Table S1: Study groups and treatment protocols

Study Group	Treatment protocol	Period
AIEOP-BFM ALL Study Group	AIEOP-BFM ALL 2009	2010 – 2017
ALL-BFM-Austria Study Group	ALL-BFM-A 2000	1999 – 2009
DCOG	DCOG ALL10	2004 – 2012
	DCOG ALL11	2012 – 2020
ALL-IC BFM Study Group	ALL-IC BFM 2009	2010 – 2018
MRC UKALL	UKALL2003	2003 – 2011
EORTC-CLG	EORTC-CLG 58951	1998 – 2008
COALL Study Group	COALL 07-03	2003 – 2010
	COALL 08-09	2010 – 2016

Abbreviations: ALL, acute lymphoblastic leukemia; AIEOP-BFM, Associazione Italiana Ematologia e Oncologia Pediatrica (AIEOP) – Berlin-Frankfurt-Münster ALL Study Group; DCOG, Dutch Childhood Oncology Group; ALL-IC (Intercontinental) BFM Study Group; MRC UKALL, Medical Research Council United Kingdom ALL; EORTC-CLG, European Organization for Research and Treatment of Cancer – Children's Leukemia Group; COALL, Cooperative Study Group for ALL.

Table S2: Number of patients with B-ALL overall, HHD B-ALL and non-HDD B-ALL across the eight trials

Treatment protocol	No. of HHD B-ALL pts.	No. of non-HHD B-ALL pts.	all pts.
AIEOP-BFM ALL 2009*	843	1791	2634
ALL-BFM-A 2000	119	331	450
DCOG ALL10	151	470	621
DCOG ALL11	162	516	678
ALL-IC BFM 2009	559	2325	2884
UKALL2003	712	1513	2225
EORTC-CLG 58951	442	832	1274
COALL 07-03 & 08-09	243	521	764
	3231	8299	11530

Abbreviations: HHD, hyperdiploid; ALL, acute lymphoblastic leukemia; No,,number, pts., patients.

* without AIEOP ALL patients for whom no conventional karyotypes were available.

Table S3: 5-year event-free survival rates of the HHD B-ALL according to MCN 54–67 and UK-profile across the eight trials

AIEOP-BFM ALL 2009

	N	%	EFS	CI 95% LL	CI 95% UL
MCN / UK-profile					
MCN <54 & UK poor	45	5.3	73.1	57.5	83.7
MCN <54 & UK good	93	11.0	89.1	80.7	94.0
MCN 54-67 & UK poor	102	12.1	91.9	84.5	95.9
MCN 54-67 & UK good	603	71.5	91.3	88.7	93.3

ALL-BFM-A 2000

	N	%	EFS	CI 95% LL	CI 95% UL
MCN / UK-profile					
MCN <54 & UK poor	6	5.0	83.3	27.3	97.5
MCN <54 & UK good	15	12.6	86.7	56.4	96.5
MCN 54-67 & UK poor	27	22.7	78.6	54.6	90.9
MCN 54-67 & UK good	71	59.7	86.8	76.1	92.9

DCOG ALL10

	N	%	EFS	CI 95% LL	CI 95% UL
MCN / UK-profile					
MCN <54 & UK poor	12	7.9	58.3	30.5	86.1
MCN <54 & UK good	13	8.6	84.6	65.0	100.0
MCN 54-67 & UK poor	17	11.3	88.2	72.9	100.0
MCN 54-67 & UK good	109	72.2	91.7	86.6	96.8

DCOG ALL11

	N	%	EFS	CI 95% LL	CI 95% UL
MCN / UK-profile					
MCN <54 & UK poor	12	7.4	90.9	73.8	100.0
MCN <54 & UK good	16	9,9	85.2	66.0	100.0
MCN 54-67 & UK poor	10	6.2	90.0	71.4	100.0
MCN 54-67 & UK good	124	76.5	92.6	87.5	97.7

ALL-IC BFM 2009

	N	%	EFS	CI 95% LL	CI 95% UL
MCN / UK-profile					
MCN <54 & UK poor	67	11.5	69.3	55.6	79.5
MCN <54 & UK good	40	6.9	76.7	59.9	87.1
MCN 54-67 & UK poor	255	43.8	80.4	74.2	85.2
MCN 54-67 & UK good	220	37.8	83.6	77.6	88.1

UKALL2003

	N	%	EFS	CI 95% LL	CI 95% UL
MCN / UK-profile					
MCN <54 & UK poor	51	7.2	86.2	73.2	93.2
MCN <54 & UK good	75	10.5	90.7	81.4	95.4
MCN 54-67 & UK poor	93	13.1	83.6	74.3	89.8
MCN 54-67 & UK good	493	69.2	93.3	91.3	95.7

