Children with hyperdiploid but not triple trisomy (+4,+10,+17) acute lymphoblastic leukemia have an increased incidence of extramedullary relapse on current therapies: A single institution experience

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To evaluate the outcome of children with high hyperdiploid acute lymphoblastic leukemia (hHDALL) treated at the author's institution. One hundred thirty-five consecutive children with B-precursor ALL were diagnosed between 1991 and 2002: 38 (28.1%) hHDALL and 97 (71.9%) non-hHDALL. In the hHDALL group, 11/38 (28.9%) relapsed at a median interval of 2.8 years (range: 0.8–5.0 years) with 9/11 relapses occurring at the end or after the completion of therapy. Three (27.3%) relapses were isolated hematopoietic (BM), while eight (72.7%) were either isolated extramedullary (EM) relapses (n = 6; Testis: 4; CNS: 2) or combined hematopoietic and extramedullary relapses (n = 2; BM + CNS: 1; BM + Testis: 1). For the non-hHDALL group, 29/97 (29.9%) relapsed. Unlike the hHDALL group, the non-hHDALL group experienced hematopoietic relapses (62%; n = 18) more frequently than isolated extramedullary (27.5%; n = 8: Testis: 1; CNS: 7) or combined hematopoietic and extramedullary relapses (10.3%; CNS + BM: 3), with 24/29 (82.8%) of the relapses occurring on therapy. Relapses in hHDALL frequently involved EM sites (P = 0.053). Presence of triple trisomy of +4,+10,+17 at diagnosis had a protective effect against relapse (P < 0.05). Five-year EFS for the hHDALL and non-hHDALL patients was similar, 70.5 ± 7.5% and 66.4 ± 4.9%, respectively. Five-year OS for the hHDALL patients was significantly higher than for the non-hHDALL patients, 92 ± 4.5% vs. 74.1 ± 4.5%, P = 0.038. Biologically significant differences exist between relapse patterns of hHDALL and non-hHDALL cases related to relapse sites and time periods when relapses occur. hDALL relapses continue to be chemo-sensitive. Am. J. Hematol. 83:34–40, 2008. © 2007 Wiley-Liss, Inc.

Introduction

Identification of recurring chromosomal abnormalities including ploidy has a major impact on risk assignment for treatment of childhood acute lymphoblastic leukemia (ALL) [1–6]. Among the distinguishing cytogenetic features of acute lymphoblastic leukemia (ALL), a high hyperdiploid karyotype with a modal chromosome number (MCN) of 51–65 chromosomes is one of the most reliable predictors of a favorable clinical outcome [1–6]. It is present in ~25% of pediatric B-precursor ALL cases [2–6] and ~85% of children with this form of ALL can be cured with contemporary treatments [1]. High hyperdiploid ALL (hHDALL) can also be identified by the DNA content of blast cells measured by flow cytometry as the DNA index (DI). The most common chromosomes present in extra copy number in hHDALL are 21, 6, X, 14, 4, 18, 17, 10, 8, and 5 [7–10]. Patients within the hHDALL category typically have several favorable prognostic features at diagnosis including: (i) age between 2 and 10 years, (ii) low initial white blood cell count, and (iii) expression of the stem cell marker (CD34) in addition to common ALL antigen (cALLA) expression.

In this article, we report our institutional experience of treating a cohort of children with hHDALL with an emphasis on their relapse patterns compared with non-hHDALL cases. We observed that the relapse pattern of hHDALL is different than the other subsets of childhood ALL with a predisposition to extramedullary relapse sites.

Results

From January 1991 to December 2002, 135 children (age > 1 year to 18 years) were diagnosed with B-precursor ALL at Children's Hospital of Michigan (CHM). A total of 38 children had hHDALL (MCN 51–65 chromosomes). Thus, the hHDALL group constituted 38 (28.1%) and the non-HD group included the remaining 97 (71.9%) children. Median follow-up for the entire cohort was 7.6 years (range: 1.2 months to 15.2 years). Descriptive demographic features of both the groups are shown in Tables I and II.

