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Three distinct subgroups of hypodiploidy in acute lymphoblastic leukaemia

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Summary

This study of children and adults with acute lymphoblastic leukaemia (ALL) is the largest series of patients with hypodiploidy (<46 chromosomes) yet reported. The incidence of 5% was independent of age. Patients were subdivided by the number of chromosomes; near-haploidy (23–29 chromosomes), low hypodiploidy (33–39 chromosomes) and high hypodiploidy (42–45 chromosomes). The near-haploid and low hypodiploid groups were characterized by their chromosomal gains and a doubled hyperdiploid population. Structural abnormalities were more frequent in the low hypodiploid group. Near-haploidy was restricted to children of median age 7 years (range 2–15) whereas low hypodiploidy occurred in an older group of median age 15 years (range 9–54). Patients with 42–45 chromosomes were characterized by complex karyotypes involving chromosomes 7, 9 and 12. The features shared by the few patients with 42–44 chromosomes and the large number with 45 justified their inclusion in the same group. Survival analysis showed a poor outcome for the near-haploid and low hypodiploid groups compared to those with 42–45 chromosomes. Thus cytogenetics, or at least a clear definition of the modal chromosome number, is essential at diagnosis in order to stratify patients with hypodiploidy into the appropriate risk group for treatment.

Keywords: acute lymphoblastic leukaemia, cytogenetics, hypodiploidy, event free survival.

In spite of continually improving event-free (EFS) and overall survival (OS) for acute lymphoblastic leukaemia (ALL), particularly in children, a number of patients on current therapies will relapse and die. The challenge remains of how to accurately identify these patients in order to classify them into the appropriate risk group for treatment. In addition to age, white blood cell count (WBC) and early response to therapy, cytogenetics has been shown to be an independent risk factor in both childhood and adult ALL (Chessels *et al*, 1997; Secker-Walker *et al*, 1997). Changes in chromosome number have been linked to outcome, with high hyperdiploidy (51–65 chromosomes) related to a good prognosis and hypodiploidy of <46 chromosomes regarded as an adverse risk factor (Secker-Walker *et al*, 1978; Pui *et al*, 1987; Trueworthy *et al*, 1992; Heerema *et al*, 2000).

Hypodiploidy is a rare finding occurring in approximately 6% of ALL patients, of which the majority (80%) have 45 chromosomes [Third International Workshop on Chromosomes in Leukemia (IWCL3), 1981, 1983; Pui *et al*, 1987]. This indicates a small proportion of cases with <45 chromosomes, which have been subdivided in various ways (Callen *et al*, 1989; Pui *et al*, 1990; Heerema *et al*, 1999; Raimondi *et al*, 2003). Earlier studies reported a particularly poor outcome for children with near-haploidy (23–29 chromosomes) (Gibbons *et al*, 1991; Chessels *et al*, 1997). Survival analysis carried out on a cohort of children with ALL treated on the Children's Cancer Group (CCG) intensive protocols concluded that patients with hypodiploidy had a progressively worse outcome with decreasing chromosome number (Heerema *et al*, 1999), thus raising the possibility that subsets of hypodiploid patients have different treatment outcomes.

This study of children and adults treated according to the UK ALL trial protocols presents the largest single series of ALL patients with hypodiploidy so far reported. It has permitted us to redefine cases with <46 chromosomes into three distinct subgroups according to patterns of chromosomal abnormalities, age distribution and other clinical features and so determine the relationship to outcome.

Patients and methods

Patients

Patients in this study with a hypodiploid karyotype, defined as 45 chromosomes or less, were identified among a series of children and adults with a diagnosis of ALL entered to one of the Medical Research Council (MRC) ALL treatment trials: Infant 92 protocol, for infants up to 1 year of age (January 1992 to July 1999), UKALL XI and subsequently ALL97 for children aged 1–18 years inclusive (October 1990 to June 2002), or UKALL XII for adults aged 15–55 years (June 1993 to present). In this study adolescents (15–18-years old) were categorized either as children or adults according to the trial on which they were treated. Cell counts, immunophenotyping and morphological classification were carried out in the local referral centres. All data, including follow-up information, were collected by the Clinical Trial Service Unit (CTSU), Oxford. Informed consent for cytogenetic analysis was obtained from the patients or their guardians.

