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ORIGINAL ARTICLE

Clinical and genetic characterization of de novo double-hit B cell precursor leukemia/lymphoma

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Abstract

The 2016 revised World Health Organization (WHO) classification of lymphoid neoplasms included the category of high-grade B cell lymphomas (HGBLs) with combined *MYC* and *BCL2* and/or *BCL6* rearrangements (double-hit, DH). However, the clinical features of B cell precursor leukemia (BCP-ALL) that harbor DH genetics remain widely unknown. We performed a retrospective analysis of the German Multicenter Study Group for Adult ALL registry and a literature search for de novo DH-BCP-ALLs. We identified 6 patients in the GMALL registry and 11 patients published in the literature between 1983 and June 2018. Patients of all ages (range, 15–86 years) are affected. There is a high incidence of meningeal disease and other extramedullary disease manifestations. Current treatment approaches are mainly ALL-based and are sufficient to induce first complete remissions, but progression-free survival is only 4.0 months (95% CI, 1.5–6.5 months) and all patients succumb to their disease, once relapsed, with a median survival of 5.0 months (95% CI, 3.1–6.9 months), despite intensive salvage and targeted therapy approaches. Of all patients, only two that attained an initial complete remission were alive at data cutoff. In all cases, the *BCL2* gene was rearranged to be in proximity to the *IGH* locus, whereas *MYC* had various translocation partners juxtaposed. There was no significant survival difference between IG and non-IG translocation partners (HR, 1.03; 95% CI, 0.33–3.2; $p = 0.89$). In conclusion, de novo DH-BCP-ALL is an aggressive B cell malignancy with deleterious outcome. Physicians have to be aware of this rare disease subset due to the atypical clinical behavior and especially because latest classification systems do not cover this sub-entity.

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Introduction

Approximately 5–10% of all newly diagnosed aggressive B cell lymphomas harbor *MYC* rearrangements that are accompanied by simultaneous *BCL2* and/or *BCL6* rearrangements [1, 2] and the presence of these dual (or triple) translocations is commonly referred to as "double-hit" (DH) or in the case of three rearrangements (*MYC*, *BCL2*, *BCL6*) "triple-hit" (TH) lymphomas [3]. Large retrospective series have linked DH/TH lymphomas to distinct clinical features compared to their non-DH/TH counterparts, such as a more advanced disease stage at first diagnosis, a higher rate of central nervous system (CNS) involvement and other extramedullary manifestations as well as inferior survival with standard R-CHOP therapy, independent of established risk factors [1, 4].

In recognition of these data, the 2016 revised World Health Organization (WHO) classification of lymphoid neoplasms included the category of high-grade B cell lymphomas (HGBLs) with combined *MYC* and *BCL2* and/or *BCL6* rearrangements. Aside from diffuse large B cell lymphomas, all large B cell lymphomas with DH or TH rearrangements, except for cases that fulfill the criteria for follicular or lymphoblastic lymphoma, are likewise pooled into this new category [5]. Recently, Miyaoka et al. published a comprehensive clinicopathological review on 25 patients with follicular lymphoma (FL) harboring DH genetics [6]. Although DH-FL tended to be high-grade (grade 3), the overall clinical behavior seemed to be less aggressive than DH/TH-HGBLs and therefore the authors conclude that the DH/TH-HGBLs category does not apply to DH-FLs, as consistently proposed by the WHO 2016 classification.

However, other morphological and clinical entities that harbor DH/TH rearrangements are not considered in this classification, and it is ambiguous how to classify them [7]. Systematic information on the clinical characteristics of other DH/TH-B cell malignancies is lacking, especially on B cell precursor lymphoblastic leukemia (BCP-ALL), which obviously rarely falls within the DH/TH genetic category.