Relapse characteristics of high hyperdiploid ALL

Eleven of 38 patients in the hHDALL group (28.9%; 95% CI: 13.72–42.17) experienced a leukemia relapse (Tables I and II) at a median interval from diagnosis to relapse of 2.8 years (range: 0.8–5.0 years). Two (18%) relapses occurred prior to therapy completion at week 39 (UPN 29) and week

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TABLE I. Characteristics of Children With Hyperdiploid and Non-Hyperdiploid B-Precursor ALL

Characteristics	High hyperdiploidy ALL	Non-high hyperdiploidy ALL	*P* value
Total number of patients	38 (28.1%)	97 (71.9%)	
Median age at diagnosis (yrs)	3.8 (1.2–16)	6.5 (1.5–18)	0.002
Male: female	22 (58%): 16 (42%)	55 (56.7%): 42 (43.3%)	NS
Age >10 yrs	5 (13%)	33 (34%)	0.005
Race: C/AA/O	27 (71%)/6 (16%)/5 (13%)	57 (58.2%)/28 (28.6%)/12 (12.2%)	
Median WBC /mm^3 count at Dx	8,942 (range: 900–41,700)		
Total no of events (relapse + death)	11 (28.9%)	33 (34%)	NS
Total no of relapse	11 (28.9%)	29 (29.9%)	NS
Relapse characteristics			
Mean age at relapse (yrs)	6.9 (1.6–16)	9.5 (1.5–18)	NS
Male/female	8 (72.7%)/3 (27.3%)	20 (69%)/9 (31%)	NS
Age >10	4/11 (36.4%)	14/29 (48.3%)	NS
Race: C/AA/O	10 (91%)***/0 (%)/1 (9%)	10 (34.5%)***/14 (48.3%)/5 (17.2%)	0.005
Median follow up (yrs)	8.8 ± 3.9 (0.8–15.2)	7.1 ± 4.4 (0.1–15.2)	
Pattern of relapse			
Hematopoietic (H)	3 (27%)***	18 (62.1%)***	0.053
Total extramedullary relapse	8 (73%)***	11 (37.1)***	
Isolated CNS relapse	0	7	
Isolated testis relapse	4	1	0.02♣
CNS + H	3	2	
Testis + H	1	1	
Mortality	3 (0.07%)****	26 (26.8%)****	0.009
Deaths due to relapse	3 (100%)***	22 (75.9%)***	0.009¥
Non-relapse deaths	0	7 (24.1 %)	
Survival			
Event free survival (yrs)	70.5% ± 7.5%	66.4% ± 4.9%	NS
Overall survival (yrs)	92.9% ± 4.5%	74.1% ± 4.5%	0.038

NS: not significant.
***: *P*-value indicates chi-square analysis including hematopoietic versus nonhematopoietic relapses in hyperdiploid and nonhyperdiploid group.
♣: *P*-value indicates χ^2 analysis between testicular versus all other relapses in hyperdiploid and non-hyperdiploid group.
****: χ^2 analysis comparing relapses versus no-relapses in hyperdiploid and nonhyperdiploid group.
¥: *P*-value indicates χ^2 analysis between deaths due to relapse versus survival after relapses in hyperdiploid and nonhyperdiploid group.

109 (UPN 35), while the majority of relapses (82%) occurred at either the end of therapy ($n = 1$; UPN 36) or after therapy completion ($n = 8$). The median interval between therapy completion and relapse was 0.3 years (range: 42 days to 2.6 years) for the eight off-therapy relapses. Thus, early relapses (occurring within 6 months after completion of therapy) occurred in eight children, while three children had late relapses.

Among the 11 patients who relapsed, 3 (27.3%) children experienced an isolated hematopoietic relapse, while 8 (72.7%) children experienced either isolated extramedullary (EM) relapses ($n = 6$; Testis: 4; CNS: 2) or combined hematopoietic and EM relapses ($n = 2$; BM + CNS: 1; BM+ Testis: 1). Four (UPN 28, 32, 34, and 35) of the five (80%) children in the hHDALL group who had NCI high-risk criterion (age > 10 years), [11] experienced a relapse. One teenage patient (UPN 34) who experienced a testicular relapse, had monosomy 7 and *t*(1;19)(q23;p13) besides a high hyperdiploid karyotype (MCN 61-64). Patient (UPN 28) with a 9p deletion at diagnosis experienced a hematopoietic relapse (MCN 57). Among the 14 (40%) children with triple trisomies of +4/+10/+17, only one teenage male (UPN 35) experienced an on-therapy bone marrow relapse. The remaining 10 relapses in hHDALL group occurred in patients who did not have triple trisomies of +4/+10/+17. Thus, the presence of triple trisomies of +4/+10/+17 appeared to be associated with a lower risk of relapse ($P < 0.05$). There was no significant difference between the frequency of relapses in the hHDALL group between patients [24% (7/29)] who received repetitive courses of VCR/corticosteroid pulses during maintenance compared to patients [44.4% (4/9)] who did not receive these treatment pulses.

