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Research paper

Microarray testing as an efficient tool to redefine hyperdiploid paediatric B-cell precursor acute lymphoblastic leukaemia patients

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ABSTRACT

The aim of our study was to characterize genetic alterations in a cohort of paediatric patients with B-cell progenitors (BCP-ALL) and a hyperdiploid karyotype. In our study, we analysed 55 childhood hyperdiploid BCP-ALL patients using single nucleotide polymorphism (SNP) microarray testing. The group consisted mostly of patients with the modal number of chromosomes between 54 and 58 (34 cases). Within this group, Trisomy 4 and Trisomy 10 (30 cases) were the most frequent cases. Additionally, a total of 93 structural abnormalities mainly affecting chromosomes 1, 6, 9, 12, and 17 as well as 68 copy number alterations (CNAs) were identified. The microarray testing revealed a loss of *ETV6, IKZF1, CDKN2A/CDKN2B, PAX5,* and *RB1*. Moreover, chromosomal abnormalities resulting in the loss of heterozygosity (LOH) were also observed. Currently, patients with hyperdiploidy constitute a genetically heterogeneous group, and therefore, it is insufficient to rely only on banding cytogenetic analysis for the identification of hyperdiploid karyotype. Microarray testing has been proven an effective and satisfactory tool for the analysis of molecular karyotypes and to redefine the prognostic criteria in hyperdiploid patients.

1. Introduction

Acute lymphoblastic leukaemia (ALL) is one of the most common malignancies in paediatric patients. The most common type of leukaemia in children (over 80% of all cases) derives from B-cell progenitors (BCP-ALL). Currently, the prognostic factors for childhood ALL are patients' response to steroid therapy, remission duration, the status of minimal residual diseases (MRD), and the results of cytogenetic as well as molecular tests [1].

The use of both numerical and chromosomal abnormalities as prognostic tools is not new. Therefore, since the mid-1980s, hyperdiploidy, which is understood as the gain of one or more chromosomes in a non-random fashion, has been recognized. It is a characteristic subgroup of childhood ALL diagnosed by karyotyping. What is more, high-hyperdiploid (HeH) karyotype, described by the presence of 51–65 chromosomes, is detected in 30% of BCP-ALL cases [2–4]. There are numerous clinical features, such as a median age of 4 years or low white blood cell count (WBC) at the time of HeH diagnosis, that result in an overall survival rate of approximately 90%. Combined trisomy of chromosomes 4, 10 and 17 is associated with a particularly excellent prognosis in patients with standard-risk BCP-ALL. A childhood trial conducted by the UK Medical Research Council revealed that Trisomy 18 is an informative marker with the most favourable outcomes [5]. Unfortunately, the prognosis still remains vague for patients with low hyperdiploidy (47–50 chromosomes). As most studies suggest, hyperdiploidy 47–50 has been associated with an intermediate prognosis or a worse outcome compared with high hyperdiploid patients [6,7]. Additionally, it has also been demonstrated that the gain of chromosome 5 and the occurrence of structural abnormalities may have a negative effect on the outcome [7]. The mechanism involved in the generation of hyperdiploidy and its role in leukaemogenesis remains unclear [8,9].

Sometimes, good prognoses for hyperdiploid patients can be disturbed by hidden genetic alterations. Nevertheless, such alterations cannot be detected by targeted fluorescence *in situ* hybridization (FISH) or by banding cytogenetic analysis.

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The aim of our study was to characterize the genetic alterations in a cohort of paediatric patients with BCP-ALL and hyperdiploid karyotype, as well as in patients with an unsuccessful bone marrow cell culture.

2. Materials and methods

2.1. Patients

This study included 55 children with BCP-ALL and without well-known chromosomal translocations: t(9;22)(q34;q11), t(12;21)(p13;q21), 11q23 rearrangement and t(1;19)(q23;p13). Hyperdiploid karyotype was diagnosed by microarray testing. All children were treated according to the ALL IC BFM 2009 protocol, which was a randomized trial of the International Berlin-Frankfurt-Munster Study Group (I-BFM-SG) for the therapy of childhood ALL. The trial was conducted in the Department of Pediatric Hematology, Oncology and Transplantology of the Children's University Hospital in Lublin in the years 2011 and 2017. The group consisted of 23 girls and 32 boys. The age of the patients ranged from 1.06 to 15.44 years (with a median age of 5.31). The range of the follow-up was 0.5–5.0 years, and the median was 2.38 years. The clinical and laboratory details of patients with ALL are presented in Table 1.

2.2. Molecular analysis

The microarray analyses were performed with the use of the CytoScan HD array (2 670 000 probes including 750 000 SNPs; Applied Biosystems, Thermo Fisher, Waltham, MA). The genomic DNA isolated from mononuclear cells of the bone marrow constituted the research material. The isolation procedure of the mononuclear cells was performed in a concentration gradient using Ficoll-Paque PLUS and in an aqueous solution of density 1.077 + 0.001 g/ml (Amersham Biosciences). The median leukaemic cell count in collected bone marrow at diagnosis assessed by flow-cytometry was 87.90% (the range 52.50–98.70). The DNA isolations were completed with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The concentration and quality of isolates were determined by spectrophotometry (NanoDrop 8000, Thermo Scientific, Waltham, MA). In total, 250 ng of the genomic DNA was analysed in accordance with the manufacturers' protocols. The study was based on an analysis of scanned data files that was generated with the Chromosome Analysis Suite v 3.3 (ChAS, Thermo Fisher, Waltham, MA). Furthermore, the copy number of altered regions (CNAs) were calculated, and the data were normalized to a reference model (Thermo Fisher) of baseline reference intensities NA 33 (hg19/CRCh37). The copy number states (CNS) and their breakpoints were determined with the use of the hidden Markov model (HMM) software package. The threshold level of $\log_2$ ratio ≥ 0.5 and ≤ 0.5 were used respectively for the categorization of the altered chromosomal regions as copy number variation (CNV) gains and losses. The identification of normal diploid markers in the cancer samples constituted an essential part of the algorithm, which was particularly significant in highly sample-induced *aberrations*. Furthermore, unaltered diploid markers were used for the calibration of signals, and they resulted in a $\log_2$ ratio of 0 (e.g., copy number 2). The algorithm also indicated that the identified unaltered diploid markers could correspond with CN = 4. In such a case, the $\log_2$ ratio was readjusted, and the chromosomal ploidy of 4 was reported. The obtained data were analysed based on two different criteria: genome-wide CNVs and leukaemia-associated region/gene-specific CNAs (leukaemia genes_all_20150505; Fullerton Overlap Map_hg19). The minimal number of probes was applied to determine the CNAs: 50 probes for duplication (gain); 25 probes for deletion (loss). To further identify the genes involved in the CNVs, two databases were applied: the UCSC database (http://genome.ucsc.edu) and Ensemble (http://www.ensembl.org).