Single rearrangements involving the *MYC* translocation occur in approximately 5–10% of BCP-ALL patients and are associated with hypodiploidy and *TP53* mutations [8, 9]. Presence of *MYC* rearrangements were independently associated with inferior survival in multivariate analysis (hazard ratio, 2.1; $p = 0.25$) in a large cohort of BCP-ALL, Burkitt Leukemia, and T-lineage ALL [8], limiting the validity for BCP-ALL patients only. Single rearrangements involving the 14q32 (*IGH*) locus are present in approximately 2–5% of all BCP-ALL cases and are probably likewise associated with inferior survival compared to patients with no cytogenetic alterations (hazard ratio, 1.13; 95% CI, 1.06–1.21). The presence of *IGH/BCL2* rearrangements, however, accounts for less than 1% and is considered to be sporadic [10–12].

To date, there is no valid data on the frequency and clinical features of combined rearrangements in terms of DH/TH in patients with BCP-ALL. We therefore sought to determine the clinical characteristics, treatment approaches, and overall prognosis of patients with de novo BCP-ALL that harbors DH or TH genetics and put our findings into the context of the new WHO 2016 and current lymphoblastic leukemia classifications.

Patients and methods

Eligible patients were identified within the registry databases of the German Multicenter Study Group for Adult ALL (GMALL). Patients with a diagnosis of BCP-ALL or BCP lymphoblastic lymphoma between 2004 and 2018 (data cutoff June 2018) according to standard criteria based on cytology and immune-phenotyping were included if they harbored a rearrangement of *BCL2* and/or *BCL6* simultaneously with a rearrangement of *MYC* irrespective of the translocation partner. Patients with a known history of a malignant B cell disorder such as DLBCL or FL were excluded, as were patients harboring the *t*(9;22)(q34;q11) Philadelphia translocation. Information on disease and patient characteristics in addition to information on treatment strategies were obtained from the GMALL database and included results from bone marrow biopsies, blood counts, and cytogenetic and molecular genetic analyses using standard banding techniques and PCR-based gene analysis, fluorescence activated cell sorting (FACS), and minimal residual disease (MRD) status. Genetic analyses, FACS, and MRD quantification were performed in GMALL reference laboratories. Patients were risk-stratified according to the respective GMALL protocols.

In addition, a systematic and exhaustive MEDLINE database search for patients with the stated eligibility criteria was performed. Patients with immunoglobulin surface expression B cell (Burkitt) leukemia and a previous history of B cell malignancy were likewise excluded from this analysis. Authors were contacted for additional information on the published patients' disease history and treatment regimens. We assumed intrathecal prophylaxis to have been performed, if it was explicitly mentioned by the authors, or when intrathecal prophylaxis was included in the patient's treatment protocol.

Progression-free survival (PFS) and overall survival (OS) were determined for all patients. PFS was defined as the time from diagnosis to disease progression, relapse, or death of any cause. OS was defined as the time from first DH/TH-BCP-ALL diagnosis to death from any cause. Patients who were still alive at data cutoff (01.06.2018) were censored with regard to overall survival analysis at the date of last contact. Alive patients without disease progression at the time of the data cutoff were likewise censored with regard to PFS analysis at the date of last contact. Informed consent was obtained from all individual participants from the GMALL registry included into this analysis. The GMALL registry is registered on www.clinicaltrials.gov: NCT02872987 and was approved by the responsible Institutional Review Boards.

Statistical analysis

The number of all included patients and recorded variables were reported using descriptive statistics. Survival analyses were performed using the Kaplan-Meier method for estimation. Differences in survival distribution were evaluated by log-rank test reporting the 95% confidence interval (95% CI) and hazard ratio (HR). p values ≤ 0.05 were considered as statistically significant. GraphPad Prism Version 6.01 (GraphPad software Inc., USA) and SPSS, Version 25.0.0 (IBM Corp., USA) were used for statistical analysis and reporting of the data collected for this study. Due to the small number of cases, multivariate analyses were not performed.

Results

GMALL

Patients and treatment approach

We identified 6 patients with de novo BCP-ALL with co-occurrence of *MYC* and *BCL2* and/or *BCL6* rearrangements that were confirmed by fluorescence in situ hybridization (FISH) in 5 cases ($n = 1$ unknown) in the GMALL registry between 2004 and 2018. One patient suffered from B cell precursor lymphoblastic lymphoma without evidence of bone marrow infiltration.