Relapse characteristics of nonhigh hyperdiploid ALL

Among the 97 children in the non-HD ALL group, 57 (58.8%) children qualified for low or standard risk criteria, while 40 (41.2%) qualified for high risk criteria based on age at diagnosis, WBC count at diagnosis or presence of the Philadelphia chromosome. Median age at diagnosis for the non-hHDALL cohort was 6.5 years (range: 1.5–18 years). Twenty-nine (29.9%; 95% CI 22–38) children experienced a leukemia relapse. Unlike the hHDALL group, the non-hHDALL group experienced hematopoietic relapses (62%; $n = 18$) more frequently than isolated EM (27.5%; $n = 8$: Testis: 1; CNS: 7) or combined hematopoietic and EM relapses (10.3%; CNS + BM: 3). In the non-hHDALL group, 82.8% ($n = 24$) of the relapses occurred on-therapy, while 17.2% ($n = 5$) of the relapses occurred off-therapy. The median interval between diagnosis and relapse was 1.6 years (range: 0.28–7.72 years). The median follow-up for this cohort was 7.4 ± 4.4 years (range: 0.28–15.2 years).

Summary of relapse characteristics and mortality for the entire cohort

In summary, there was a higher frequency of EM relapses in hHDALL (8/11; 73%) compared with non-hHDALL patients (11/29; 37.9%) ($P = 0.011$). Relative risk of developing EM relapses in non-HD ALL was 0.27 implying a protective effect against EM relapses. Caucasian children were more predisposed to relapse if they had high hyperdi-

TABLE II. Characteristics of Children With Hyperdiploid ALL at Diagnosis

UPN	Race/ sex	Age	Initial WBC/mm^3	Karyotypes	DNA index	Protocol used	Site of relapse	Interval: therapy completion and relapse (yrs)	Interval (OS): Dx and FU (yrs)	Status
1	O/F	4.6	3,200	56,XX,+X,+X,+4,+8,+9, +10,+14,+18,+18, +21[17]/46,XX [3]	1.2	9904♣	NA	NA	5.0	Alive:CR1
2	C/M	2.8	4,400	56,XY,+X,+4,+10,+14, +14,+18,+21,+22, +2mar[cp12]/46,XY[8]	1.2	9005	NA	NA	15.2	Alive:CR1
3	AA/M	3.3	18,400	56,XY,+X,+4,+6,+8,+10, +14,+17,+18,+21, +21[15]/46,XY[15]	1.19	9201♣	NA	NA	11.7	Alive:CR1
4	C/M	8.5	2,300	5557[cp4]/46,XY[23]	1.22	9201♣	NA	NA	8.6	Alive:CR1
5	C/M	3.3	1,700	60∼62,XY,+X,+4,+5,+6, der(6)t(1;6)(q12;q21), +9,+10,+11,+14, +14,+17,+18,+18, +19,+21,+21, +22[cp6]/46,XY[14]	1.29	9904♣	NA	NA	4.6	Alive: CR1
6	C/M	6.0	3,100	58∼62,XX,-Y,+4,+5,+6, +7,+8,+10,+11,+14, +15,+17,+18,+21,+21, +22[cp13]/46,XY[3]	1.34	9005	NA	NA	14.3	Alive:CR1
7	AA/F	1.3	18,600	55∼57,XX,+X,+4,+6,+7, +9,+14,+16,+18,+21, +21[cp11]/46,XX[9]	1.19	9904♣	NA	NA	5.7	Alive:CR1
8	AA/F	2.9	12,600	52∼53,XX,dup(1)(q21q32), +6,+8,+10,+14,+18, +21,+21[cp4]/46,XX[16]	1.29	9904♣	NA	NA	3.6	Alive: on therapy
9	O/F	3.9	3,500	53,XX,+4,+6,+10,+14,+17, +21,+21[8]/46,XX[12]	1.22	9904♣	NA	NA	4.5	Alive-CR1
10	C/F	10.2	2,800	52,XX,+i(X)(p10),+4,−7, ins(7;?)(q22;?), t(10;21) (q22;q22),+14, +der(15)t(9;15)(q12;p11.2), +21,+21,+mar[11]/46,XX[9]	1.13	9005	NA	NA	14.0	Alive-CR1
11	C/M	3.3	7,600	59,XY,+X,+2,del(2)(q35),+4, +6,del(6)(q15q25),+8,+10, +12,+14,+17,+18,+21, +21,+22[cp10]]/46,XY[2]	1.18	9201♣	NA	NA	7.2	Alive-CR1
12	C/M	3.9	1,500	54,XXcY,+4,+8,+9,+14,+16, +18,+21 [cp6]/46,XY[28]/47, XXY [18]	1.14	9201♣	NA	NA	12.9	Alive-CR1
13	O/M	3.6	5,600	58∼63,XY,+X,+Y,dup(1)(q12q22), +4,+5,+5,+6,+7,+8,+10, +11,+14,+17,+18,+18, +21,+21,+22[cp5]/46, XY[cp27]	1.12	9201♣	NA	NA	10.2	Alive-CR1
14	AA/M	5.0	3,300	55∼56,XY,+X,+4,+6,+9,+10, +14,+17,+18,+21, +21cp7]/46,XY[13]	1.27	9201♣	NA	NA	8.4	Alive-CR1
15	C/M	4.4	2,400	56,XY,+X,+4,+6,+7,+10, +14,+17,+18,+21, +21[16]/46,XY[4]	1.17	9201♣	NA	NA	9.2	Alive-CR1
16	AA/M	1.9	3,500	57∼61,XY,+X,+4,+5,+6,+9, +10,+10,+12,+14,+14, +17,+17,+18,+21, +21[cp13]/46,XY[8]	1.18	9005	NA	NA	14.8	Alive-CR1
17	C/F	5.4	2,300	55,XX,+X,+4,+6,+10,+14, +17,+18,add(19)(p13), +21,+21[cp7]/46,XX[13]	1.36	9005	NA	NA	15.1	Alive-CR1
18	C/M	3.6	6,700	53∼55,XY,+X,+4,+6,+10, +14,+17,+18,+21, +21[cp18]/46,XY[5]	1.35	9201♣	NA	NA	12.9	Alive-CR1
19	C/M	5.0	5,700	Failed	1.19	9201♣	NA	NA	11.5	Alive-CR1