Table 1
Clinical characteristics of patients.

Clinical details of patients	number of patients (%) 55 (100%)
Sex	
Female	23 (40%)
Male	32 (60%)
Median age/years (range)	5.31 (1.06 -15.44)
Immunophenotype	
pre B ALL	40
common positive	15
Median WBC at diagnosis [ul]	17 834 (680 - 137 010)
Median % blast (PB) at diagnosis	32 (20.2 - 94.04)
Median % blast (BM) at diagnosis	87.90 (52.50 - 98.70)
Predisone response	
Good	52 (94%)
Poor	3 (6%)
BM morphology at day 15	
M1	43
M2	9
M3	3
BM morphology at day 33	
M1	55
M2	0
M3	0
MRD-FC status at 15 day	
M1	42
M2	10
M3	3
MRD-FC status at 33 day	
M1	55
M2	0
M3	0
ALL IC 2009 Risk Group	
HRG	9
IRG	35
SRG	11
Follow up (years)	2.38 (0.5 - 5.0)
Relapse	3

Abbreviations: WBCwhite blood cells; MRD-FCMinimal residual disease flow cytometry; SRGstandard-risk group; IRGintermediate-risk group; HRGhigh-risk group; CNScentral nervous system; BMbone marrow; PBperipheral blood; M1– ; blasts < 5% blasts; M2– ; blasts ≥ 5 to < 25%; M3– blasts ≥ 25%.

3. Results

Hyperdiploidy was revealed only in 32 out of 55 patients who were initially tested with banding cytogenetics. The remaining 23 cases presented either bone marrow cell culture failure or normal karyotype (Table 2A). Additionally, the distribution of molecular karyotypes investigated in the group of 55 paediatric patients with SNP array indicated seven low hyperdiploid cases and 48 high hyperdiploid cases. The research did not indicate any cases of masked hypodiploidy or any near haploid cases.

The trisomy and tetrasomy distribution of individual chromosomes for HeH and HeL groups is presented in Fig. 1. Moreover, only chromosome 13 did not occur as trisomy in the examined group.

In all, 24 patients presented only numerical aberrations, whereas 93 structural abnormalities were identified in the remaining patients including: 32 losses and 61 gains. The most common structural abnormality that was potentially visible in the cytogenetic analysis was the gain or loss of chromosomal regions > 10-15 Mb. These findings are presented in Table 2B and Table 1S (Supplementary material).

The study showed 68 CNAs (an average of 1.2 CNAs per case), including microdeletions and microduplications. There were 49 deletions (not including the *TCRG* and/or *TCRA* deletion) and 19 gains. Single gene abnormalities are presented in Table 2S. (Supplementary material)

3.1. Low hyperdiploidy

Following the revision based on the microarray, seven cases of low hyperdiploid karyotype (47–49 chromosomes) were revealed in the

Table 2A
Original karyotypes and revisions based on SNP microarray analysis in 55 cases of childhood hyperdiploid BCP-ALL.