Five male and one female patient with a median age of 54 years (range, 41–79 years) presented with a median white blood cell count of 9×10^9/L (range, 6–19 $\times 10^9$/L), a median lactate dehydrogenase of 2892 U/L (range, 1386–6938 U/L), and clinically suffered from severe malaise, night sweats, and weight loss. One patient suffered from severe anemia (5.3 mmol/L) and thrombocytopenia (16×10^9/L), whereas two patients each presented with isolated thrombocytopenia (13×10^9/L, 53×10^9/L) and anemia (5.3 mmol/Lg/dL, 6.5 mmol/L), respectively. Exact bone marrow blast count was documented in three patients, two of which suffered from a massive infiltration with nearly complete replacement of the normal hematopoiesis (see Table 1).

Two patients suffered from meningeal infiltration at first consultation and in one patient other extramedullary manifestations (gastric infiltration) were present at initial diagnosis (Table 2). In line with inclusion criteria, all patients were diagnosed with terminal deoxynucleotidyl transferase $(TdT)^+$ and $CD10^+$ BCP-ALL in the absence of surface immunoglobulin evaluated by standardized central flow cytometry in a GMALL reference laboratory ($n = 1$, location of flow cytometry not known).

Five patients were treated according to German standardized ALL protocols for younger patients [21]. One patient received best supportive care due to frailty and poor performance status and died 4 days after first diagnosis. One patient was switched to a Burkitt lymphoma treatment protocol [22] after his first induction cycle and review of cytogenetic findings. All mentioned treatment protocols throughout the manuscript are specified in the Online Resource 1 (Supplement).

As all patients were identified retrospectively and comprehensive cytogenetics are not mandatory for most patients included into the GMALL registry, we are not able to define the overall incidence of DH/TH genetics in BCP-ALL.

Treatment response, salvage strategies, and survival

All five patients who were treated with induction therapy achieved a complete hematologic remission (hCR) within the first 46 days of treatment and three patients ($n = 1$ not available) achieved a concomitant deep reduction of MRD below 2×10^{-4} by day 71 after treatment initiation. All treated patients received standard intrathecal prophylaxis. Accompanying cranial radiation as part of the meningeal disease, prophylaxis was administered to all patients except one due to thrombocytopenia-related spontaneous subdural hematoma. Table 2 gives detailed information on treatment specifications, strategies, and treatment responses.

One patient in hCR died due to therapy-related sepsis 3 months after treatment initiation. In four patients, disease relapse occurred early after a median PFS of only 4.0 months (95% CI, 2.1–5.9 months).

Two patients in our cohort received 2nd-line and further-line antineoplastic therapy including allogeneic stem cell transplantation (HCT): One patient (Pat ID 002, see Table 3) with slow-proliferative leukemic disease achieved a second hCR following salvage therapy with R-FLAG-IDA and subsequent allogeneic stem-cell transplantation (SCT) as a 2nd-line treatment. The patient progressed 2 months after SCT and was resistant to further salvage therapy with donor lymphocyte infusion and one course of dose-reduced R-FLAG-IDA. The patient received treatments with steroids and pentostatin

Table 1 Patient and disease characteristics ($n = 17$)