TABLE II. (Continued)

UPN	Race/ sex	Age	Initial WBC/mm^3	Karyotypes	DNA index	Protocol used	Site of relapse	Interval: therapy completion and relapse (yrs)	Interval (OS): Dx and FU (yrs)	Status
20	AA/F	9.1	900	55∼57,XX,+X,+4,+6,+8, +10,+11,+14,+18,+21, +21[cp8]/46,XY[12]	1.23	9904♣	NA	NA	5.6	Alive-CR1
21	C/F	2.87	15000	53,XX,+6,+9,del(9)(q21q34), +10,+14,+18,+21, +21[17]/46,XX[3]	1.13	9605	NA	NA	6.3	Alive-CR1
22	O/F	1.21	21200	56,XX,+X,+X,+4,+6,+10, +14,+17,+18,+21, +21[2] 57,idem,+9[4]/46,XX[4]	1.18	9605	NA	NA	6.7	Alive-CR1
23	C/F	5.55	6200	52,XX,+3,+4,+6,+15, +18,+21[5]/46,XX[2]	1.16	9202	NA	NA	12.0	Alive-CR1
24	C/F	2.43	13100	54,XX,+X,+6,+10,+14,+17, +18,+21,+21[cp16]/46,XX[6]	1.14	9905	NA	NA	4.7	Alive-CR1
25	C/F	4.09	41700	52∼54,XX,+X,+4,+6,+10, +14,+17,+21, +21[cp13]/46,XX [12]	1.16	9605	NA	NA	5.1	Alive-CR1
26	C/F	2.62	8400	54,XX,+X,+X,+4,+6,+14,+17, +18,+21[cp26]/46,XX[3]	1.16	9605	NA	NA	7.4	Alive-CR1
27	C/M	4.13	11200	56∼59,XY,+X,+Y,+4,+6, +9,+10,+11,+14,+18, +21,+21[cp10]/46,XY[3]	1.14	9905	NA	NA	4.7	Alive-CR1
28	C/M	13.6	6,900	57,XY+X,+4,+8,+del(9)(p21), +10,+11,+14,+18,+21,+21, +mar[cp11]/46,XY[9]	1.2	9005	BM	2.4	14	Alive-CR2
29	C/F	2.2	32,000	51,XX,+X,+6,+14,+17, +21[16]/46,XX[4]	1.12	9605♣	BM	On therapy	1.1	Died
30	O/M	3.4	8,500	54,XY,+X,+4,+6,+10,+14,+14, +21,+21[3]/55,idem,+17, del(17)(p11)[6]/46,XY [2]	1.19	9201♣	BM	1.2	7.3	Alive-CR2, Post BMT
31	C/M	3.3	8,600	53∼55,XY,+X,+Y,+6,+10, +14,+17,+18,+21, +21[cp7]/46,XY[13]	1.31	9405	Testis	0.14	10.5	Alive: CR2, Post BMT
32	C/M	16.0	2,300	56,XY,+X,+4,+6,+8,+10,+14, +18,+20,+21,+21[19]/46,XY[1]	1.2	9906♣	Testis	0.08	2.88	Died
33	C/M	3.2	31,000	54,XY,+X,+4,+6,+14, +17,+18,+21,+21[20]	1.17	9005	Testis	End of therapy	14.7	Alive-CR2
34	C/M	14.7	1,900	61∼64,XY,+X,+4,+5,−7,+8, +9,+10,+11,+12,+14, +14, add(14)(q32),+17,+17, +18,+20,der(19)t(1;19)(q23;p13), +21,+21[cp16]/46,XY[4]	1.19	9605♣	Testis	0.4	8.8	Alive-CR2
35	C/M	15.6	4,300	57,XY,+X,dup(1)(q21q42),+4,+6, +9,+10,+14,+dup(14)(q31q32), +17,+18,+21,+21[cp20]	1.21	9005	Testis, BM	On therapy	3.9	Died**
36	C/M	1.5	3,500	Failed	1.16	9605♣	CNS	End of therapy	8.8	Alive-CR2
37	C/F	2.6	10,400	55,XY,+X,+4,+5,+6,+8,+13, +14,+18,+22[20]	1.20	9605♣	CNS, BM	1	9.0	Alive- CR2, Post BMT
38	C/F	4.0	3,500	51,XX,+X,+6,+14,+21,+21[25]	1.10	9605♣	CNS, BM	0.25	8.5	Alive-CR2, OT