Cytogenetics

Cytogenetic analysis of diagnostic bone marrow or peripheral blood samples was carried out in the UK regional cytogenetics laboratories. The G-banded slides were reviewed at the Leukaemia Research Fund UK Cancer Cytogenetics Group (UKCCG) Karyotype Database in Acute Leukaemia (Harrison *et al*, 2001). Karyotypes were described according to the International System of Human Cytogenetic Nomenclature (ISCN, 1995). The one modification to this system was to describe the karyotypes of patients with 30–39 chromosomes as gains to the haploid chromosome complement (1n) rather than losses from the diploid one (2n). Fluorescence *in situ* hybridization (FISH) studies were carried out on a number of patients as part of an interphase FISH screening programme to search for chromosomal abnormalities of prognostic significance, as previously described (Harrison *et al*, 2001; Harewood *et al*, 2003). This included testing for the presence of three specific abnormalities, the *TEL/AML1* and *BCR/ABL* fusions and rearrangements involving the *MLL* gene. In addition, hidden numerical chromosomal changes were searched for in patients with a normal karyotype and a failed cytogenetic result using the Chromoprobe Multiprobe-I^{TM} system (Multiprobe-I) (Cytocell, Banbury, UK) as described elsewhere (Jabbar Al-Obaidi *et al*, 2002). Metaphase FISH studies were used to clarify the G-banded karyotypes in a small number of cases: Multiplex-FISH (M-FISH) on four samples (patients 3754, 4120, 4949 and 5639); whole chromosome paints and/or specific probes on four samples (patients 2402, 2616, 3676 and 4471).

Survival analysis

Event-free survival was defined as the time from diagnosis to either relapse, death or second tumour, and included those patients who did not enter complete remission. Continuous complete remission was measured up to the most recent patient contact. Those patients who did not suffer an adverse event were censored at the time of last contact. Survival analysis by means of Kaplan–Meier life tables was performed on patients entered to the childhood trials, UKALL XI and ALL97, and separately for the adult trial, UKALL XII, according to the log rank method described by Peto *et al* (1977), using Intercooled Stata v7.0 (Stata Corporation, TX, USA).

Results

Incidence

A total of 226 patients with hypodiploidy of 45 chromosomes or less were identified among 3534 childhood and adult patients with a successful cytogenetic result, treated on MRC ALL treatment protocols over a period of 12 years. Among them, 46 patients known to have established chromosomal abnormalities [t(12;21)(p13;q22) (23 patients), t(9;22)(q34;q11) (14 patients), t(1;19)(q23;p13) (five patients), 11q23 rearrangements (three patients), t(10;14)(q24;q11) (one patient)] were excluded from further study. The remaining 180 patients showed an overall incidence for hypodiploidy of 5%, which appeared to be independent of age [infants <1 year, four of 94 (5%); children 1–14 years, 140 of 2872 (5%); adults ≥15 years, 36 of 568 (6%)].

Comparison of the patient karyotypes indicated three distinct hypodiploid subgroups: 28 patients, 14 (8%) in each group, had 25–29 and 33–39 chromosomes, defined as near-haploid and low hypodiploid respectively; 152 patients had 42–45 chromosomes (high hypodiploidy) of which 144 (80%) had 45 chromosomes and eight patients (4%) 42–44 chromosomes. There were no patients with 30–32, 40 or 41 chromosomes. A further 209 childhood and adult patients from the trial series with a normal or a failed cytogenetic result were tested with Multiprobe-I, which did not reveal any near-haploid or low hypodiploid clones in interphase cells.

Cytogenetics and clinical features

Table I shows the clinical, laboratory, cytogenetic and survival data for the 36 patients with <45 chromosomes in this study.

25–29 Chromosomes

Among the 14 near-haploid patients, nine had 26 chromosomes. The most frequent additions to the haploid chromosome set were of chromosomes 21 (all patients), X (six of eight females), Y (four of six males), 14 (12 patients) and 18 (seven patients). Nine patients had an accompanying population of hyperdiploid cells with double the chromosome number, which proved to be a duplication of the near-haploid cell line. In patient 4601, although there was only one hyperdiploid cell by cytogenetics, there was underlying evidence from interphase FISH of a 25% population with this chromosome number. These 14 patients had a median age of 7 years (range 2–15 years). They had common/pre-B immunophenotypes and all but four of them had a low WBC count of $<50 \times 10^9$/l.

33–39 Chromosomes

The 14 patients with a low hypodiploid chromosome complement tended to be older than the near-haploids, with a median age of 15 years (range 9–54 years), 11 of them being in the range 9–20 years. All patients in this subgroup had a common/pre B immunophenotype, apart from one described as 'Null' and all but one of them had a low WBC count of $<50 \times 10^9$/l.

The pattern of chromosomal gains was, not surprisingly, distinct from that of the near-haploid cases, except for additions to the haploid set of chromosomes 21 (all patients), X (six of seven females), Y (four of seven males), 14 (eight patients) and 18 (10 patients), which the two groups had in common. Other chromosomes for which second copies were most frequently observed were chromosomes 1, 11 and 19 (all patients); 10 and 22 (13 patients); 5, 6 and 8 (10–12 patients); 2 and 12 (eight patients), while two copies of chromosomes 3, 4, 13, 15, and 16 were seen in one or two cases only. Chromosomes 7 and 17 were always monosomic. In common with the near-haploid group, nine patients showed cytogenetic evidence of a population with duplication of the hypodiploid chromosome number. Although this was observed by cytogenetics in only a single cell in two cases (1050, 4574), FISH confirmed the same chromosomal gains among the non-dividing cells. Structural abnormalities occurred in eight cases in this group as compared to the single near-haploid case in which they were observed.