Cytogenetic abnormalities	ID	Karyotype	Revision based on molecular karyotype studied by SNP microarray [GRCh37]
Low hyperdiploidy	1	Failure	arr[GRCh37](5)x2~3,6q12q22.32(75897006_126874323)x1~2,(8)x2~3
	2	Failure	arr[GRCh37] (10)x3,12p13.33p12.1(191242_23556041)x1~2,13q31.2q33.3(86098869_109584213)x2~3, 15q22.31q25.3(40420673_81041938)x3,17q21.2q25.3 (40420673_81041938)x3(21,X)x2~3
	3	Failure	arr[GRCh37] 6q14.1q22.31(82287928_120941705)x1~2,12p13.33p13.2(173786_12027012)x4,(16,18)x2~3, 19p12q13.12(22607620_37598208)x1~2,21q11.2q22.13(15006457_38850256)x4
	4	Failure	arr[GRCh37](10)x2~3, 19p13.3(1319319_4028159)x1, (20)x1,(21)x2~3
	5	47,XY,+mar	arr(21)x3(X)x3
	6	47,XY,+mar	arr[GRCh37] 5p15.33q32(113576_149487007)x3, 5q33.3q35.3(149487007_158129108)x3
	7	47,XY,+mar	arr[GRCh37] 6q12q22.32(75897006_126874323)x1~2,(14)x3
High Hyperdiploidy with trisomy 4, 10, 17	8	Failure	arr[GRCh37] (1)x3~4,(2)x2~3,(3,4,5,6,7)x3~4,(8)x2~3,(9)x2 mos hmz,(10,11,12)x3~4,13q31.1q31.3(80668777_93519171)x1~2,(15)x2~3, 16p13.3q12.2(85880_55816692)x3,17p13.3p11.2(525_17512768)x1~2 mos hmz,(17q)x3,(18,19,20)x3~4,21q22.13q22.3(38854499_48097372)x2~3,(22)x3~4,(X,Y)x1~2
	9	Failure	arr(1q)x2-3,(3,4,6,8,10)x2~3,(11)x2 mos hmz,(14)x2~3,(15)x2 mos hmz,(16,17,18)x2~3,(19,20)x2 mos hmz,(21)x3,(X)x1~2,(Y)x2
	10	Failure	arr[GRCh37] 1q21.1q32.2(146843841_208449788)x2~3,(4,6)x2~3,(8)x2~3, (10)x2~3,(11)x2 mos hmz,(12,14,17,18)x2~3,(20)x2 mos hmz,(21,X)x3
	11	Failure	arr[GRCh37](4,6,8,10)x3,(14)x3,17p13.3p11.2(525_17512768)x1~2,(17q)x3,(18,19,20)x3~4, (21)x2~3,(18)x3,(21)x4,(X)x3
	12	Failure	arr[GRCh37](4,6,8)x2~3,9p24.3p13.1(192128_40087758)x2 mos hmz,(10,14,17,18)x2~3,(21)x3,(X)x2
	13	Failure	arr[GRCh37] 1q21.1q32.2(146843841_208449788)x2~3(4,5)x3,(6)x2~3,(8,10,11,12)x3,(14,17)x2~3,(18)x3, (21)x4,(22)x2~3,(X)x2
	14	Failure	arr(4,6,10,14,17,18)x2~3,(21)x4,(X)x3
	15	Failure	arr[GRCh37] 1q41q44(220272704_246917797)x1,(4,10,14,17,18)x2~3,(21)x4,(X)x2
	16	Failure	arr[GRCh37] (4,5,6)x2~3,(7p)x1,(7q)x2~3,(10)x3,12p13.33p13.2(493923_10285984)x3,(14)x2~3, (17p)x2~3,(17q)x2~3 mos hmz,(21)x4,(X)x2
	17	Failure	arr[GRCh37] 1q21.1q44(145669267_249224684)x2~3,(4,6)x2~3,9p24.3p13.1(203861_38418449)x2~3,(10,14,17,18)x2~3,(21)x4,(X)x2
	18	Failure	arr[GRCh37](4,6)2~3,(10,14)x2~3,16q11.1q24.3(35118853_90155062)x2~3,(17,18)x2~3,(21,X)x3
	19	Failure	arr[GRCh37] 1q21.1q32.2(146843841_208449788)x3,(4,6)x2~3,(9)x2 hmz,(10,11,12)x2~3,(14,17,18)x2~3,(21)x3,(22)x2~3,(X)x3
	20	46,XY	arr[GRCh37] (1q)x3,(2)x3,(4)x4,(5,6)x2~3,(8)x3,9p24.3q13(203861_68679244)x2~3, (10-12)x3,(14,17,18)x2~3,(21)x4,(22)x2~3(X)x2,(Y)x2
	21	53~56,XY,+X,+4,+6,+10,+14,+17,+18,+20,+21,+21	arr(4,6,10)x3,(14)x4,(17,18)x3(21)x4,(X)x2
	22	53~55,XX,+X,+4,+6,+10,+17,+18,+1-3mar	arr(4,6,10,14,17,18)x2~3,(21)x4,(X)x3
	23	56~57,XX,+X,+4,+6,+8,+10,+15,+16,+17,+18,+21,+21 inc	arr(4-6,)x2~3,(8)x3(10,14,16,17)x2~3, (18,21)x3,(X)x2~3
	24	56,XY, +X,+4,+6,+10,+14,+15,+17,+18,+21,+21	arr(4)x3,(5)x2 hmz,(6)x2~3,(9)x2 hmz,(10)x3,(14,15,17)x2~3,(18)x3,(21)x4,(X)x2
	25	53~55,XY,+X,+4,+6,+10,+17,+18,+21,+21,+21	arr(4,6,10,14,17,18)x2~3,(21)x4,(X,Y)x2
	26	58,XX,+X,+X,+4,+6,+8,+10,+14,+14 + 17,+18,+21,+21	arr[GRCh37] 1q21.1q42.13(145515117_227772171)x2~3,(4,6,8,10)x2~3,14q11.2q24.2(22978375_740001111)x3,14q24.3q32.33(74029886_106342662)x4, (17,18)x2~3,(20)x2 hmz,(21)x4,(X)x4
	27	50~57,XY,+X,+4,+6,+10,?del(12)(p12p13),+14,+17,+18,?+18,+21, inc	arr[GRCh37] 1q21.1q42.13(145515117_227772171)x2~3,2q35q37.3(221209246_242783384)x1,3q25.31q29(155487451_197851986)x2~3,(4,6,10)x2~3,11q24.3q25(130126670_134938470)x2~3 12p13.33p12.1(191242_23556041)x1,(14,17)x2~3,(18)x4, (21)x2~3,(X)x2
	28	56,XY,+X,+4,+6,+8,+10,+14,+17 + 18,+21,+21	arr[GRCh37] (4,6,8,10,14)x2~3,15q11.2q13.1(22770421_29837539)x2-3,(17,18)x2~3,(21)x3,(X)x2
	29	56~57,XY,+5,+6,+8,+10,+13,+14,+15,+17,+19,+21,+21	arr(4,6)x2~3,9p24.3p13.3(192128_33728780)x2 hmz,(10,14,15,17,18)x2~3,(21)x4,(X)x2,(Y)x2
	30	60-61,XXY, -1,-2,-3,del(6)(p23),-9,-10,-12,del(12)(p13p13),-15,+21 × 4-5,-22	arr[GRCh37] (1q,4,5)x2~3,6q22.2q27(26176542_170919148)x3,(7)x2 hmz,(8,10,11)x2~3,(14,15)x2~3,16p13.3q12.2(85880_55816692)x2~3,16q12.2q24.3(55883882_90163275)x2 mos hmz,(17,18)x2~3,(21)x4,(X)x2,(Y)x2
	31	55,XY,+X+4,+6,+10,+14,+17,+18,+21,+21	arr[GRCh37](4,6)x2~3,(9)x2hmz,(14,17,18)x2~3,(21)x4,22q11.1q11.21(16888899_22043899)x3,22q13.33(50464963_51197838)x3,(X)x2
	32	54~55,XX,+X,dup(1)(q21q31),+4,+5,+6,+8,+14,+17,+21,+21	arr[GRCh37] 1q21.2q32.1(146843841_206329071)x3,(4,6)x2~3,(9)x2 hmz,(10,11,12)x2~3,(14,17,18)x2~3,(21)x3,(22)x2~3,(X)x3
	33	56~57,XY,+X,+4,+6,+der(9)t(1;9)(q21;q22),+10,+14,+17,+18,+21,+22,+mar	arr[GRCh37](1q)x2~3,(2)x2 hmz,(4,6)x3,9p24.3q31.1(203861_103922328)x2~3,(10)x3,(14)x2~3,(15)x2 hmz,(17)x3,(18)x2~3,(19)x2 hmz,(21)x4,(X)x2,(Y)x2
	34	57,XX,+?X,+4,+6,+7,+10, +12,+17,+18,+21,+21,+22 [3]/46,XX [5]	arr(4,6,7,10,12)x2~3,(13)x2 hmz,(14)x2~3,(16)x2 hmz,(17,18)x2~3,(21)x3,(22) x2~3,(X)x2~3
	35	Failure	arr(6)x2~3,(10)x3,(14,18)x2~3,(21)x3,(X,Y)x2
	36	Failure	arr(4,6,8,14,17,18)x2~3,(21)x3,(X)x0