Dx: Diagnosis, yrs: years; Int: Interval; C: Caucasian; AA: African American; O: Others; Y: Yes; N: No; NA: Not applicable; H, Hematopoietic (bone marrow); EM: Extra-medullary; CNS: Central nervous system; CR: Complete Remission; BMT: Bone marrow transplantation.

**: Died after second hematopoietic relapse.

♣: Protocols which included repetitive courses of vincrisitne and prednisone/dexamethasone during maintenance phase. All karyotypes were written according to the International System for Human Cytogenetics Nomenclature (ISCN) 2005. The gains or losses of chromosomes were expressed in relation to diploidy. (Reference: ISCN 2005: An International System for Human Cytogenetics Nomenclature. Shaffer L.G. and Tommerup N. (eds); S. Karger, Basel 2005.)

ploid cytogenetics ($P = 0.05$; Relative risk: 2.17). Male children with hHDALL experienced more (5/22; 23%) testicular relapses compared with the non-hHDALL group (2/55; 3.6%; $P = 0.003$; relative risk: 6.30). Additionally, older age (age $>$ 10 years) at diagnosis of hHDALL was a risk factor for relapse in both the hHDALL and non-hHDALL groups (4/5 vs. 14/29; $P = 0.007$; relative risk: 1.66). Although the proportion of relapses appeared higher for hHDALL (11/37) than non-HD ALL (29/97), the relative risk of relapse was almost the same for both groups (Relative risk: 0.97).

Overall mortality for the hHDALL and non-hHDALL groups was 7.9% (3/38) and 26.8% (26/97), respectively.

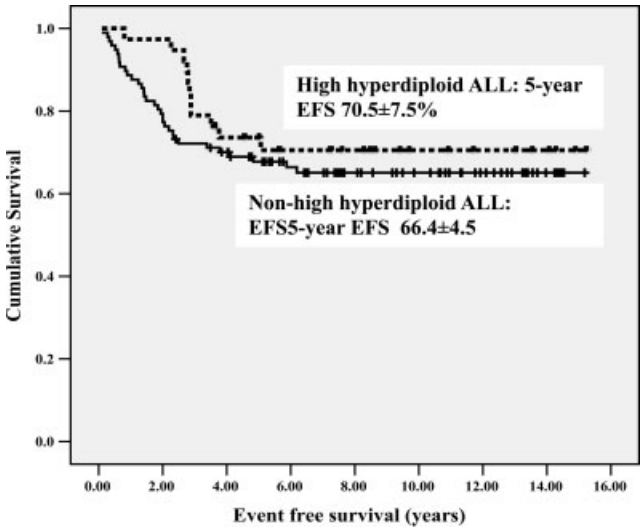

Figure 1. Event free survival for high hyperdiploid and non-high hyperdiploid ALL.