Case 4471 was unusual in that the karyotype, shown in Table I, was seen at relapse rather than at diagnosis, when the cytogenetics was normal. However, interphase FISH testing of the diagnostic sample, with the commercial probes routinely employed for the detection of prognostically significant gene fusions, revealed the presence of additional copies of the *ABL, MLL, TEL, AML1* and *BCR* genes, indicating gains of chromosomes 9, 11, 12, 21 and 22 respectively in over 50% of interphase nuclei. Further testing with Multiprobe-I revealed a pattern of centromere gains consistent with a duplication of the low hypodiploid clone of relapse. A further observation in this case was monosomy for the centromeres of chromosomes 7, 15 and 17 in approximately 20% of the interphase nuclei in the diagnostic sample. This was reflected in the low hypodiploid karyotype of the relapse sample and was consistent with the tendency to monosomy for these three chromosomes, seen in the other cases in our series. This provides evidence for a low hypodiploid clone in a small population of cells at diagnosis.

42–45 Chromosomes

A total of 153 patients were clustered within a high hypodiploid group having 42–45 chromosomes. They were clinically and cytogenetically distinct from the near-haploid and low hypodiploid groups. The age range was 1–52 years with a similar incidence across all ages. The group included patients with T-lineage ALL in addition to those with pre-B/common immunophenotypes. The overall reduction in chromosome number in this group was attributable either to whole chromosome loss (monosomy) (77 patients), or to unbalanced translocations resulting in a dicentric chromosome (dicentric)

Table I. Age, sex, outcome and karyotype for 36 patients with <45 chromosomes.