		GMALL ($n = 6$)	Literature ($n = 11$)	Summary ($n = 17$
Age (years)		($n = 6$)	($n = 11$)	($n = 17$)
	Median, range	54 (41–79)	44 (15–86)	44 (15–86)
	Mean ± SD	57 ± 15.0	43 ± 22.4	45 ± 22.7
Gender		($n = 6$)	($n = 11$)	($n = 17$)
	Male	5 (83.3%)	5 (45.5%)	10 (58.8%)
	Female	1 (16.7%)	6 (54.5%)	7 (41.2%)
Hematology (median, range)		($n = 6$)	($n = 6$)	($n = 12$)
	WBC (×10E9/L)	9 (6–19)	19 (3–92)	10 (3–92)
	Thrombocytes (×10E9/L)	34 (13–143)	62 (20–266)	55 (13–266)
	Hemoglobin (mmol/L)	7.4 (5.6–9.9)	6.8 (3.7–9.3)	6.8 (3.7–9.9)
	LDH (U/L)	2892 (1386–6938)	1164 (646–28,000)	2892 (646–28,000)
	Blast count BM (%)	90 (60–90)	90 (30–95)	90 (30–95)
FACS		($n = 6$)	($n = 6$)	($n = 12$)
	CD38/CD34 positivity	0 (0%)	3 (50%)	3 (25%)
Manifestation at first diagnosis		($n = 6$	($n = 11$)	($n = 17$)
	CNS involvement	2 (33.3%)	1(9.1%)	3 (17.6%)
	Extramedullary/extranodal manifestation	1 (16.7%)	4 (36.4%)	6 (35.3%)
Relapse location		($n = 6$)	($n = 11$)	($n = 17$)
	CNS involvement	2 (33.3%)	4 (36.4%)	6 (35.3%)
	Extramedullary manifestation	1 (16.7%)	1 (9.1%)	2 (11.8%)
Treatment protocol at first diagnosis		($n = 6$)	($n = 10$)	($n = 16$)
	ALL-based regime	4 (66.7%)	8 (80%)	12 (75%)
	B-NHL/Burkitt-based regime	1 (16.7%)	1 (10%)	2 (12.5%)
	BSC	1 (16.7%)	1 (10%)	2 (12.5%)
Best treatment response on first-line therapy		($n = 5$, BSC $n = 1$)	($n = 10$)	($n = 15$)
	CR	5 (100.0%)	5 (45.5%)	10 (62.5%)
	RD	0 (0%)	5 (45.5%)	5 (31.3%)

SD standard difference, *WBC* white blood cell count, *LDH* lactate dehydrogenase, *BM* bone marrow, *FACS* fluorescence activated cell sorting, *CNS* central nervous system, *B-NHL* B cell non-Hodgkin lymphoma, *BSC* best supportive care, *CR* complete remission, *RD* resistant disease, *ALL* acute lymphoblastic leukemia

for severe chronic graft versus host disease and died 11 months after the last salvage regime with meningeal and peritoneal disease manifestations. The second patient (Pat ID 003, see Table 3) achieved three subsequent hematologic CRs following cycle B1, Burkitt lymphoma protocol (2nd line), DHAP (5th line), and venetoclax (7th line) treatment, but rapidly progressed after each course of salvage therapy and died 16 months after first diagnosis.

Overall, typical manifestations of disease progression in our cohort were peripheral re-occurrence of blasts, isolated meningeal involvement, or other extramedullary manifestations (see Table 3).

Median OS in all six patients was 8.5 months (95% CI, 0.0–23.7 months). Only one patient (female, 45 years of age at first diagnosis) who was switched to a Burkitt lymphoma protocol (Pat ID 004, Table 2) was still alive at data cutoff and was lost to follow-up in CR after 127 months.

None of the patients was upfront planned for HCT either due to frailty ($n = 1$) or stratification into standard risk group ($n = 5$) according to GMALL criteria.

Review of literature

We identified 11 additional cases (five male, six female) with de novo DH/TH-BCP-ALL that were published between 1983 and the first of June, 2018 (data cutoff). Detailed summarized information on disease and patient characteristics are shown in Table 1. At initial diagnosis, meningeal infiltration was present either isolated or with other extramedullary disease manifestations in one and five patients, respectively. In addition to disease-defining lymphoblastic precursor surface marker expression, flow cytometric co-occurrence of CD38 and CD34 was documented in 3 cases.

Generally, treatment strategies were mainly based on ALL protocols ($n = 8$, 80%; $n = 1$ unknown), which are specified in

Table 2 Detailed information on treatment and response ($n = 17$)