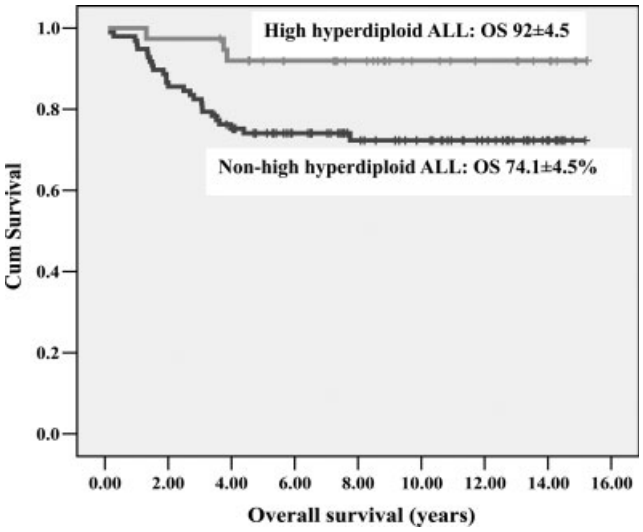

Figure 2. Overall survival for high hyperdiploid and non-high hyperdiploid ALL.

The relapse related mortality was 27.3% (3/11) for the hHDALL group and 88% (22/29) for the non-hHDALL group, ($P = 0.007$; relative risk: 0.36). Thus hHDALL had protective effect towards improving survival. An equal proportion of hHDALL (22%; 3/11) and non-hHDALL patients (22%; 6/27) received stem-cell transplantation as a part of their salvage therapy. The etiology of nonrelapse related deaths in five non-hHDALL patients was: gram negative sepsis ($n = 3$), ascending myelitis ($n = 1$), Epstein-Barr virus-related lymphoproliferative disorder in a child with Down syndrome ($n = 1$). The 5-year EFS and OS rates for the hHDALL group was 70.5 ± 7.5% and 92 ± 4.5%, respectively, while for the non-hHDALL group, 5-year EFS and OS were 66.4 ± 4.9% and 74.1 ± 4.5% respectively (Figs. 1 and 2).

Discussion

Our study identified differences in sites and frequency of relapses between children with hHDALL and non-hHDALL. Children with hHDALL had a higher frequency of EM relapses, while children with non-hHDALL had predominantly hematopoietic relapses. The majority of relapses in the hHDALL group occurred at the end of scheduled therapy or after completion of therapy, while the majority of non-hHDALL relapses occurred on therapy. Although hHDALL is associated with favorable prognosis, the proportion of relapses was the same for both hHDALL (28.9%) and non-hHDALL (29.9%). The significant finding was that a higher proportion of hHDALL children (8/11) could be salvaged successfully with second line therapy compared with non-hHDALL (7/29). The 5-year OS rate for the hHDALL group was (92 ± 4.5)% compared with (74 ± 4.5)% for the non-hHDALL group, which is comparable with the literature experience [12,13].

There is limited published literature on the relapse patterns of hHDALL. A study from St. Jude Children's Research Hospital reported the outcome of 182 children with hHDALL (51–67 chromosomes) treated on several different protocols [10]. Twenty-nine (16%) patients relapsed (bone marrow: 20; CNS: 6; testicle: 2; EM: 1), while an additional nine (5%) patients developed either secondary AML/MDS, had induction failures or died in remission. A report by the Children's Cancer Group 1900 series ALL studies suggested that relapses in patients with triple trisomies were primarily EM [14]. The Medical Research Council (MRC) United Kingdom Acute Lymphoblastic Leukemia XI (UKALL) group reported the outcome of 807 children with hHDALL, though did not elaborate on the relapse characteristics [13]. The 5-year OS for this cohort was 91% (95% CI, 88–93%). A recent review of the BFM (Berlin-Frankfurt-Muenster) ALL studies also identified that the majority of relapses occurred in good risk patients [15]. The present study has shown that hHDALL had a trend towards EM relapses ($P = 0.053$) compared with non-hHDALL. However, this analysis is limited by a large difference in sample size between two groups and selection bias towards non-HD group, as children who did not have high hyperdiploid cytogenetics formed the non-HD group. Limited sample size of hHDALL group has further limited the ability to do meaningful statistical analysis. Above all, the prognostic role of TEL/AML1 translocation on hHDALL outcome was not examined, as the diagnostic screening for this translocation had only been performed in the more recent era of POG studies and not in earlier studies. Since a large proportion of the patients from the current study were diagnosed and enrolled on earlier POG studies, the present study did not have ability to evaluate if TEL/AML1 protects against adverse outcome.