(*continued on next page*)

Table 2A (*continued*)

Cytogenetic abnormalities	ID	Karyotype	Revision based on molecular karyotype studied by SNP microarray [GRCh37]
High Hyperdiploidy without both trisomy 4, 10, 17	37	Failure	arr(4,6,14,17,18)x2~3,(21,X)x3
	38	Failure	arr(4,6,14,17,18)x2~3,(21)x3,(X)x2~3
	39	Failure	arr[GRCh37](4)x2~3,6p25.3q13(156974_70912244)x2~3, 6q22.33q27(128883402_170919482) x2~3,13q22.3q31.1(77224328_84643143)x1~2, 14q11.2q21.3(20511672_50315725)x2~3,(21,X)x2~3
	40	Failure	arr[CRCh37] (7)x2~3,9p24.2q13(203861_67983174)x1,(10,18)x2~3,20p13p11.21(61568_25441834) x2~3, 20q11.21q13.33(29448795_62913996)x2 mos hmz,(21)x2~3,(X)x4
	41	Failure	arr[GRCh37] (5)x2~3,(14)x2~3,(21)x4,(X)x3
	42	46,XX	arr[GRCh37] 1q21.1q44(145795625_249224684)x2~3,3q25.31q29(155487451_197851986)x2~3,(5,6) x2~3, 9p24.2q13(203861_68679244)x2~3,(9q)x2 mos hmz,(10)x3,(11,12)x2~3,(14)x3,(17,18)x2~3,(21) x4,(X)x2~3
	43	55,XY,+X,+4,+6,+14, +17,+18,+21,+21,+21, +21,+mar	arr(4,6,14,17,)x2~3,(18)x3,(21)x4,(X)x3,(Y)x3
	44	54 ~ 55,XX,+X, +4,+6,+8, +9,+14,+18,+21,+21	arr(4,6,9,14,17,18)x2~3,(21)x4,(X)x3
	45	54,XY,+X,+6,+10,+10, +14,+18,+21,+21	arr(6)x2~3,(10)x4,(14,18)x2~3(21)x4,(X)x2
	46	55,XX,+X,+6,+10,+14, +17,+18,+21,+mar	arr(6,10,14,17,18)x2~3,(21)x4,(X)x3
	47	56,XY,+4,+6,+8,+9,+10, +14,+17 + 18,+21,+21	arr[GRCh37] 1q21.2q44(147933972_249224684)x2~3,(4)x2~3,5p15.33q11.2(113576_52612589)x2~3, 6p25.3q16.1(156974_93971627)x2~3,(7-10)x2~3,(13)x1,(14)x2~3,(18,21)x3,(X)x2~3
	48	56,XX,+X,+6,+10,+14, +17,+18,?+20,+21,+22, +mar	arr[GRCh37] 3p26.2p26.3(2824659_6619410)x1,(4,6,10)x2~3,11q13.1q25(64123188_134938470) x2~3, 12p13.33p13.2(493923_10285984)x3,12q15q21.1(67291855_77057559)x1, 12q23.2q23.3(99907459_108104063)x1,(14)x4,17p12q25.3(10741894_81041983)x2~3, (18)x2~3,(21) x4,(X)x3
	49	54~57,XY,+X,+Y,+4,+10, +14,+14,+15, +21 + 21,inc	arr(4)x3,(6)x3,(9)x2 hmz,(10)x3,(14)x4,(15)x2~3,(18)x3,(21)x4,(X)x2,(Y)x2
	50	52~58,XY,+6,+8,+11,+12, +13,+14,+17,+18,+20, +21,+21,+22	arr(6)x2~3,(7)x2 mos hmz,(8)x2~3,(10,11,12,14)x2~3,(16)x2 mos hmz,(17,18)x2~3,(21)x3,(22)x2~3,(X) x1~2
	51	53~55,XX,+X,+4, +6,+14, +17,+18,+21,+21,+mar	arr(4,6)x2~3,(14,17,18)x2~3,(21)x4,(X)x3
	52	56,XY,+X +4, +6, +8,+14, +17,+18,+21,+21,+22	arr(4,6-8)x2~3,(14,17,18)x2~3,(21)x4,(22)x2~3,(X)x3
	53	56~58,X,der(X)t(X;1) (q13;q21),+4,+5,+6,i(7) (q10),+8,+10,+?13,+14, +17,+18,+20,+21,+21	arr[GRCh37](1q)x2~3,2q22.1q33.1(138174359_168735065)x1~2,3p12q13.33(73812977_121253989) x1~2, 3q23q26.33(140031049_182842663)x1~2, (5,6)x2~3,(7p)x1,(7q)x2~3,(8) x2~3,9p24.2p21.1(21387434_32393835)x1 (10)x2~3,13q21.1q31.1(58125283_79019147)x1~2, (14,17,18)x2~3,(21)x3
	54	54,XX,+X,+8,+10,+14, +18,+21,+mar,inc [13] / 46,XX [7]	arr[GRCh37] (5)x2 hmz,6p25.3q14.3(156974_86857389)x2~3,6q22.2q27(132872357_170919482)x2~3, (8,14)x2~3, (18)x4,(21)x3,(X)x3
	55	55~56,XY,+X,+6,+10,+12, +14,+17,+18,+21,+21, +mar	arr(6,10,12,14,17,18,)x3,(21)x4,(X)x3

Abbreviations: CNS-copy number states; 1 = deletion/loss; 3 or 4 = duplication/gain; dup-duplication; del-deletion; p-short arm of chromosome; q-long arm of chromosome; kbp-kilo base pairs; arr-array ; GRCh37 - Genome Reference Consortium Human Build 37; CNLOH copy number loss of heterozygosity.

study. One of the patients (case 6 in Tables 2A and 2B) presented 47 chromosomes, and the banding cytogenetics analysis did not identify the gained chromosome (the marker chromosome). The microarray analysis both defined this marker as chromosome 5 and indicated that this additional chromosome 5 had an intrachromosomal deletion occurring in the long arm (149487007_158129108). Moreover, 13 other structural abnormalities were identified within this group (Table 2B). The single gene abnormalities were revealed in five patients (Table 2B).