EORTC-CLG 58951

	N	%	EFS	CI 95% LL	CI 95% UL
MCN / UK-profile					
MCN <54 & UK poor	35	7.9	88.0	71.2	95.3
MCN <54 & UK good	51	11.5	75.1	60.2	85.1
MCN 54-67 & UK poor	119	26.9	90.9	83.7	95.0
MCN 54-67 & UK good	237	53.6	93.8	89.8	96.3

COALL 07-03 & 08-09

	N	%	EFS	CI 95% LL	CI 95% UL
MCN / UK-profile					
MCN <54 & UK poor	16	6.6	68.8	40.5	85.6
MCN <54 & UK good	20	8.2	68.6	43.0	84.5
MCN 54-67 & UK poor	53	21.8	84.6	71.5	92.0
MCN 54-67 & UK good	154	63.4	90.2	84.2	93.9

Abbreviations: N, number; EFS, event-free survival; CI, confidence interval; LL, lower limit; UL, upper limit; MCN, modal chromosome number; UK, United Kingdom; GR, good-risk; PR, poor-risk

Table S4 Stepwise statistical analysis applied for final ranking of the HHD B-ALL subtypes across the eight trials

-1- In each analysed cohort, the subtypes were ranked based on their outcome (1 is the best and 8 is the worst).

-2- For each HHD B-ALL subtype, the difference of outcome compared to the best performing subtype (_diff.) was calculated.

-3- Weighting (multiply) of the HHD B-ALL subtype ranking was done by the corresponding difference (rank+).

-4- Steps 1 to 3 were repeated for EFS, OS and CIR.

-5- The weighted rankings of all three endpoints ($EFS_{rank+} + OS_{rank+} + CIR_{rank+}$) were added.

-6- For each trial, the proportion of HHD B-ALL patients who harbor that subgroup ($Rank_{p\ HHD}$) were calculated and ranked.

-7- For each trial, the proportion of B-ALL patients who harbor that subgroup ($Rank_{p\ Total}$) were calculated and ranked.

-8- For each subgroup within the analysed cohorts, the final ranking was calculated by weighting (multiplying) its corresponding ($EFS_{rank+} + OS_{rank+} + CIR_{rank+}$) * $Rank_{p\ HHD}$ * $Rank_{p\ Total}$

i-BFM Collaborative Acute lymphoblastic leukemia Project proposal

Outcome of high-hyperdiploid ALL (as assessed by conventional methods) in a modern therapy era

Responsible Scientists: Andishe Attarbaschi & Anthony Moormann

Final Analysis Plan Version 1.0, 29.09.2024

Aims

High-hyperdiploidy, the largest and favorable subtype of childhood ALL, exhibits significant biological and prognostic heterogeneity. To establish outcome of patients treated on European-based ALL protocols, according to eight different definitions of "low-risk" high-hyperdiploidy: **a)** modal chromosome number 51-67 with 4 subcategories; **b)** United Kingdom ALL low-risk high-hyperdiploid group; **c)** single trisomy of chromosome 18; **d)** double trisomy of chromosomes 4 and 10; **e)** and triple trisomy of chromosomes 4, 10, and 17. Outcome data will serve as reference bench mark (landmark paper) for ongoing and future high-hyperdiploid ALL cohorts defined by more modern methods such as SNP arrays and treatment with the most contemporary therapy protocols.

Study population

- Completed trials where the major recruitment period was between January 1, 1998 and December 31, 2020. For example, a trial running from 1999 to 2006 is acceptable but not one running 1994 to 2001.
- Patients 1 to 18 years with **non-Down syndrome** B-ALL.
- Availability of "only" basic data on the whole reported cohort: sex (ratio of male to female), age (median and ranges), WBC counts (median and ranges), NCI group (ratio of SR vs. HR)
- Availability of 5-year event-free survival (EFS) and overall survival rates (OS) as well as cumulative incidence of relapse (CIR) of the eight categories as outlined below.
- Availability of median follow-up (and ranges) of surviving patients (at least five years)

Definitions

High-hyperdiploidy is defined as a chromosome number of 51-67 inclusive (Chr. 51-67). Diagnostic pretreatment bone marrow and/or peripheral blood samples should have been analyzed **by standard G-banded karyotyping methods**. FISH to confirm uncertain losses or gains of chromosomes is acceptable, but should not have been the sole method for detecting high-hyperdiploidy. Patients diagnosed only by DNA index, SNP arrays and other methods are excluded from the present study!