Pat ID	Age	1st-line treatment	BR1	I.th.	2nd line	BR2	3rd + line	BR3+	EM1	EMX	CNS1	CNSX	PFS	Survival [months]	Ref.
001	79	BSC											0.1	0.1	
002	42	GMALL 07/03	hCR, MRD 1×10E-4	X	$n = 4$ therapy lines, see Table 3				Intestinal	Parotid gland, intestinal	X	X	30.6	47.2	
003	41	GMALL 07/03	hCR, MRD-CR	X	$n = 7$ therapy lines, see Table 3					Humeral joint, subcutaneous		X	3.6	15.8	
004	45	GMALL 07/03 Ind. 1	hCR, MRD-CR*	X	Switch to Burkitt protocol without PD								127.0	127.0+	
005	63	GMALL 07/03 elderly	hCR	X	BSC						X		8.1	8.5	
006	67	GMALL 07/03	hCR, MRD 2×10E-4	X	Death in CR								3.1	3.1	
007	18	Hyper CVAD	RD	X	R-CHOEP	RD	Clofarabine + decitabine	RD				X	1.0	7.0	[13]
008	24	Linker regime	hCR	X	Blinatumomab	RD	EPOCH + ofatumumab + bortezomib	RD				X	6.0	13.0	[13]
009	15	AALL0232	hCR	X					Parotid gland		X		53.0	53.0+	[13]
010	27	ALL protocol, unspecified	RD	U KN	UKN				Intestinal	Pleural		X	1.0	5.0	[14]
011	57	CODOX-M/IVAC	RD	X	UKN								1.0	2.5	[15]
012	86	BSC											0.5	0.5	[15]
013	56	R-Hyper CVAD	RD	U KN	"Additional therapy"								1.0	5.0	[16]
014	60	VDLP	hCR	U KN	BSC								U KN	U KN	[17]
015	21	VDLP	RD	U KN	BSC				Subcutaneous				1.0	3.5	[18]
016	29	UKN	hCR	U KN	"Multiple salvage regimes"				Intestinal, ovaries			X	8.0	29.0	[19]
017	15	VDCP + maintenance (6-MP, MTX, VCR)	hCR	X	"Chemotherapy"								5.0	5.0	[20]

Pat ID patient identification, *BR1* best response to first-line therapy, *BR2* best response to second-line therapy, *BR3* best response to third-line therapy, *I.th.* intrathecal chemoprophylaxis, *EM1* extramedullary disease (other than CNS) manifestation at first presentation, *EMX* extramedullary disease (other than CNS) relapse during course of disease, *CNS1* central nervous system disease manifestation at first presentation, *CNSX* central nervous system disease manifestation during course of disease, *PFS* progression-free survival after first-line therapy (patients with RD are set to 1-month PFS), *Ref.* references, *BSC* best supportive care, *hCR* hematologic remission, *MRD* minimal residual disease, *MRD-CR* MRD negativity, *RD* resistant disease, + indicates that patient is still alive at data cutoff; treatment protocols (GMALL 07/03, Burkitt protocol, Hyper CVAD, Linker regime, AALL0232, CODOX-M/IVAC, R-Hyper-CVAD, VDLP, VDCP, R-CHOEP, EPOCH) are specified in the supplement section); *M* month after first diagnosis; * hCR was documented an day 11 after induction cycle I, patient was then switched to Burkitt protocol, and MRD-CR was achieved after additional cycle A1 on day 51

Table 3 Detailed information on treatment and response in patient 002 and 003

Pat ID	002	003
1st-line treatment	GMALL 07/03	GMALL 07/03-MO
BR1	hCR, MRD 1×10E-4	hCR, MRD-CR
P1	Systemic progression	Systemic progression
2nd line	R-FLAG-IDA I-M31	B1 Burkitt protocol-M3
BR2	RD	hCR, MRD 1×10E-4
P2	Isolated meningeal infiltration	(switch to blinatumomab)
3rd line	R-FLAG-IDA II + HCT-M34	Blinatumomab-M5
BR3	hCR, MRD 2×10E-3	hCR, MRD 1×10E-4
P3	Systemic progression	Isolated juxta renal mass
4rd line	R-FLAG-IDA III 50% - M36	Al Burkitt protocol-M6
BR4	RD	PD
P4	Systemic progression	Isolated parotid gland infiltration
5th line	DLIs - M37, M39	DHAP I + II + HCT-M7
BR5	RD	hCR, mixed chimerism on day 13 after SCT
P5	Systemic progression, death M47	Systemic progression, subcutaneous chloroma
6th line		Panobinostat-M10
BR6		RD
P6		Systemic progression, subcutaneous chloroma, meningeal infiltration
7th line		Venetoclax-M12
BR7		hCR
P7		Isolated subcranial lesion
8th line		Inotuzumab-M14
BR8		PD
P8		Death-M16

