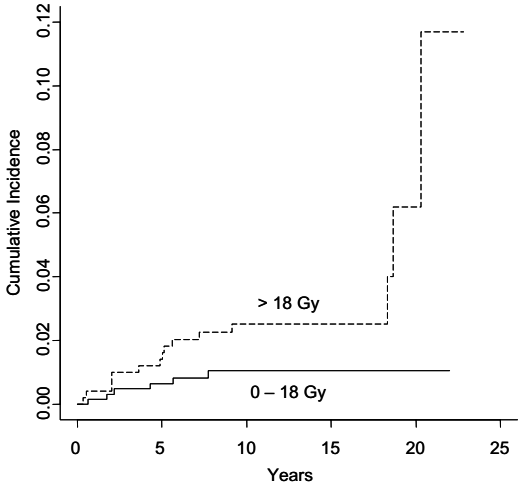


Fig. 3 – Cumulative incidence (CI) of secondary malignant neoplasm (SMN) in patients with 1st relapse of ALL, treated according to protocols ALL-REZ BFM 83–96 by cumulative dose of cranial irradiation. >18 Gy: N = 499, alive = 244, dead = 240, SMN = 15, CI (15 years) = 2.52% (±0.72% SE). 0–18 Gy: N = 631, alive = 279, dead = 346, SMN = 6, CI (15 years) = 1.06% (±0.44% SE). P (Gray) = 0.019.

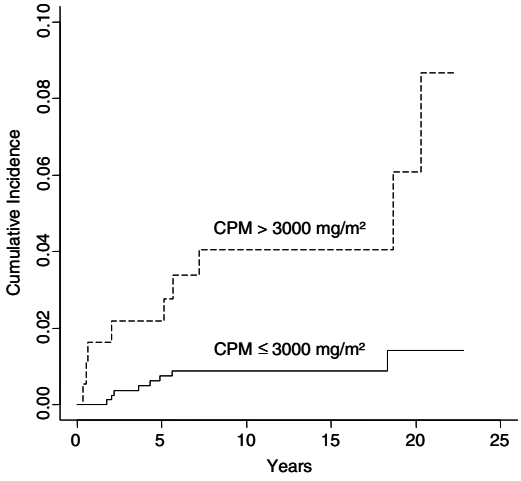


Fig. 5 – Cumulative incidence (CI, competing risks 2nd relapse, death) of secondary malignant neoplasm (SMN) in patients with 1st relapse of ALL, treated according to protocols ALL-REZ BFM 83–96 by cumulative dose of cyclophosphamide. CY $\geqslant$3000 mg/m^2: N = 184, CCR = 62, death in CR2 = 33, 2nd relapse = 80, SMN = 9, CI (15 years) = 4.12% (±1.54% SE). CY <3000 mg/m^2: N = 823, CCR = 333, death in CR2 = 38, 2nd relapse = 444, SMN = 8, CI (15 years) = .88% (±0.33% SE). P (Gray) = 0.0005.

cumulative doses of etoposide and cyclophosphamide (Figs. 4 and 5) above the median. Furthermore, SCT was significantly associated to SMN development looking at different stem cell sources, and it proved to be associated with a significantly higher cumulative incidence of SMN, when pooling the transplanted patients data (Fig. 2). In contrast, higher doses of methotrexate given intravenously (IV) were associated with a lower rate and a lower incidence of SMN, although without statistical significance. Comparing the distribution of the individual cumulative doses of cranial irradiation, VP16, cyclophosphamide and methorexate IV among patients who suffered a SMN in CR2 ($n = 17$) versus those with relapse-free survival in CR2 of at least 6 months (thus having received the complete intensive salvage therapy; $n = 528$; missing values for cumulative doses excluded), patients with SMN had received significantly higher cumulative doses of cranial irradiation (median = 24 Gray [min. 12/max. 36] versus 18 Gray [min. 0/max. 42]; $p = .009$), VP16 (median = 1350 mg/m^2 [min. 0/max. 3100] versus 0 mg/m^2 [min. 0/max. 3775]; $p = .012$) and cyclophosphamide (median = 3600 mg/m^2 [min. 0/max. 10,200] versus 3000 mg/m^2 [min. 0/max. 6600]; $p = .004$) but lower cumulative doses of IV methotrexate (median = 6500 mg/m^2 [min. 2000/max. 31,500] versus 10,000 mg/m^2 [min. 2000/max. 45,000]; $p = .044$; Fig. 6). In multivariate Cox regression for SMN-free survival including the drug and radiation doses as continuous covariates, cumulative doses of etoposide ($p < .001$) and cyclophosphamide ($p = .009$) proved to be the only parameter independently associated to the development of SMN, whereas radiation therapy, SCT, IV methotrexate, sex, time point and site of relapse, immunophenotype, age and 2nd versus 1st relapse did not improve the model.