Patient	Age	Sex	WBC	Immuno-phenotype	EFS	Karyotype
23–29 Chromosomes						
4184*	7	M	135	Com/pre-B	29+	25<1n>,X,+Y,+21[9]/50,idem,x2[3]
105*	2	F	18	Common	11	26<1n>,X,+8,+18,+21[17]/52,idemx2[5]
1167*	3	F	48	Common	24	26<1n>,X,+14,+18,+21[22]
2764*	15	M	41	Com/pre-B	17	26<1n>,X,+14,+18,+21[3]/52,idemx2[8]
1601*	12	F	35	Common	14	26<1n>,X,+X,+14,+21[7]
3056*	5	F	27	Com/pre-B	55+	26<1n>,X,+X,+14,+21[7]/52,idemx2[6]
3256*	6	F	116	Com/pre-B	12	26<1n>,X,+X,+14,+21[28]/52,idem,x2[19]
3897*	3	F	172	Com/pre-B	11	26<1n>,X,+X,+14,+21[5]/52,idemx2[4]
3959*	8	M	243	Com/pre-B	38+	26<1n>,X,+Y,+14,+21[23]
4120*	14	M	NA	NA	NA	26<1n>,X,+Y,+del(14)(q?),+21[3]/27,idem,+14,-del(14)(q?),+9[3]/52,idemx2,+14,-del(14)(q?)[3]
2888*	3	M	15	Com/pre-B	19	27<1n>,X,+Y,+14,+18,+21[6]
5613*	10	M	16	Com/pre-B	6+	27<1n>,X,+8,+14,+18,+21[8]
4601*	6	F	5	Com/pre-B	18+	28<1n>,X,+X,+10,+14,+18,+21[8]/56,idemx2[1]
2402*	10	F	4	Common	25	29<1n>,X,+X,add(1)(q4),+10,t(10;14)(q21;q11),+14,+15,+18,+21[8]/58,idem,x2[4]
30–39 Chromosomes						
3273†	42	F	2	Common	10	33<1n>,X,+1,+2,+6,+10,+11,+12,+18,+19,+21,+22[9]/66,idemx2[5]
4587*	12	M	13	Com/pre-B	4	33<1n>,X,+1,+5,+6,+8,+11,+14,+18,+19,+21,+22[4]/66,idemx2[5]
4949†	20	F	53	NA	4	33<1n>,X,+X,+1,+2,der(2)t(7;13;2)(p15;?;p11),+6,der(6)t(6;7)(q2;q22),+8,der(9)t(9;18)(p1;p1), +del(9)(p2),+10,+11,+12,dic(12;22)(p1;p1),der(12;14)(q10;q10),+14,+19,+21[8]
3676*	13	F	8	Com/pre-B	40+	34<1n>,X,+X,+1,+2,+6,+10,+11,+12,+18,+19,+21,+22[7]/72,idemx2[8]
2651*	11	M	4	Com/pre-B	13	35<1n>,X,+1,+5,+6,+8,+10,+11,+12,+14,+18,+19,+21,+22[7]
4574*	11	F	4	Com/pre-B	18+	35<1n>,X,+X,+1,+2,+6,+8,+10,+11,+12,+18,+19,+21,+22[3]/69,idemx2,-19[1]
1928†	51	F	12	Common	12	36<1n>,X,+X,+1,+4,+5,add(6)(p25),+8,+10,+11,+13,+14,+18,+19,+21,+22[8]/ 37,idem,-X,+add(X)(p22),add(19)(p13),+mar[7]/74,idemx2[13]
3108*	14	M	15	Com/pre-B	16	36<1n>,X,+Y,+1,+5,+6,+8,+10,+11,+14,+15,+18,+19,+21,+22[4]/72,idemx2[7]
4416*	15	M	6	Com/pre-B	23	36<1n>,X,+Y,+1,+5,+6,+8,+9,+10,+11,+15,+18,+19,+21,+22[2]/36,idem,del(6)(q13q15)[8]/ 68,idemx2[6]
923†	15	M	22	Common	110+	38<1n>,X,+Y,+1,+2,+4,add(4)(q21),+5,add(5)(q13),+6,+8,+10,add(10)(p?), +11,+12,+14,+18,+19,+21,+22[23]
4442*	9	F	4	Com/pre-B	20+	38<1n>,X,+X,+1,add(3)(q2),+del(4)(q2q3),+5,+6,+8,+9,+10,+11,der(13;13)(q10;q10), +14,+19,+20,+21,+22,+mar[5]
1050†	17	M	7	Null	8	39<1n>,+1,der(1)t(1;1)(p13;q21),+2,+4,+5,+6,+9,+10,+11,+11,+12,+13,+19, +20,+21,idic(21)(p11),+22,+mar1[5]/40,idem,+mar2[3]/78,idemx2[1]
4471†	54	M	3	Pre-B	18	39<1n>,X,+Y,+1,+2,+3,+5,+8,+9,+10,+11,+12,+19,+21,+22,+mar1,+mar2,+mar3[cp5]‡
5639†	18	F	2	Common	5+	39<1n>,X,+X,+1,dup(1)(q21q32),+2,+5,+6,+8,+9,der(9)del(9)(p11p24)del(9)(q22q34),+10/ der(10)t(4;10)(p?;q2),+11,t(13;15)(q3;q24),+14,+16,+18,+19,+20,+21,+22[5]/39,idem,+1,-dup(1) (q21q32), der(6)t(6;15)(p2;q26),-der(13)t(13;15)(q3;q24),+der(13)t(13;15)t(6;15)[11]/78,idem,x2[2]/78, idemx2,+1,+1,-dup(1)(q21q32)x2,der(6)t(6;15)(p2;q26)x2,-der(13)t(13;15)(q3;q2?4)x2, +der(13)t(13;15)t(6;15)x2[1]
42–44 Chromosomes						
3977*	7	M	103	T-Cell	9	42,XY,del(1)(p22p36),-4,der(7;9)(q10;q10),del(8)(p2p2),del(9)(p1p2), dic(12;17)(p11;p11),-14[3]
660*	4	F	5	T-Cell	12	43,X,-X,der(1;12)(p10;q10),-9,add(16)(q24)[5]
4813†	26	M	4	T-Cell	13	43,X,-Y,add(3)(q?),-4,add(6)(q27),del(6)(q13q25),-8,add(9)(q22),-9,-12,-13, add(17)(p1),add(18)(q2),add(21)(p1),-22,+mar1,+mar2,+mar3,+mar4[10]
47*	6	M	7	Com/pre-B	38	44,XY,der(3)t(3;15)(p14;q13),del(6)(q15q25),add(7)(p13),add(9)(p13), t(12;17)(p11;q21),-15,-21[3]
375*	6	F	4	Common	116+	44,inc[7]§
464*	7	F	43	Common	98+	44,-X,add(X)(p1),del(2)(p1),-12,add(12)(p?)[5]/44,idem,add(9)(p?)[3]/45,idem,add(9)(p?), add(5)(p?)[5]/45,idem,+add(12)(p?),-13,+mar[2]

Table I. *continued*

Patient	Age	Sex	WBC	Immuno-phenotype	EFS	Karyotype
2616*	14	M	2	Com/pre-B	8	44,XY,der(4;17)(p10;q10),dic(9;12)(p11;p11)[4]/45,idem,+der(17;21)(q10;q10)[8]
3754*	1	M	4	Com/pre-B	40+	44,XY,der(2)t(2;17)(p13;q11),der(6)t(6;8)(q2;q2),-8,dic(9;17)(p1;q11),-17, +der(13)t(2;13)(p13;q11)[4]

Age in years; M, male; F, female; WBC, white blood cell count $\times 10^9$/l; Com, Common; pre-B, pre-B immunophenotype; NA, not available; EFS, event-free survival (months); +, still in first remission.