Patients with concomitant *BCR::ABL1*, *ETV6::RUNX1*, or *TCF3::PBX1* fusions (as proven by molecular-genetic methods) or rearranged *KMT2A* should be excluded from the high-hyperdiploid group, as they are regarded as the primary genetic abnormality with prognostic and therapeutic impact potentially overriding the beneficial effect of the high-hyperdiploidy.

All karyotypes should have been curated to exclude masked haploidy / hypodiploidy from the high-hyperdiploid cohort either by molecular testing (e.g. SNP array) or by the pattern of tetrasomies (see Charrin et al. Blood. 2004 Oct 15; 104(8): 2444-51)

Incomplete karyotypes where only a chromosome count was possible or where the analysis was sub-standard and resulting in an excessive number of chromosomes (e.g. three or more) being labelled marker chromosomes should also be excluded. **In addition, if "only" a range of the modal chromosome number is reported, please take the highest value.**

For the analysis below, the "other non-HHD B-ALL" cases should only comprise cases where successful cytogenetic analysis was possible. So failed karyotypes (no abnormal cells or fewer than 20 normal metaphases) should be excluded.

Karyotypes will need to be classified into the following high-hyperdiploid subgroups:

(1) Triple trisomy (TT), defined by simultaneous trisomy of chromosomes 4, 10 and 17
(2) Double trisomy (DT), defined by simultaneous trisomy of 4 and 10
(3) Trisomy 18
(4) UKALL low-risk high hyperdiploid (UK-ALL) defined as (a) simultaneous gain of chromosome 17 and 18 or (b) the gain of chromosome 17 or 18 but no gain of chromosome 5 or 20
(5) MCN: 51-53
(6) MCN: 54-55
(7) MCN: 56-67
(8) MCN: 54-67

Statistical definitions and methods
Survival analysis – please use the Kaplan-Meier method to estimate survival probabilities (as per the definitions below) and the log-rank test to compare differences.

Event-free survival (EFS), defined as time from diagnosis (or treatment start) to relapse, second malignant neoplasm or death, censoring at date of last contact.

Overall survival (OS), defined as time from diagnosis (or treatment start) to death, censoring at date of last contact.

Cumulative Incidence of relapse (CIR) is calculated using cumulative incidence functions for competing events constructed by the method of Kalbfleisch and Prentice and compared with Gray's test.

Please use Chi-squared test to compare proportions and the Kruskal-Wallis test to compare the equality of medians.

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DATASET FORMAT

Please, send us the results in an EXCEL spreadsheet

Cohort description – Please provide the following data table for **EACH** trial submitted.

		HHD cases (n)	Other non-HHD B-ALL cases (n)	P-value HHD vs. other B-ALL
Number of cases				
Sex	Male			
	Female			
Age	Median			
	Interquartile Range			
	1 – 9 years			
	10 – 15 years			
	16 – 18 years			
WBC	Median			
	Interquartile Range			
	<10.0 G/L			
	≥10.0 – <50.0 G/L			
	≥50.0 – <100.0 G/L			
	≥100.0 G/L			
NCI Risk group	SR			
	HR			
MRD at EOI	Negative			
	Positive			
	Unknown			
Induction failure	No			
	Yes			
	Unknown			
Event-free survival at 5 years				
95% CI				
Overall survival at 5 years				
95% CI				
Cumulative incidence of relapse at 5 years				
95% CI				
Median follow-up time (years)				
Interquartile range				

High-hyperdiploid subgroup data

Please provide the following data table for **EACH of the 8 subgroups** listed above:

		Subgroup	Other HHD B-ALL cases	P-value Subgroup vs. other HHD B-ALL cases	Other non-HHD B-ALL cases	P-value Subgroup vs. other non-HHD B-ALL cases
Number of cases						
Sex	Male					
	Female					
Age	Median					
	Interquartile Range					
	1 – 9 years					
	10 – 15 years					
	16 – 18 years					
WBC	Median					
	Interquartile Range					
	<10.0 G/L					
	≥10.0 – <50.0 G/L					
	≥50.0 – <100.0 G/L					
	≥100.0 G/L					
NCI Risk group	SR					
	HR					
MRD at EOI	Negative					
	Positive					
	Unknown					
Induction failure	No					
	Yes					
	Unknown					
Event-free survival at 5 years						
95% CI						
Overall survival at 5 years						
95% CI						
Cumulative incidence of relapse at 5 years						
95% CI						