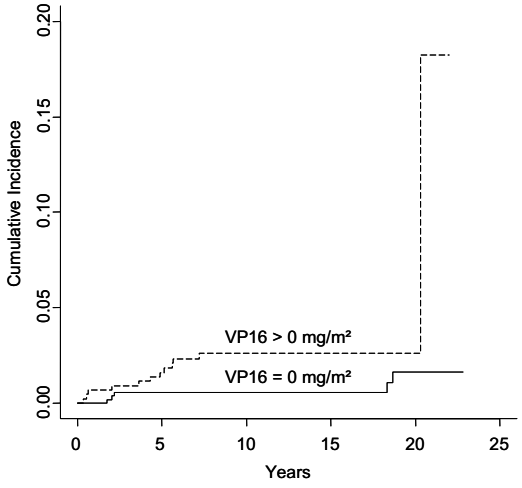


Fig. 4 – Cumulative incidence (CI; competing risks 2nd relapse, death) of secondary malignant neoplasm (SMN) in patients with 1st relapse of ALL, treated according to protocols ALL-REZ BFM 83–96 by cumulative dose of etoposide. VP16 $\geqslant$0 mg/m^2: N = 438, CCR = 169, death in CR2 = 45, 2nd relapse = 212, SMN = 12, CI (15 years) = 2.61% (±0.78% SE). VP16 0 mg/m^2: N = 526, CCR = 210, death in CR2 = 15, 2nd relapse = 296, SMN = 5, CI (15 years) = 0.58% (±0.33% SE). P (Gray) = 0.006.

The expected rate of SMN was calculated from German cancer registry data: in a total of 7283 person-years at risk, the expected incidence of a malignancy would have been 2.1, the observed incidence of SMN was 21. This represents an SIR of 10.0, i.e. there is a 10-fold greater risk of developing a malignant disease in the study population compared to the general population.

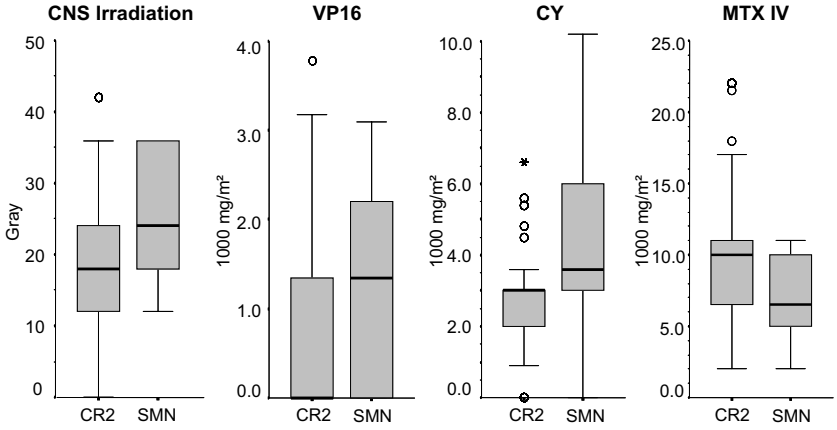


Fig. 6 – Cumulative dose of cranial irradiation and chemotherapeutic agents by secondary malignant neoplasm (SMN, *n* = 17) versus complete 2nd remission without SMN (CR2, *n* = 528, missing values for cumulative doses excluded) in patients with 1st relapse of ALL, treated according to protocols ALL-REZ BFM 83–96. Cranial irradiation, CR2 N = 429, P = .001; etoposide (VP16), CR2 N = 377, P = .002; cyclophosphamide (CY), CR2 N = 393, P = .007; methotrexate IV, CR2 N = 472, P = .011.

5.3. *Treatment and outcome of secondary malignant neoplasms*

For treatment of the SMN the patients received disease-specific protocols with individual modifications; malignant neoplasms have been treated with combination chemotherapy, whereas benign tumours were resected. The overall survival probability of patients with SMN was 0.41 ± 0.15 at 10 years. The chance of survival in patients with haematological malignancies was less (2/8 surviving, 1 CML, 1 B-NHL; pOS = .25 ± .15) as compared to the other forms of malignancies (10/13 surviving; pOS = .49 ± .23; $p = .010$). Haematological SMN occurred at significantly shorter intervals after relapse diagnosis (median = 2.03, minimum = 0.36, maximum = 9.12 years) than the other SMN (median = 5.6, minimum = .62, maximum = 20.29 years; $p = 0.010$).