*Treated on one of the childhood trials, UKALL XI or ALL97.

†Treated on the adult trial, UKALL XII.

‡Relapse karyotype.

§Chromosome count only.

(76 patients). In the majority of patients (95%) the karyotypes were complex. Only nine patients with monosomies had no visible evidence of structural abnormalities. The most frequent whole chromosome losses were of a sex chromosome (25 patients) and of chromosomes 7, 9 and 13 in 17, eight and seven patients respectively. Chromosome 9 was the one most often involved in the formation of dicentrics (50 patients), among which a dic(9;20)(p11∼13;q11) was the most common rearrangement (25 patients). Chromosomes 7 and 12 were the next most frequently involved in the formation of dicentrics, in 14 and 10 patients respectively. The most commonly observed deletions were of the short arms of chromosomes 9, 12 and 7 in 84, 38 and 33 patients respectively. Populations with duplication of the hypodiploid chromosome number were not seen.

As other studies have segregated patients with <45 from those with 45 chromosomes, the eight patients in this study with 42–44 chromosomes were examined separately, as shown in Table I. Their chromosomal changes appeared to have more in common with the 45 chromosome group than with those with <40 chromosomes.

Patient follow up

Follow-up data was available for a total of 141 patients (1–18-years old) with hypodiploidy treated on UKALL XI and ALL97 (Fig 1). The survival of children with 25–39 chromosomes was extremely poor, with a 3-year EFS of only 29% [95% confidence interval (CI): 10–53%]. Among the near-haploid childhood patients, there was a single, long-term, event-free survivor at 55 months, as indicated in Table I. Of the seven patients with 33–39 chromosomes treated on the childhood trials, four had relapsed within two years of diagnosis. The presence of a duplicated hyperdiploid population did not appear to have an effect on EFS.

The 3-year EFS for children with 42–45 chromosomes was 66% (95% CI: 56–74%, $P = 0{\cdot}0002$) as shown in Fig 1. No difference in survival was noted between the monosomic and dicentric subgroups of these children (data not shown). The EFS of the seven children with 42–44 chromosomes was highly

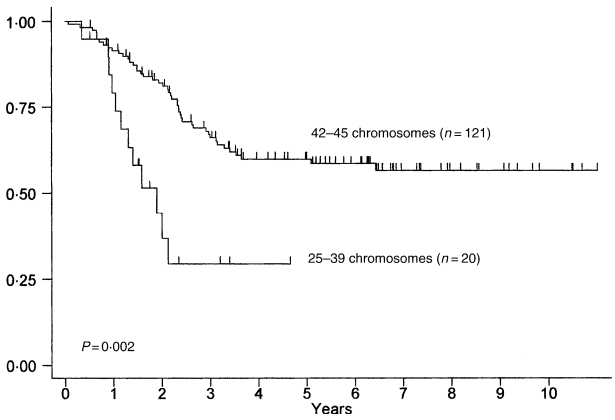

Fig 1. Event-free survival of 141 children with hypodiploid acute lymphoblastic leukaemia by modal chromosome number.

variable. Three remained in long-term remission, one relapsed at 38 months while three patients relapsed within a year of diagnosis. Three patients in this group with T-cell ALL (T ALL) died within 13 months.

Follow up data was available for 32 patients with hypodiploidy entered to UKALL XII. An extremely poor outcome was indicated for those with <40 chromosomes. Five of the seven patients in the low hypodiploid group treated on the adult trial relapsed within 2 years of diagnosis (Table I). The only long-term event-free survivor in this group (110 months) received an autologous bone marrow transplant. The 5-year EFS for the 25 adults with hypodiploidy of 42–45 chromosomes was 45% (95% CI: 27–61%) with a median follow-up of 70 months (6–111 months).

Discussion

This large consecutive series of children and adults from MRC treatment trials confirmed the rarity of hypodiploidy in ALL. The consistent incidence across all age groups was demonstrated for the first time in this study, as other reports included only children (Pui *et al*, 1990; Heerema *et al*, 1999; Raimondi *et al*, 2003). Based on chromosome number and characteristic

cytogenetic features, the patients clustered into three distinct subgroups: near-haploidy, low hypodiploidy and high hypodiploidy. Patients with near-haploidy showed common chromosomal gains, rare structural abnormalities and a co-incident doubled hyperdiploid population, which compared with 43 previously reported near-haploid cases with complete karyotypes (Gibbons *et al*, 1991; Mitelman *et al*, 2003; Raimondi *et al*, 2003). Our study has provided further evidence to define near-haploidy as a unique patient group.