Pat ID patient identification, *BR1–7* best response to treatment line 1–7, *P1–7* side of progression on first-seventh relapse, *hCR* hematologic complete remission, *MRD* minimal residual disease, *MRD-CR* MRD negativity, *RD* resistant disease, *HCT* allogeneic stem cell transplantation; treatment protocols (GMALL 07/03, FLAG-IDA, DHAP, Burkitt protocol [A1, B1] are specified in the supplement section); administration of single doses of vincristine, cyclophosphamide, and concomitant irradiation of single lesions is not explicitly mentioned

Table 2. Only one patient was upfront treated with a regimen for aggressive B cell/Burkitt lymphoma (CODOX-M/IVAC, see Supplement), suffered from resistant disease, and died after a survival time of 2.5 months. Data on MRD status/level were overall not available. A total of five patients received 2nd-line and further-line systemic antineoplastic treatment. Salvage options included anthracycline-based polychemotherapy (R-CHOEP, EPOCH) and combination or monotherapy consisting of clofarabine, decitabine, ofatumumab, and blinatumomab (see Table 2). No second hCRs were documented after disease relapse and median OS was 5.0 months (95% CI, 3.5–6.5). One patient at the age of 15 with meningeal and parotid gland infiltration at first diagnosis who was treated according to the pediatric ALL protocol "AALL0232" was lost to follow-up in CR after 53.3 months.

One potential case of a 10-year-old girl was only available in Japanese language and could not be considered in this analysis. She was diagnosed with CD10, TdT, and HLA-DR-positive BCP-ALL, achieved a complete remission with dose-intensive chemotherapy, and subsequently relapsed in the bone marrow and CNS. She died 44 days after first diagnosis after cord-blood transplantation in non-remission status (English abstract) [19]. Another case was mentioned in a retrospective series, but there were no further information on disease history and course of disease and could therefore not be considered in this analysis [23].

Survival analysis

Median PFS of all patients (data available for 16 patients [94.1%]) was 4.0 months (95% CI, 1.5–6.5 months) (Fig. 1a) and median OS was 5.0 months ($n = 16$ [94.1%]; 95% CI, 3.1–6.9 months). Only two patients were alive at data cutoff (lost to follow-up in CR after 53.3 and 127.4 months,

respectively; 11.8%) (Fig. 1b) and two patients died in hCR due to infectious complications (survival, 3.1 months; $n = 1$ unknown). After first diagnosis, 9 patients (56.3%) died within 6 months, 7 patients (43.8%) survived 12 months, and 5 patients (31%) were alive after 2 years. Median survival time after disease relapse was 2.5 months ($n = 14$ [82.3%]; 95% CI, 0.0–5.6 months). There was no significant survival difference between patients identified in the GMALL registry and in the literature (HR, 0.69; 95% CI, 0.23–1.96).

Genetic findings

Standard cytogenetic approaches of all patients ($n = 17$) identified *IGH* as the only translocation partner of the *BCL2* oncogene. Immunoglobulin heavy chain (*IGH*) and immunoglobulin light chain (*IGL*) were the translocation partners for *MYC* in six and five patients, respectively. The chromosomal region 9p13 was found to be juxtaposed to *MYC* in five cases. In one case, the origin of the chromosomal translocation partner for *MYC* could not be determined. Two patients showed additional translocations of the *BCL6* gene locus (3q27, "triple hit") with 10q11 and 12p11 in one case each. All but one patient (with data on cytogenetic findings only available from a patient-derived cell line) had a complex karyotype. For additional information on the patients' karyotype and translocation partners, see Online Resource 2 (Supplement). There was no significant survival difference between patients harboring IG or non-IG *MYC* rearrangements (HR, 1.03; 95% CI, 0.33–3.2; $p = 0.89$). Data on the surface expression of MYC and BCL2/BCL6 was only irregularly assessed and could therefore not be considered in this analysis.