6. Discussion

We investigated the cumulative incidence of secondary neoplasms in children with relapsed ALL treated according to ALL-REZ BFM trials since 1983. We found a cumulative incidence of 1.26% which was significantly associated with SCT and higher cumulative doses of cranial irradiation in univariate analysis, and with the cumulative dose of cyclophosphamide and etoposide above the median in uni- and multivariate analysis.

With the contemporary conventional multidrug chemotherapy regimen for children with ALL, high initial complete remission rates of 90–100% and long-term remission rates of almost 80% can be achieved.[24] As survival rates continue to improve, SMN and other long-term side effects of therapy will become increasingly important in the long-term treatment of children with cancer. The influence of antineoplastic treatment on the development of SMN is well described in the literature. It appears that the risk of developing SMN may also be subject to individual patient factors such as age and genetics.[8,9,25–28,30] Our hypothesis was that the cumulative incidence of SMN after treatment for ALL-relapse would be substantially higher than after treatment for primary ALL, because of the burden of cumulative drug doses, radiation therapy and the higher rate of patients with SCT.

Löning et al. found a 2.8 ± 0.8% cumulative risk at 15 years after diagnosis of 5006 patients in first CR after ALL frontline therapy according to ALL-BFM protocols. The time interval between the diagnosis of ALL and the occurrence of SMN ranged from 11 months to 15 years (median: 6 years). The cumulative risk for SMN was 3.5% at 15 years for children who received radiotherapy and 1.2% for children who received the same chemotherapy according to the ALL BFM regimen but without irradiation. Using Cox stepwise regression, three parameters reached statistical significance: initial CRT, CNS involvement and administration of epipodophyllotoxins in frontline therapy.[30] Other groups have reported comparable or higher incidences between 2.1% and 5.1% of SMN at 10–20 years in patients with primary ALL or with different primary malignancies.[31,32,8,9,25,26,29] Most commonly SMN occurred in the CNS, followed by haematological malignancies.[8,9] Thus, the incidence in our more intensively pre-treated cohort was lower than in most studies on patients with primary ALL or other primary malignancies. One reason may be the high rate of subsequent competing failures, namely death after 2nd relapse. Also the end of the observation of most surviving patients between 10 and 20 years after relapse diagnosis led to a constant decrease of patients at risk for SMN over time, with less than 30% of the total patient cohort at risk after 10 years and less than 5% after 20 years (Fig. 1). All except three SMN occurred within 10 years after relapse diagnosis. At that point in time there is a comparatively larger comparison group of patients at risk. In contrast, very late SMN increases the cumulative incidence at much higher steps, because they refer to a much smaller cohort of surviving patients with long-term follow-up.

With respect to basic clinical parameters, so far only the younger age group at diagnosis of leukaemia has been identified with an increased risk for SMN, as confirmed by the Childhood Cancer Survivor Study.[33] Our study did not confirm this finding, but age at relapse is known to interact with other

important risk factors such as duration of 1st remission and may be superposed by them.

The association of radiotherapy with an increased risk of developing a SMN has been published by several authors.[28,34–37] An age younger than 5–6 years at exposure has again been shown to be a significant risk-factor in the context of irradiation.[8,9,30,38] In our study, 44% of the patients had received cumulative doses of cranial irradiation of more than 18 Gray. This was significantly associated with the occurrence of SMN in univariate analysis. In multivariate analysis including drug and radiation doses as continuous covariates, however, the cumulative dose of cranial irradiation was not independently related to SMN ($p = 0.08$). Nevertheless, when a conditioning regime is planned in pre-irradiated children, the risk of SMN warrants particular clinical consideration. A continual increase of SMN beyond 20 years was reported by Relling et al. from the St. Jude Total Therapy Studies.[44] Among the 10-year event-free survivors, the cumulative risk of SMN was 23.4% from 10 years to 30 years after initial diagnosis of ALL. Almost all of these late SMN occurred in patients exposed to cranial or craniospinal irradiation and most were low-grade malignancies such as basal cell carcinoma, meningioma, or carcinoma of the thyroid or parotid gland. Among the patients who did not receive CNS irradiation, there was no increased risk of second neoplasm beyond 10 years after diagnosis of ALL.