Previous reports of patients with 30–39 chromosomes are rare, with the karyotypes of only 31 cases described in the literature (Callen *et al*, 1989; Pui *et al*, 1990; Heerema *et al*, 1999; Mitelman *et al*, 2003; Raimondi *et al*, 2003). Consistent numerical chromosomal changes demonstrated that this group was distinct from near-haploidy. This evidence originally came from a small study of seven patients described as 'severe hypodiploidy' (Callen *et al*, 1989). Our study confirmed that this low hypodiploid group showed a number of chromosomal gains onto the haploid chromosome set in common with the near-haploid patients, but with additional gains specific to them. There were significantly more patients with structural chromosomal abnormalities than in the near-haploid group, as also noted by others (Pui *et al*, 1990).

In common with previous reports, the majority of patients with low hypodiploidy in our series showed cells with doubling of the chromosome number. Cytogenetic evidence has indicated that endoreduplication is the mechanism responsible for this related population (Pui *et al*, 1990; Gibbons *et al*, 1991; Ma *et al*, 1998). Routine interphase FISH screening for numerical chromosomal abnormalities in patients with a normal or a failed cytogenetic result did not reveal any hidden near-haploid or low hypodiploid clones. This confirms that these abnormalities are indeed as rare as they have been proved to be by cytogenetic studies (Pui *et al*, 1990; Chessels *et al*, 1997; Heerema *et al*, 1999). However, it has previously been shown that apparent hyperdiploidy, manifesting with a doubling of gained chromosomes, may be masking a near haploid or low hypodiploid clone (Stark *et al*, 2001). The one case in our series, in addition to three near-haploid cases detected only at relapse by retrospective FISH analysis (Stark *et al*, 2001), emphasizes the need for accurate diagnosis of near-haploidy and low hyperdiploidy by FISH and/or flow cytometric analysis of DNA content (Raimondi *et al*, 2003).

These two groups of patients with <40 chromosomes showed different age distributions. Near-haploidy was restricted to childhood patients, as previously noted (Gibbons *et al*, 1991), while patients with low hypodiploidy included adults and children ≥9-years old. The majority were clustered between 9–20 years, as observed in 29 previously reported cases with ages provided (Callen *et al*, 1989; Pui *et al*, 1990; Mitelman *et al*, 2003; Raimondi *et al*, 2003). Patients with low hypodiploidy were originally described as having 'age restricted leukaemia': the male predominance seen in this small series of six patients has not been subsequently confirmed (Callen *et al*, 1989).

Hypodiploidy is rare in both childhood and adult ALL. Our study, the largest to date, results from karyotype collection over a 12-year period, during which time period the patients were treated on different protocols. To minimize the effect of different treatments and any selection bias arising from the low recruitment to adult trials, formal survival analysis was restricted to the two consecutive childhood trials. These trials were sufficiently similar to allow their outcome data to be combined (Hann *et al*, 2000). In order to examine the effects of hypodiploidy on survival, patients with established chromosomal changes, some with known prognostic associations, were excluded from our study. Such patients were included in the earlier childhood studies (Pui *et al*, 1990; Heerema *et al*, 1999; Raimondi *et al*, 2003). Children in both the near-haploid and low hypodiploid groups had an extremely poor outcome. These findings were validated by their agreement with previous reports (Pui *et al*, 1990; Heerema *et al*, 1999; Raimondi *et al*, 2003). Furthermore our study indicated a similar poor outcome for the few patients with <40 chromosomes treated on the adult trial.

The karyotypes of the high hypodiploid group with 42–45 chromosomes differed significantly from the two groups with <40 chromosomes. They had a high incidence of complex chromosomal abnormalities, with preferential involvement of chromosomes 7, 9 and 12 and no duplicated hyperdiploid population, as previously observed (Pui *et al*, 1990; Heerema *et al*, 1999). In agreement with Heerema *et al* (1999) there was no obvious association between the type of chromosomal abnormality and outcome in this group of patients. The 3-year EFS for children with 42–45 chromosomes from UKALL XI and ALL97 was 66%. This compares to an overall 5-year EFS of 63% (95% CI: 61–65%) for the 2090 childhood ALL patients analysed from UKALL XI, which included all chromosomal abnormalities as well as normal and failed cytogenetic results (Hann *et al*, 2001). The 5-year EFS of 45% for adults with 42–45 chromosomes in this series compares favourably with the poor overall 5-year EFS of 28% (95% CI: 23–32%) reported for adult patients from all cytogenetic subgroups entered to the previous UK adult trial, MRC UKALL XA (Secker-Walker *et al*, 1997).