Male sex and older age were associated with an adverse outcome in the current study, while triple trisomies of +4/+10/+17 was protective against relapse. The influence of older age and gender as a predictor of poor outcome has been reported already in the MRC UKALL XI study [13]. This study has shown that children younger than age 10 years with both +4 and +10 had an improved survival compared with those older than 10 years with both +4 and +10 (96% vs. 84% at 5 years, $P < 0.0001$) [15]. These findings imply that male gender and older (> 10 years) children might benefit from more intensive chemotherapy regimens as currently used in frontline COG ALL protocols while young children with triple trisomy of +4,+10,+17 can be treated with low intensity chemotherapy regimens. The salvage therapy used in relapsed cases was similar between hHDALL and non-hHDALL cases, including the proportion of patients who underwent allogeneic bone marrow transplant in our study. Despite this, the OS was significantly higher in the hHDALL compared with non-hHDALL cases due to a high successful salvage rate in hHDALL cases. This finding may suggest that the mechanism of

relapse in hHDALL cases may not entirely be related to drug resistance.

Since hyperdiploid blasts are sensitive to cell cycle specific drugs and antimetabolites viz. methotrexate and 6-MP [16–18], the relationship between anti-metabolite dose intensity/therapy adherence and relapse was analyzed. Although the chemotherapy protocols have changed over the decade, there was no difference in cumulative antimetabolite treatment intensity and treatment interruptions between hHDALL patients who relapsed and those who remained in first complete remission [data not shown], further supporting the hypothesis that the relapse mechanisms in hHDALL maybe unrelated to anti-metabolite drug resistance in the majority of cases.

It is interesting to note that earlier studies during an era of evolution of ALL therapy from our own institution reported a lower incidence of testicular relapse [19]. The important component of leukemia therapy in these legacy studies included a repetitive reinduction strategy with VCR/ PDN along with oral 6-MP and methotrexate every 10 weeks over 5 years [19]. An early study from St. Jude Children's Research Hospital, showed a lower incidence of testicular relapse in regimens that included frequent VCR/PDN pulses [20]. A recent report from Children's Cancer Study Group (CCSG) further underscores the importance of repetitive courses of VCR/PDN for maintaining durable remissions [21]. In the CCG-161 study, 631 children with ALL at low risk for relapse were randomized to receive monthly pulses of VCR/PDN during maintenance therapy in addition to standard therapy with 6-MP and methotrexate, and either cranial irradiation during consolidation or intrathecal methotrexate every 3 months during maintenance. This study showed a superior 5-year relapse-free survival for the children who were randomized to receive VCR/PDN irrespective of randomization to cranial irradiation during consolidation (76.7% vs. 63.9%; $P = 0.002$). The important reason for the difference in relapse-free survival was primarily due to less bone marrow relapses ($P = 0.0008$), and in boys, due to fewer testicular relapses ($P = 0.003$). These observations may imply that repetitive courses of VCR/PDN may be protective against both hematopoietic and testicular relapse. In contrast, several standard/low risk POG protocols did not incorporate VCR/PDN pulses during maintenance therapy, which may have contributed to the higher EM relapses observed in our study. In the present study, relapse free survival was not statistically different between those who received VCR/PDN pulses versus those who did not. However, it is interesting to note that among five children who had testicular involvement at relapse, three (60%) did not receive VCR/PDN pulses during maintenance therapy (Table II). This finding may support the potential benefit of this treatment strategy in preventing testicular relapse in hHDALL. Hence, based on the literature experience, the protective efficacy of VCR/PDN pulses in preventing testicular relapse needs further exploration in clinical and pharmacologic studies in children with hHDALL.

Our study suggests that the biology of high hyperdiploid and non-high hyperdiploid lymphoblasts is different. Although the majority of hHDALL blast cells are in S-phase, paradoxically they have greater propensity to undergo apoptosis [22]. Since chemotherapy medications achieve higher concentrations in the bone marrow, blast cell populations in the bone marrow can potentially be eradicated. However, blast cells localized in EM sites like testis or CNS may not be eradicated easily as chemotherapy drugs cannot achieve adequate cytotoxic concentrations due to the so called "blood-brain barrier" or "blood-testicular barrier." In addition, it has been shown that intra-testicular biochemical regulation is known to be capable of decreasing the proliferation of leukemic cells [23]. Since most of the cytotoxic drugs used in the treatment of ALL have a poor effect on nondividing cells, these nondividing "hidden" leukemia cells may not be eliminated by chemotherapy. The blast cells at sanctuary sites retain their ability of uncontrolled proliferation to produce locally invasive disease once they escape immune surveillance mechanisms. As the majority of EM relapses occurred within 6 months of completion of therapy and 80% of those children responded to second line therapy, this may indicate that the original leukemia cells were transiently suppressed at EM sites without altering the primary biology of the disease.