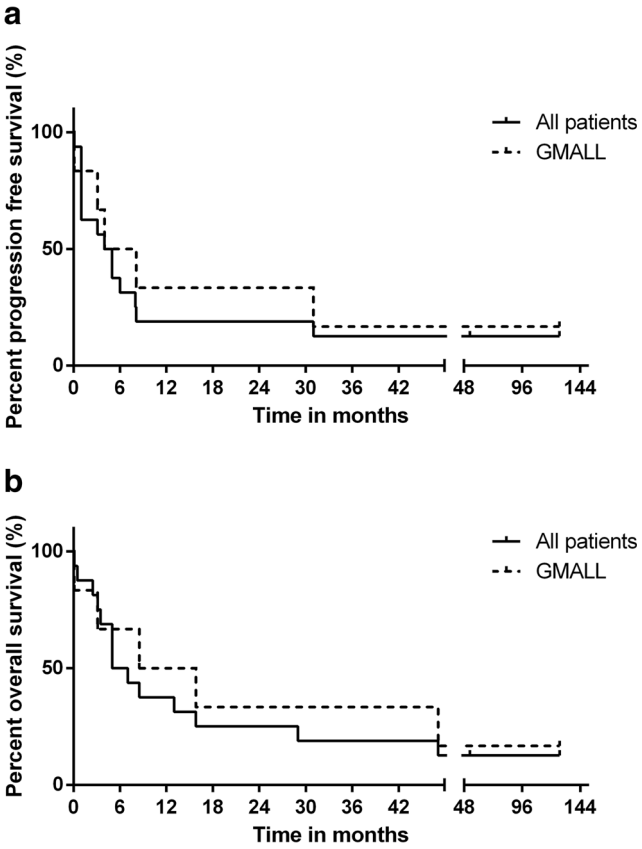

Fig. 1 **a** Progression-free survival of de novo DH-ALL patients identified in the GMALL registry (dotted pattern) and all adult de novo DH-B-ALL patients (solid pattern). **b** Overall survival of de novo DH-ALL patients identified in the GMALL registry (dotted pattern) and all adult de novo DH-B-ALL patients (solid pattern)

Discussion

High-grade B cell lymphomas, with *MYC* and *BCL2* and/or *BCL6* rearrangements, are a morphologically heterogeneous group of B cell neoplasms. Here, we report the clinical and genetic characteristics of 6 cases of de novo acute B cell precursor leukemia that harbored combined *MYC* and *BCL2* and/or *BCL6* rearrangements (DH/TH-BCP-ALL) that were identified in the GMALL registry between 2004 and 2018 in addition to 11 published cases in international databases.

Patients of all ages (range, 15–86 years) were diagnosed with DH/TH-BCP-ALL and usually presented with severe symptomatic disease burden and a massive bone marrow infiltration. Approximately 20% of all patients suffered from CNS involvement at first diagnosis and one third of all patients showed meningeal infiltration during their course of disease despite CNS prophylaxis. Retrospective series report a similar CNS involvement rate between 4 and 23% in DH/TH-HGBLs [4, 24, 25], whereas CNS involvement at disease relapse in DH/TH-HGBL seems to be a rare event in patients who had achieved a first CR and was reported to be less than 5% [26], which is comparable to the rate of CNS relapse in non-DH/TH-BCP-ALL patients [27].

There was a high incidence of other extramedullary manifestations at initial diagnosis, a tendency well recognized in DH-HGBLs [25, 28]. Disease manifestations were mainly located in the gastrointestinal tract including the salivary glands, but also paraosseal and in the ovaries in one case and patients with initial extramedullary manifestations were at high risk for extramedullary relapses during their course of disease. This remains true in patients with hCR and low or undetectable MRD levels and underscores the need for alertness regarding potential (and atypical) symptoms of disease relapse. Of interest, extramedullary relapse was not seen in patients who presented with medullary and lymph node disease only, at initial diagnosis. On the contrary, extramedullary relapse in non-DH/TH-BCP-ALL can be found in less than 10% in larger series and these cases mostly show concomitant systemic progression with a re-occurrence of blasts in peripheral blood or bone marrow [27, 29].