Patients with 40–44 chromosomes are extremely rare. Pui *et al* (1990) initially classified them separately. However, in an updated series from the St Jude Children's Research Hospital (Raimondi *et al*, 2003), they were combined with the low hypodiploid group, as were those from another series from the CCG study (Heerema *et al*, 1999). The reported patients with 42–44 chromosomes (Pui *et al*, 1990; Heerema *et al*, 1999; Raimondi *et al*, 2003) appeared to have similar cytogenetic features and the same variable EFS as those with 45 chromosomes. It thus seemed more appropriate to us to put our cases with 42–44 chromosomes in the high hypodiploid group. The variable survival among patients with 42–44 chromosomes may be linked to features other than cytogenetics, as for example, the three patients with T ALL and a particularly poor prognosis, may represent a rare but genuine, biological entity.

Despite the rarity of cases with hypodiploidy, a picture is beginning to emerge. There are three distinct hypodiploid subgroups in ALL, defined by chromosome number, which are related to prognosis. A consistently poor outcome was observed in the two subgroups that included patients with <40 chromosomes. At the present time, cytogenetic analysis in adult and childhood ALL, at least to determine chromosome number, is vital to ensure that patients with <40 chromosomes are accurately identified and classified into the appropriate high risk group for treatment. However, patients with 42–45 chromosomes appear to be heterogeneous, both in terms of karyotypic abnormalities and clinical features. For the time being the variable outcome within this group translates into an overall standard risk. Further collaborative studies of the rare 40–44 chromosome group will clarify their relationship to those patients with 45 chromosomes. Additional contributing factors, possibly at the genetic level, need to be sought to accurately classify these patients in terms of outcome and improve the understanding of their therapeutic requirements.

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References

Callen, D.F., Raphael, K., Michael, P.M. & Garson, O.M. (1989) Acute lymphoblastic leukemia with a hypodiploid karyotype with less than 40 chromosomes: the basis for division into two subgroups. *Leukemia*, **3,** 749–752.

Chessels, J.M., Swansbury, G.J., Reeves, B., Bailey, C.C. & Richards, S.M. (1997) Cytogenetics and prognosis in childhood lymphoblastic leukaemia: results of MRC UKALL X. Medical Research Council Working Party in Childhood Leukaemia. *British Journal of Haematology*, **99,** 93–100.

Gibbons, B., MacCallum, P., Watts, E., Rohatiner, A.Z.S., Webb, D., Katz, F.E., Secker-Walker, L.M., Temperley, I.J., Harrison, C.J., Campbell, R.H.A., Nash, R., Broadbent, V. & Chessells, J.M. (1991) Near haploid acute lymphoblastic leukemia: seven new cases and a review of the literature. *Leukemia*, **5,** 738–743.

Hann, I., Vora, A., Richards, S., Hill, F., Gibson, B., Lilleyman, J., Kinsey, S., Mitchell, C. & Eden, O.B. (2000) Benefit of intensified treatment for all children with acute lymphoblastic leukaemia: results from MRC UKALL XI and MRC ALL97 randomised trials. UK Medical Research Council's Working Party on Childhood Leukaemia. *Leukemia*, **14,** 356–363.

Hann, I., Vora, A., Harrison, G., Harrison, C., Martineau, M., Moorman, A.V., Secker Walker, L.M., Eden, O., Hill, F., Gibson, B. & Richards, S. (2001) Determinants of outcome after intensified therapy of childhood lymphoblastic leukaemia: results from Medical Research Council United Kingdom acute lymphoblastic leukaemia XI protocol. *British Journal of Haematology*, **113,** 103–114.

Harewood, L., Robinson, H., Harris, R., Jabbar Al-Obaidi, M.S., Jalali, G.R., Martineau, M., Moorman, A.V., Sumption, N., Richards, S., Mitchell, C. & Harrison, C.J. (2003) Amplification of AML1 on a duplicated chromosome 21 in acute lymphoblastic leukemia: a study of 20 cases. *Leukemia*, **17,** 547–553.

Harrison, C.J., Martineau, M. & Secker-Walker, L.M. (2001) The Leukaemia Research Fund/United Kingdom Cancer Cytogenetics Group Karyotype Database in acute lymphoblastic leukaemia: a valuable resource for patient management. *British Journal of Haematology*, **113,** 3–10.

Heerema, N.A., Nachman, J.B., Sather, H.N., Sensel, M.G., Lee, M.K., Hutchinson, R., Lange, B.J., Steinherz, P.G., Bostrom, B., Gaynon, P.S. & Uckun, F. (1999) Hypodiploidy with less than 45 chromosomes confers adverse risk in childhood acute lymphoblastic leukaemia: a report from the Children's Cancer Group. *Blood*, **94,** 4036–4046.