There is a substantial risk for the occurrence of a secondary malignant neoplasm after SCT[6,7]: Bhatia et al. studied 2150 patients who received autologous, allogeneic family or matched unrelated donor stem cell transplantation for either leukaemia or severe aplastic anaemia. Fifty-one patients developed 53 SMN, with a cumulative incidence of 9.9 ± 2.3% at 13 years. Of the 51 patients, 22 developed B-cell lymphoproliferative disorder (BLPD). The incidence reached a plateau 4 years after SCT. Factors independently associated with increased risk included T-cell depletion, HLA mismatch, use of antithymocyte globulin (ATG) and primary immunodeficiency. In 11 patients with MDS/AML the plateau of SMN incidence was achieved at 9 years and in 17 patients with a secondary solid tumour at 13 years.[6] The phenomenon that reduced immune surveillance following SCT enables (EBV associated) uncontrolled B-cell proliferation which is an increasing and important problem in the context of transplantation. In our patient cohort, the association of SCT with SMN development could be confirmed. However, there was no significant association to SMN in multivariate analysis.

A number of chemotherapeutic agents have been implicated in the development of SMN. The drugs most frequently reported have been the alkylating agents,[6,39] namely cyclophosphamide,[43] DNA topoisomerase II inhibitors,[40,41] namely epipodophyllotoxins,[33] and anthracyclines.[42] In our study, VP16 and CY doses above the median were significantly associated to SMN development and proved to be independently predictive for SMN in multivariate analysis. Since these drugs are widely used components of conditioning regimens, we hypothesise, that at least in part the effect of SCT on the development of SMN derives from the cytostatic agents and radiation therapy given for myeloablative conditioning therapy. We would encourage other authors to review their data with respect to this question. Nevertheless it is widely accepted that the development of EBV-associated B-cell lymphoproliferative diseases is mainly related to the severe T-cell depletion in some transplantation settings.

An important question is whether these results should have consequences on the design of frontline and relapse therapy to reduce SMN in surviving patients. In fact, high cumulative doses of epipodophyllotoxins as given in the early total therapy regimens of the St. Judes group are eliminated from contemporary protocols.[40] The same is true for cyclophosphamide given in high cumulative doses for maintenance therapy in some early ALL-regimens.[48] Cranial radiation therapy has been confined to specific indication in frontline therapy, mainly due to other late side effects. Cranial irradiation in patients with bone-marrow relapse of ALL, however, has been shown to significantly improve prognosis.[49] Similarly, the conditioning regimen TBI/VP16/ ± CY has been proven to result in better disease-free survival rates, than other regimen.[4] These treatments will continue to be important cornerstones of antileukaemic therapy as long as the favourable effects are not more than counterbalanced by the SMN rates.

The treatment of SMN after at least two intensive antineoplastic regimens is difficult and has to be tailored to the individual considering cumulative dosages, individual organ damage and the psychosocial context. All patients with AML/MDS as SMN died in the course of the disease. In contrast, most of the patients with solid tumours and those with non-malignant SMN survived. The low number of observed SMN does not allow performing subgroup analyses to determine risk factors for the particularly fatal disease group.

In our patient cohort, two non-malignant neoplasms are included, one basal cell carcinoma and one meningioma. Whereas the three cited German cancer registries have agreed to register basal cell carcinomas,[22,23] only one out of the registers includes meningeoma.[22] All events, however, are additionally registered at the disease-specific registries. Furthermore, the inclusion of a patient with malignant histiocytosis as SMN caused debate, as a differential diagnosis of secondary lymphoproliferative histiocytosis was proposed. Analysing the data after exclusion of these 3 patients, SCT loses statistical significance with respect to association to SMN ($p = 0.061$), whereas the cumulative dose of CNS radiation therapy ($p = 0.024$) and the dose of cyclophosphamide ($p = 0.033$) and VP16 ($p = 0.006$) above the median remain significantly predictive. The total cumulative incidence of SMN is reduced to 1.29% ± .32%.

In summary, we have found a comparably low rate of SMN in patients with relapsed ALL at 15 years with an association to higher doses of VP16 and CY, and to a lesser degree to cranial irradiation and SCT. The influence of SCT appears to be mediated at least partially by the components of the conditioning regimes. This hypothesis requires verification from larger patients cohorts derived from SCT registries. Further prospective studies with long-term follow-up of all surviving children with malignancies treated with chemo/radiotherapy are warranted. Such studies can only be realised with central registries for children and adults such as the German Childhood Cancer Registry (GCCR).[45,46] Success strongly depends on a good cooperation with the local and

general physicians as well as effective communication across the hospital and community settings.

Conflict of interest statement

None declared.

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