Although hematologic remission was achieved in more than 60% of the overall DH/TH-BCP-ALL population, the

majority of patients relapsed within 12 months. Primary refractory or relapsed disease was inevitably linked to an unfavorable prognosis and all of these patients subsequently succumbed to their disease. Median OS after disease relapse was only 2.5 months, which is markedly inferior compared to relapsed or refractory non-DH/TH-BCP-ALL patients who achieve a second CR in approximately 40% of all cases and show a median survival of 5.8 months (95% CI, 5.5–6.2 months) after salvage therapy [30]. Patients with DH/TH-HGBL achieve similar CR rates with more intensive first-line treatments (such as DA-EPOCHR), but OS is notably superior with a reported overall 2-year survival exceeding 50% in retrospective studies [24, 31]. In addition, a proportion of patients with relapsed or refractory DH/TH-HGBLs can successfully be treated with salvage regimes and autologous stem cell transplantation (ASCT) achieving a 4-year OS of 25% [32]. Nevertheless, adverse risk factors such as CNS involvement, high disease burden, and elevated lactate dehydrogenase levels severely impact survival rates in DH-HGBL and are regularly seen in patients with DH/TH-BCP-ALL [1], reasoning that these risk factors may evenly contribute to the inferior survival of DH/TH-BCP-ALL.

There is emerging evidence that intensified treatment protocols (such as DA-EPOCHR, CODOX-M/IVAC) may overcome the adverse prognosis of DH-HGBLs seen with standard R-CHOP therapy; however, consolidation with ASCT in first CR does not seem to improve survival [24, 31, 33]. In DH/TH-BCP-ALL, intensive treatment regimens were capable to induce CRs in the majority of the entire cohort; still, the high relapse rate emphasizes the need for potent consolidation strategies in patients with DH/TH-BCP-ALL in first remission. Based on our findings, HCT in first CR should be considered as a consolidative treatment option in eligible patients with DH or TH genetics, a treatment approach that was not considered upfront based on current risk classifications according to the GMALL. However, due to the few numbers of survivors, conclusions regarding optimal treatment approaches remain highly speculative.

Altogether, three different translocation partners of *MYC* were identified namely *IGH*, *IGL*, and the region 9p13. These partners accounted for approximately one third of the cases each. Although suggested previously [34], we saw no OS benefit for non-IG-translocation partners of *MYC*. Prognostic impact of other risk factors (e.g., white blood cell count, performance status, CD38 positivity) has been previously described, but owing to the limited and heterogeneous patient population, we do not believe that it is appropriate to statistically define independent factors that might contribute to the overall prognosis of DH-BCP-ALL. As *IGH* was the only translocation partner of *BCL2*, FISH screening for *IGH* rearrangements may serve as a highly sensitive screening method for DH/TH-BCP-ALLs.

In summary, de novo DH/TH-BCP-ALL is a highly aggressive B cell malignancy that comprises of unique clinical characteristic. There is a high incidence of meningeal and other extramedullary disease manifestations at first diagnosis and during the course of disease and prognosis is markedly inferior compared to patients without DH/TH genetics. Patients who achieve a first (and deep) CR after induction treatment are at profound risk from early disease relapse and all patients, once relapsed, eventually succumb to their disease, despite intensive salvage strategies. Our data therefore emphasizes the need for effective consolidation strategies in this rare disease sub-entity. We believe that physicians need to be aware of this entity due to its uncommon clinical behavior and because it is not covered by latest classification systems. Further studies are needed to more precisely define this disease's behavior and the overall incidence, and to optimize the treatment approaches in patients with this rare and aggressive B cell malignancy.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Disclosures of conflict of interest Prof. Haferlach reports reimbursements for diagnostic services from the MLL Munich Leukemia Laboratory during the conduct of this study; and being a part owner of the MLL Munich Leukemia Laboratory; The other authors declare no conflicts of interest.

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