The fundamental question is, why there are differences in relapses at sanctuary sites between hHDALL and non-hHDALL cases. One potential mechanism could be related to the role of chemokines especially stromal cell-derived factor-1 (SDF-1) and tissue specific expression of its receptor CXCR4 and its influence on lymphoblast trafficking [24]. SDF1 plays an important role in hematopoiesis, development, and organization of the immune system [25]. There is evidence that CXCR4 receptors are uniformly expressed at high density on all B-precursor lymphoblasts and bone marrow stromal cells, explaining bone marrow involvement in ALL [24–26]. Recent animal studies have shown that these receptors are also expressed in the testis and the CNS [27,28]. Liu et al. have shown the increased expression of CXCR4 receptors on leukemia blasts with EM relapses [29]. Hence, further clarification on CXCR4 expression on hHDALL blasts may shed more light in predicting EM relapses in hHDALL.

In conclusion, biological differences exist between relapse patterns of hHDALL and non-hHDALL cases related to relapse sites and time periods when relapses occur. Despite relapses, hHDALL continues to be responsive to second line therapy with high overall survival rates.

Patients and Methods

Patient selection

All consecutive children greater than 1 year of age with newly diagnosed B-precursor ALL at CHM from January 1991 to December 2002 were included in this retrospective cohort study. The diagnosis of ALL was based on standard morphological, cytochemical, and immunophenotype criteria. The DI was determined by flow-cytometry as the ratio of DNA content in leukemic cells G0/G1 cells versus normal G0/G1 lymphocytes. Karyotype and immunophenotype analysis of leukemic cells was performed at our institution, while the DI was analyzed in the Pediatric Oncology Group/Children's Oncology Group ALL Biology Reference Laboratory. Children whose karyotype revealed a MCN of 51–65 were included in the hHDALL group, while all the remaining children without a high hyperdiploid karyotype were included in the non-hHDALL group. This retrospective review was approved by the Wayne State University Human Investigation Committee.

Treatment details

All children were enrolled on Pediatric Oncology Group (POG) protocols and were classified as low-risk, standard-risk, and high-risk for ALL based on their age at diagnosis, WBC count at diagnosis, and leukemia cytogenetic subgroups as outlined by cooperative group protocol criteria according to the treatment era they were being treated in. The low-risk protocols included POG 9201, 9005, and 9904, the standard-risk protocols included POG 9202, 9405, 9605, and 9905 and the high-risk protocols included 9006, 9203, 9406, and 9906. Concise information of these protocols have been published earlier [30] and detail information is available at http://www.acor.org/ped-onc/diseases/ALL. All hHDALL patients in this study were treated with either low-risk or standard-risk protocols except for two teenage patients who received induction with high-risk protocol. All treatment schedules included a phase of induction (weeks 1 to 4), consolidation phase (weeks 5 to 24/33) and maintenance phase (weeks 25/34-130). In general, three-drug induction therapy (vincristine, L-asparaginase, and prednisone [PDN]/or dexamethasone) was used for the low or standard risk patients. Two teenage patients in the hHDALL group received induction therapy

which also included daunorubicin according to the high-risk protocol based on their age (>10 years) at diagnosis and were subsequently treated on standard-risk protocols for consolidation and maintenance therapy. Central nervous system (CNS) directed therapy included intravenous methotrexate (1–2.5 g/m^2), with intrathecal methotrexate or triple therapy. All treatment protocols incorporated maintenance therapy with daily 6-mercaptopurine (6-MP) and weekly methotrexate (oral or intramuscular), intrathecal chemotherapy and vincristine (VCR)/corticosteroid pulses every 8–16 weeks except for the POG 9005 and 9405 protocols.

Statistical analysis

The primary end point of the study was event free survival (EFS). An "event" was defined as induction failure, death due to any etiology (e.g., infection) or disease relapse at any site. The EFS was calculated from the date of diagnosis to the date of first event or last follow-up whichever occurred first. Overall survival (OS) was defined as time interval between date of diagnosis to the last follow up. Kaplan–Meier life tables and survival curves were constructed using the log-rank method [31]. The log-rank test was used to test the independent influence of the following variables on the EFS: sex, race, and immunophenotype. Patterns of relapse in hHDALL were compared with non-hHDALL children diagnosed during the same period. To assess the differences between the groups, χ^2 or Fisher's exact test was used for the categorical data and t-tests were used for continuous data. A P value < 0.05 was considered significant to confirm the association between variables. Analyses were performed using SPSS version 13 statistical software.

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