3.2. High hyperdiploidy

The comparison of individual combinations of gained chromosomes is presented in Fig. 2. In the case of HeH patients, the modal chromosome number (MCN) 55 was the most frequent.

One of the groups categorized within high hyperdiploidy was high hyperdiploidy with Trisomy 4, 10, 17. Triple Trisomies (+4, +10, +17) were found in 27 cases with a median mode number of 57 (range 54–64). Within this group, partial gain of 1q was the most common abnormality, and it was observed in 11 cases. The common region overlap was observed to be 1q21.2-1q32.2. Five patients presented the gained chromosome 5 with partial duplication 1q, which constituted a significant correlation coefficient. Duplication 1q was observed with a partial duplication 9p in 3 cases. In nine cases, only numerical aberrations were displayed, and there were no large structural abnormalities or microdeletions. Abnormalities concerning a single gene were not revealed in three patients (Tables 2A and 2B). There was 1 patient (case 10) with an intragenic deletion of 2–7 exons of *IKZF1* and a co-occurring deletion in *CDKN2A/CDKN2B*. Another patient (case 16) presented with deletion of the whole short arm of chromosome 7 and with a deletion of *CDKN2A/CDKN2B* and a duplication of 1q. One patient with biallelic losses (case 29) in genes *CDKN2A/CDKN2B, IFNA1,* and *MLLT3* was observed within this group. Loss of heterozygosity (LOH) was found in whole chromosomes 9 (7.2%) and 20 (5.4%) as well as in the case of chromosomes 11, 15, 19, 2, 5, 13, and 16. On the whole, LOH was observed more frequently in this group of patients.

The other group included all other high hyperdiploidy cases in which the combination of Trisomies 4, 10, and 17 did not occur together. The research indicated 21 cases of high hyperdiploidy with the abovementioned combination of trisomies (51–58 chromosomes). The gain of chromosome 21 was the most common type of trisomy observed in this group, and it was identified in all cases, together with the gains of chromosomes 18, 14, X and 17. In ten cases, only numerical

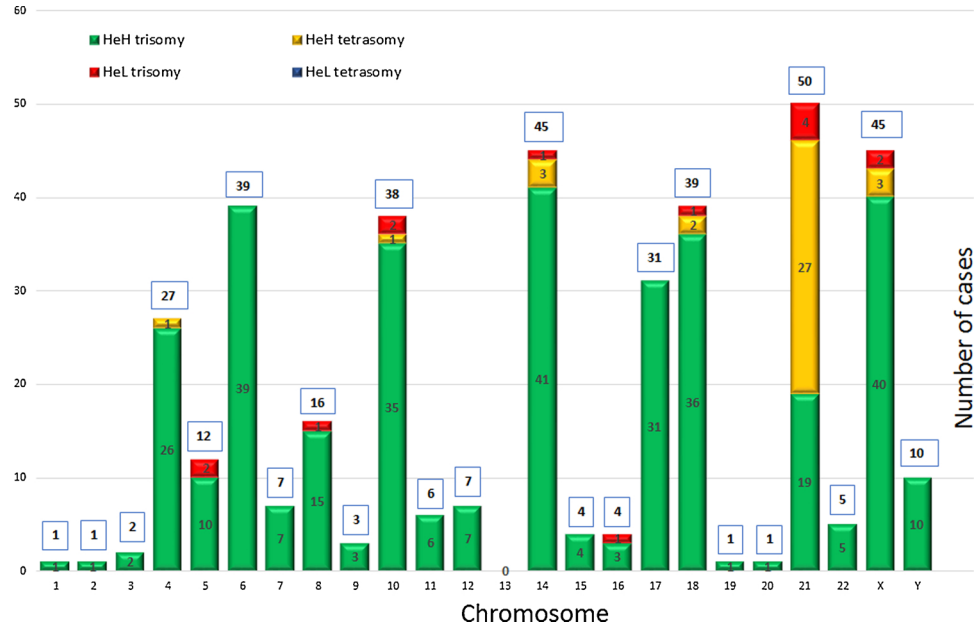

Fig. 1. Gains of whole chromosomes detected by SNP microarray analysis in 55 cases of childhood hyperdiploid BCP-ALL.

aberrations were displayed, and they had no large structural abnormalities or microdeletions. Moreover, duplication of 1q was observed in 3 patients. One patient with biallelic losses (case 39) in the following genes *CDKN2A/CDKN2B, IFNA1, MLLT3* was observed within this group of patients. There was one patient (case 53) with a cytogenetic banding result that revealed a deletion of the whole short arm of chromosome 7 and a duplication of the whole long arm of chromosome 7. What is more, the SNP microarray revealed an additional deletion of gene *CDKN2A/CDKN2B*. There were no structural abnormalities observed in 13 cases. LOH of whole chromosomes (5, 7, 9, 16) was observed in 3 cases, and LOH of partial chromosomes was observed in 2 cases (9q and 20q).

3.3. Cases with Culture Failure

When re-examined with microarray, the patients with cell culture failure (23 cases for which conducting banding cytogenetics was not possible) proved to have high hyperdiploidy in 19 cases and low hyperdiploidy in four cases. The results are detailed in Table 2A.

4. Discussion

The cytogenetic analysis of malignancies has become both an integral part of the diagnostic process, a prognostic tool and an indicator of responsiveness to therapy. Recent publications describe microarrays as an excellent tool to conduct genome-wide screening for copy number aberrations that are undetectable by G-banding or FISH [2,10,11]. During the past decade, many array platforms have been developed and optimized, and as a consequence, arrays containing polymorphic probes (SNP microarrays) have been widely utilized for molecular karyotyping of malignancies [12]. High hyperdiploidy has been very well described in the literature; however, 20% of affected patients still relapse [13].

Patients with low hyperdiploidy are very rarely discussed in the literature, and they are, in fact, often excluded from analyses, which makes it difficult to determine the prognostic significance of this abnormality. Two relapses were observed within this group of patients in our study (case 2 and case 6), who are still alive after the allogeneic stem cell transplantation. Our study proved, however, that it would be worth expanding the analysis of such patients. Further investigation into the abnormalities they present could prove to have a significant prognostic value.

The most frequent modal chromosome number (MCN = 55) in the HeH group in our study corresponded to a similar patient group in the study conducted by Raimondi et al. [10]. The patients included in our study group presented gained chromosomes and followed a pattern commonly reported in other studies [5,7,10,14–17], which is detailed in Table 3.