Heerema, N.A., Sather, H.N., Sensel, M.G., Zhang, T., Hutchinson, R.J., Nachman, J.B., Lange, B.J., Steinherz, P.G., Bostrom, B.C., Reaman, G.H., Gaynon, P.S. & Uckun, F.M. (2000) Prognostic impact of trisomies of chromosomes 10, 17, and 5 among children with acute lymphoblastic leukemia and high hyperdiploidy (>50 chromosomes). *Journal of Clinical Oncology*, **18,** 1876–1887.

ISCN (1995) *An International System for Human Cytogenetic Nomenclature*, 1 edn., pp. 1–114. S. Karger, Basel.

IWCL3 (1981) Third International Workshop on Chromosomes in Leukemia (1980). Chromosomal abnormalities in acute lymphoblastic leukemia: structural and numerical changes in 234 cases. *Cancer Genetics and Cytogenetics*, **4,** 101–110.

IWCL3 (1983) Third International Workshop on Chromosomes in Leukemia (1980). Chromosome abnormalities and their clinical significance in acute lymphoblastic leukemia. *Cancer Research*, **43,** 868–873.

Jabbar Al-Obaidi, M.S., Martineau, M., Bennett, C.F., Franklin, I.M., Goldstone, A.H., Harewood, L., Jalali, G.R., Prentice, H.G., Richards, S.M., Roberts, K. & Harrison, C.J. (2002) ETV6/AML1 fusion by FISH in adult acute lymphoblastic leukemia. *Leukemia*, **16,** 669–674.

Ma, S.K., Chan, G.C., Wan, T.S., Lam, C.K., Ha, S.Y., Lau, Y.L. & Chan, L.C. (1998) Near-haploid common acute lymphoblastic leukaemia of childhood with a second hyperdiploid line: a DNA ploidy and fluorescence in-situ hybridization study. *British Journal of Haematology*, **103,** 750–755.

Mitelman, F., Johansson, B. & Mertens, F. (2003) *Mitelman Database of Chromosome Aberrations in Cancer*. (WWW document). URL http://cgap.nci.nih.gov/Chromosomes/Mitelman.

Peto, R., Pike, M.C., Armitage, P., Breslow, N.E., Cox, D.R., Howard, S.V., Mantel, N., McPherson, K., Peto, J. & Smith, P.G. (1977) Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. *British Journal of Cancer*, **35,** 1–39.

Pui, C.H., Williams, D.L., Raimondi, S.C., Rivera, G.K., Look, A.T., Dodge, R.K., George, S.L., Behm, F.G., Crist, W.M. & Murphy, S.B. (1987) Hypodiploidy is associated with a poor prognosis in childhood acute lymphoblastic leukemia. *Blood*, **70,** 247–253.

Pui, C.-H., Carroll, A.J., Raimondi, S.C., Land, V.J., Crist, W.M., Shuster, J.J., Williams, D.L., Pullen, J., Borowitz, M.J., Behm, F.G. & Look, A.T. (1990) Clinical presentation, karyotypic characterization, and treatment outcome of childhood acute lymphoblastic leukemia with a near-haploid or hypodiploid <45 line. *Blood*, **75,** 1170–1177.

Raimondi, S.C., Zhou, Y., Mathew, S., Shurtleff, S.A., Sandlund, J.T., Rivera, G.K., Behm, F.G. & Pui, C.H. (2003) Reassessment of the

prognostic significance of hypodiploidy in pediatric patients with acute lymphoblastic leukemia. *Cancer*, **98,** 2715–2722.

Secker-Walker, L.M., Lawler, S.D. & Hardisty, R.M. (1978) Prognostic implications of chromosomal findings in acute lymphoblastic leukaemia at diagnosis. *British Medical Journal*, **2,** 1529–1530.

Secker-Walker, L.M., Prentice, H.G., Durrant, J., Richards, S., Hall, E. & Harrison, G. (1997) Cytogenetics adds independent prognostic information in adults with acute lymphoblastic leukaemia on MRC trial UKALL XA. *British Journal of Haematology*, **96,** 601–610.

Stark, B., Jeison, M., Gobuzov, R., Krug, H., Glaser-Gabay, L., Luria, D., El Hasid, R., Harush, M.B., Avrahami, G., Fisher, S., Stein, J., Zaizov, R. & Yaniv, I. (2001) Near haploid childhood acute lymphoblastic leukemia masked by hyperdiploid line: detection by fluorescence in situ hybridization. *Cancer Genetics and Cytogenetics*, **128,** 108–113.

Trueworthy, R., Shuster, J., Look, T., Crist, W., Borowitz, M., Carroll, A., Frankel, L., Harris, M., Wagner, H. & Haggard, M. (1992) Ploidy of lymphoblasts is the strongest predictor of treatment outcome in B-progenitor cell acute lymphoblastic leukemia of childhood: a Pediatric Oncology Group study. *Journal of Clinical Oncology*, **10,** 606–613.