The majority of available publications describes the incidence of chromosome 4 or 10 as well as their combination (30/48 cases HeH in our study). Mousa et al. described that the group of Egyptian children with B-ALL and Trisomies 4 and 10 (17.5%) had good prognostic indicators [11]. Sutcliffe et al. described the studies conducted independently in a Paediatric Oncology Group (POG) and Children's Cancer Group (CCG) and showed that a simultaneous occurrence of chromosomes 4, 10 and 17 trisomies was strongly indicative of a favourable prognosis [18]. Kawamata et al. reported that children with HeH ALL without gained chromosome 17 or 18 had a worse prognosis than those who presented +4, +10, +17, +18 [19]. Sharathkumar et al. confirmed this observation in a single institution experiment [20]. The study implemented by Vojacek et al. proved that HeH was not an independent variable. It was overridden by isolated trisomy of chromosome 4 instead [21]. In our study, we observed that the combinations of individual trisomies occurred with a similar frequency (Fig. 2), and they were related to HeH. None of the patients with these combinations had a relapse, which suggested a good prognosis for patients with HeH and triple trisomy (+4, +10, +17) or quadruple trisomy (+4, +10, +17, +18).

The co-occurrence of the hyperdiploid karyotype and the structural changes, such as 1q duplication, 6q deletion, 9p deletion, and isochromosome 17q, are quite common. In our study, the frequency of duplication of 1q achieved the level of 29% (14/48 cases), which was higher than the frequency described in the literature. All of those cases were associated with high hyperdiploidy. There was also a strong association of trisomy of chromosome 5 with the 1q duplication (6 cases in our study), which was similar to other reported results [15,16]. Interestingly, a partial gain of 14q was observed in 1 case (case 26). The co-occurrence of a fragment of trisomy chromosome and a second fragment in tetrasomy were revealed by the SNP array analysis.

Among monoallelic losses, in our study, there were three noteworthy cases with losses that encompassed *IKZF1* (5.4%): the first patient (case 10 Tables 2A and 2B) spanned 119 Kb (loss of exons 2e7); the other 2 patients (cases 16 and 53 Tables 2A and 2B) spanned 62,394

Table 2B
The summary of genetic data in the studied patients: whole chromosome gains or loss, whole chromosome arm (p) or (q), segmental duplications and deletions, single gene abnormalities and LOH.

Cytogenetic abnormalities	ID	whole chromosome		whole chromosome arm (p) or (q)		segmental abnormalities		single gene abnormalities		CNLOH		
		gain	loss	gain	loss	duplication	deletion	gain	loss	whole chromosome UDP	whole short (p) arm UDP	whole long (q) arm UDP
Low hyperdi-ploidy	1	5, 8					6q12q22.32	*L1CAM, RPL10*	*UBA2, WTIP, ZNF146*			
	2	10, 21, X				13q31.2q33.3 15q22.31q25.3 17q21.2q25.3	12p13.33p12		*PAX5, GNA11, DAZAP1, TCF3*			
	3	16, 18				(12p13.33-p13.2)x4; (21q11.2-q22.13)x4	6q14.1q22.3 19p12q13.12		*SPINT1, ITPKA, TYRO3, RB1*			
	4	10, 21	20				19p13.3	*L1CAM*				
	5	21, X							*MRC1, CEBPG, CEBPA,*			
	6					5p15.33q32; 5q33.3q35.3						
	7	14					6q12q22.32					
High Hyperdi-ploidy with trisomy 4, 10, 17	8	1,2,3,4,5,6,7,8-,10,11, 12,15,18,19,2-0,22, X,Y		17q	17p13.3p11.2	16p13.3q12.2; 21q22.13q22.3	13q31.1q31.3		*MTAP, CDKN2A/ CDKN2B, IGFR1,*	9	17p	
	9	3,4,5,6,8,9,10,-14,16, 17,18,21,X,Y		1q21.1q44				*PRDM16*	*IGFR1*	11,15, 19,20		
	10	4,6,8,10,12,14-,17,18, 21,X				1q21.1q32.2		*TSC1*	*IKZF1, MTAP, CDKN2A/ CDKN2B, ATP10A*	11, 20		
	11	4,6,8,10,14,18-,21, 21,X		17q	17p13.3p11.2			*TSC1*	*NOTCH2, MTAP, CDKN2A/ CDKN2B, ETV6*			
	12	4,6,8,10,14,17-,18, 21,X							*MTAP, CDKN2A/ CDKN2B*		9p	
	13	4,5,6,8,10,11,-12,14, 17,18,21,21,2-2,X				1q21.1q32.2		*TFG, NOTCH1 CBFA2T3, STK11, JAK2*				
	14	4,6,10,14,17,1-8,21, 21,X						*TFG*	*RB1*			
	15	4,10,14,17,18,-21,21,X					1q41q44		*ETV6*			
	16	4,5,6,10,14,17-,21,21,X		7q	7p	12p13.33p13.2			*FIP1L1, MTAP, CDKN2A/ CDKN2B,GPI*			17q
	17	4,6,10,14,17,1-8,21,21,X		1q21.1q44		9p24.3p13.1						
	18	4,6,10,14,17,1-8,21,X			16q11.1q24.3			*TSC1, NOTCH1, JAK2*	*PSEN2, ETV6, FLI1*			
	19	4,6,10,11,12,1-4,17,18,21,22,-X				1q21.1q32.2				9		
	20	2,4,4,5,6,8,10,-11,12,		1q21.1q44		9p24.3q13		*TSC1*	*FIP1L1*			

(*continued on next page*)

Table 2B (*continued*)

Cytogenetic abnormalities	ID	whole chromosome		whole chromosome arm (p) or (q)		segmental abnormalities		single gene abnormalities		CNLOH		
		gain	loss	gain	loss	duplication	deletion	gain	loss	whole chromosome UDP	whole short (p) arm UDP	whole long (q) arm UDP
		14,17,18,21,2-1,22,X,Y										
	21	4,6,10,14,14,1-7,18,21,21, X						*TSC1, EP400*	*MIXL1, ENAH, MRC1, TSPAN8, ASC1*			
	22	4,6,10,14,17,1-8,21, 21,X							*FLI1*			
	23	4,5,6,8,10,14,-16,17,18,21, X						*TSC1*	*FLI1*			
	24	4,6,10,14,15,1-7,18,21,21,X							*FIP1L1*	5, 9		
	25	4,6,10,14,17,1-8,21,21,X,Y							*JAZF1, FLI1*			
	26	4,6,8,10,17,18-,21,21,X,X				1q21.1q42.13; (14q11.2q24.2) x3 (14q24.3-q32.33)x4				20		
	27	4,6,10,14,17,1-8,18,21,X				1q21.1q42.13; 3q25.31q29; 11q24.3q25	2q35q37.3; 12p13.33p12.1		*FIP1L1, FLI1,*			
	28	4,6,8,10,14,17-,18,21,X				15q11.2q13.1		*TSC1, NOTCH1*	*EGLN1, ARL8B*			
	29	4,6,10,14,15,1-7,18,21,21,X,Y							*MTAP, CDKN2A/ CDKN2B, IFNA1, MLLT3*		9p	
	30	4,5,8,10,14,17-,18,21, 21,X,Y		1q21.1q44		6q22.2q27; 16p13.3q12.2		*TSC1, CBFA2T3, NOTCH1*	*FIP1L1, JAZF1, ETV6, FXYD5*			
	31	4,6,10,14,17,1-8,21,21,X				22q11.1q11.21		*TFG*	*ETV6, UBE3A, APT10A, FLI1*	9		
	32	4,6,10,14,17,2-1,21,X, X				1q21.2q32.1		*CTLCL1, SEPT5, CBFA2T3, APRT, CDT1*	*IGF1R*			
	33	4,6,10,14,17,1-8,21,21,X,Y		1q21.1q44		9p24.3q31.1			*MEF2C*	2, 15, 19		
	34	4,6,7,10,12,14-,17,18, 21,22,X							*NOTCH2, PAX5*	13, 16		
High Hyperdi-ploidy without both trisomy 4, 10, 17	35	6,10,14,18,21,-X,Y							*ENAH, PDE4DIP, IGFR1, MRC1*			
	36	4,6,8,14,17,18-,21	X					*CRTC1*	*POU4F1,MRC1*			
	37	4,6,14,17,18,2-1,X						*NOTCH1,*	*PAX5, MRC1*			
	38	4,6,14,17,18,2-1,X							*FIP1L1, MRC1*			
	39	4, 21, X		6p25.3q13		6q22.33q27; 14q11.2q21.3	13q22.3q31.1		*MTAP, CDKN2A/ CDKN2B, IFNA1, MLLT3*			

(*continued on next page*)

Table 2B (*continued*)

Cytogenetic abnormalities	ID	whole chromosome		whole chromosome arm (p) or (q)		segmental abnormalities		single gene abnormalities		CNLOH		
		gain	loss	gain	loss	duplication	deletion	gain	loss	whole chromosome UDP	whole short (p) arm UDP	whole long (q) arm UDP
	40	7,10,18,21,X,X		20q11.21-q13.33			9p24.2q13		*FIP1L1, MRC1*			20q
	41	5, 14,21,21,X						*NOTCH1,*	*ETV6, MRC1*			
	42	5,6,10,11,12,1-4,17,18,21,21, X		1q21.1q44		3q25.31q29; 9p24.2q13			*FIP1L1*			9q
	43	4,6,14,17,18,2-1,21,X,Y							*FIP1L1*			
	44	4,6,9,14,17,18-,21,21,X							*RB1*			
	45	6,10,10,14,18,-21,21,X										
	46	6,10,14,17,18,-21,21,X							*FIP1L1, c20orf94, FLI1, IGF1R, MRC1*			
	47	4, 7,8,9,10, 14,18,21,X	13	5p15.33q11		1q21.2q44; 6p25.3q16.1		*NOTCH1, VANC*	*EBF1, CREBBP*			
	48	4,6,10,14,14,1-8,21,21,X				11q13.1q25; 12p13.33-p13.217p12-q25.3	3p26.2p26.3; 12q15q21.1; 12q23.2q23.3	*NOTCH1*	*PDE4DIP, ETV6, MRC1*			
	49	4,6,10,14,14,1-5,18,21,21,X,Y							*MRC1*	9		
	50	6,8,10,11,12,1-4,17,18,21,22,-X						*NOTCH2, TSC1*	*MRC1*	7, 16		
	51	4,6,14,17,18,2-1,21,X							*JAZF1, ETV6, RB1, MRC1*			
	52	4,6,7,8,14,17,-18,21,21,22,X							*NOTCH2, PDE4DIP, JAZF1, MRC1*			
	53	5,6,8,10,14,17-,18,21		1q21.1q44; 7q	7p		2q22.1q33.1; 3p12q13.33; 9p24.2p21.1; 13q21.1q31	*MAPK12*	*ETV6, MRC1*			
	54	8,10,14,18,18,-21,X		6p25.3q14.3		6q22.2q27			*PAX5, FLI1, MRC1*	5		
	55	6,10,12,14,17,-18,21, 21,X						*NOTCH2, TSC1*				

Abbreviations: CNS-copy number states; 1 = deletion/loss; 3 or 4 = duplication/gain; dup-duplication; del-deletion; p-short arm of chromosome; q-long arm of chromosome; kbp-kilo base pairs; arr-array ; GRCh37 - Genome Reference Consortium Human Build 37; CNLOH copy number loss of heterozygosity.

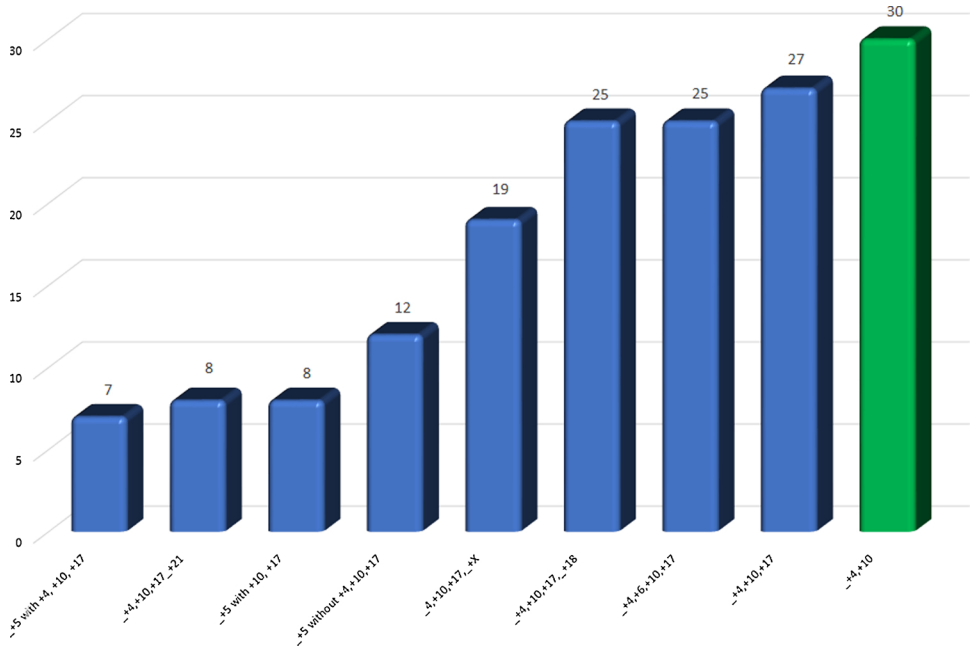

Fig. 2. Frequency of combinations of trisomies detected by SNP microarray analysis in 48 cases of childhood high hyperdiploid BCP-ALL.

Table 3
Comparison of chromosome gains in HeH found in our study vs references.

Chromosome	Our study [%]	Moorman 2003 [5] [%]	Heerema 2000 [7] [%]	Reismüller 2016 [10] [%]	Moorman 1996 [14] [%]	Paulsson 2010 [15] [%]	Paulsson 2013 [16] [%]	Baughn 2015 [17] [%]
21	**100**	100	98	97.1	99	100	98.4	100
14	**95.6**	90	81	76.7	84	91	82.8	90
X	**93.5**	95	88	79.6	89	76.7	90	95
6	**84.7**	86	85	76.2	85	91	83.2	90
4	**58.7**	75	74	71.4	78	80	77.3	100
10	**78.3**	61	63	58.7	63	76	71.2	100
18	**82.6**	78	73	71.4	76	86	77.7	90
17	**67.7**	65	67	64.1	68	77	73.1	70
8	**32.6**	–	37	50	–	36	37.5	–
5	**21,7**	–	20	–	–	26	–	–

HeH - High Hiperdiploidy.

Mb from band 7p11.2 to 7p22.3. These 3 patients presented other CNAs, including the loss of *CDKN2A* and *CDKN2B*. A similar observation was made by Baughn et al., who reported that in the +4, +10 hyperdiploid cases, the *IKZF1* losses were typically appearing with an average of 2 other abnormalities other than *PAX5* or *CDKN2A/CDKN2B*. Similarly, the smaller 71-Kb *IKZF1* deletion was identified in the group in which atypical cytogenetics were studied. This study was conducted by Baughn et al., and they also found losses of *CDKN2A* and *PAX5,* as well as a loss in *ERG*, whereas the loss of *ERG* did not occur in the larger 11.2-Mb deletion [17]. *IKZF1* alterations are less frequent (15% in B-ALL; 9% in HeH-ALL in study by Schwab) than *PAX5* (30% in B-ALL; 4% in HeH-ALL in study by Schwab). Nevertheless, they are associated with an adverse outcome in both BCR-ABL1-positve and negative ALL cases [22]. Our study showed that the *ETV6* gene proved to be the gene most frequently undergoing monoallelic deletion, and this alteration occurred in 16% of cases (vs. 15% – Paulsson et al.; 7% – Schwab et al.) [15,22]. The abovementioned *CDKN2A* and *CDKN2B* genes underwent a monoallelic deletion (vs. 34% of cases – Mullighan et al.; 26.4% of cases – Braun et al.) and were deleted in 13% of cases together with the adjacent *MTAP* gene [23,24].

Moreover, our study indicated high hyperdiploid cases with hidden alterations, such as deletion *IKZF1*, coexisting with second deletions *CDKN2A* and *CDKN2B*. According to recent studies by Stanulla et al., these deletions belong to the new subgroup *IKZF1*^{plus} [25]. None of our patients with the deletion *IKZF1* had a relapse, but all of them were previously qualified for the high-risk group based on other clinical data.

The loss of heterozygosity (LOH) is a common occurrence among hyperdiploid patients, of whom 25% have LOH in one to three whole chromosomes [17]. A study conducted by Paulsson et al. determined that chromosomes 9, 15, and 16 were most frequently involved in LOH. They also proved that the loss of heterozygosity occurred in chromosomes 9 (11%), 11 (8.5%), 12 (5.4%), X and 1 (4.1%), and 7 (2.7%) [15]. In our study, LOH was observed in chromosome 9 (9%), 20 (5.4%) and 5, 7, 11, 15, 19, and X (all 3.6%), and LOH was revealed in the following 4 chromosomal regions: 9p, 17p, 17q and 20q.

5. Conclusion

The benefits of microarray analysis are unquestionable. This analysis should be taken into account, especially when the limitations of banding cytogenetic analysis related to bone marrow cell culture failure are considered. The molecular karyotype obtained by using microarrays enables the detection of the missing or hidden hyperdiploid cases among cases with failed or normal cytogenetics. In conclusion, the chromosome abnormalities discussed by the previously published studies were confirmed by our study. Interestingly, the application of SNP array allowed the

detection of microdeletions involving *IKZF1* and *CDKN2A/CDKN2B* in three high hyperdiploid patients. Moreover, these submicroscopic genetic abnormalities proved to be candidate risk stratification markers that are potentially useful for the detection of childhood ALL. The microarray method may allow to redefine the prognosis for hyperdiploid patients who also have hidden genetic alterations.

Moreover, in the case of BCP-ALL patients who had a normal karyotype or pseudohyperdiploidy (low hyperdiploidy), the use of SNP microarray was a necessary tool to obtain a good diagnostic result. The molecular karyotype results defined microdeletions in the targeted genes. Moreover, this approach both allows for a rational application of SNP microarrays and enables real financial benefits.

Authors' contribution

ML, WM and J.R.K are responsible to the conception and design of the study. JZ, A.Z.P, AP and JT shared patients' clinical data and bone marrow samples. ML, BS, DW and MB conducted laboratory work. ML, JZ, BS, AP and JT responsible for analysis and interpretation data. ML and JRK prepared final manuscript for publication. This manuscript was reviewed and approved by all authors.

Findings

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Conflict of interest statement

There is no conflict of interest to report.

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None. No founding to declare.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2019.05.013.

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