Cancer Genetics 242 (2020) 8–14

Contents lists available at ScienceDirect

Cancer Genetics

journal homepage: www.elsevier.com/locate/cancergen

Enrichment of atypical hyperdiploidy and IKZF1 deletions detected by SNP-microarray in high-risk Australian AIEOP-BFM B-cell acute lymphoblastic leukaemia cohort

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ARTICLE INFO

Article history:
Received 29 July 2019
Revised 11 January 2020
Accepted 28 January 2020

Keywords:
Microarray
Acute lymphoblastic leukemia
Risk stratification
Hyperdiploidy
IKZF1 deletion
High-risk

ABSTRACT

Acute lymphoblastic leukaemia (ALL) is the most common childhood malignancy with the majority of patients being classified as B-cell lineage (B-ALL). The sub-classification of B-ALL is based on genomic architecture. Recent studies have demonstrated the capability of SNP-microarrays to detect genomic changes in B-ALL which cannot be observed by conventional cytogenetic methods. In current clinical trials, B-ALL patients at high risk of relapse are mainly identified by adverse cancer genomics and/or poor response to early therapy. To test the hypothesis that inclusion of SNP-microarrays in frontline diagnostics could more efficiently and accurately identify adverse genomic factors than conventional techniques, we evaluated the Australian high-risk B-ALL cohort enrolled on AIEOP-BFM ALL 2009 study ($n = 33$). SNP-microarray analysis identified additional aberrations in 97% of patients (32/33) compared to conventional techniques. This changed the genomic risk category of 24% (8/33) of patients. Additionally, 27% (9/33) of patients exhibited a 'hyperdiploid' genome, which is generally associated with a good genomic risk and favourable outcomes. An enrichment of *IKZF1* deletions was observed with one third of the cohort affected. Our findings suggest the current classification system could be improved and highlights the need to use more sensitive techniques such as SNP-microarray for cytogenomic risk stratification in B-ALL.

Introduction

Acute lymphoblastic leukaemia (ALL) is the most common cancer and leading cause of cancer death in children [1,2]. Between 85% and 90% of childhood ALL cases achieve long-term cure; however, the outlook is very poor for those who are refractory or relapse [1,3–5]. The genetic profile of B-cell ALL is heterogeneous with many documented subgroups and classifications based primarily on cytogenetic findings. These subgroups have an impact on prognosis and stratification for therapy [3,4,6–8].

The continued development of molecular cytogenomic techniques, such as SNP-microarray, has seen the identification of new recurring sub-microscopic genomic changes in B-cell ALL and uncovered additional complexity in previously straight-forward classifications. These additional genomic findings have challenged the traditional classification system of B-cell ALL and impacted treatment options [1,5,9–18].

Hyperdiploidy or high hyperdiploidy B-cell ALL is a subgroup associated with a good genomic risk (GEN-GR) and a favourable

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https://doi.org/10.1016/j.cancergen.2020.01.051

outcome for paediatric patients. While a favourable outcome for patients in this subgroup is inferred, approximately 20% continue to relapse with a 10% mortality rate [19–21], indicating that 10–20% of patients do not fit the GEN-GR stratification they had been assigned at diagnosis. One explanation for this discrepancy is that patients who have hyperdiploidy according to their G-banded metaphase analysis may have poor risk genomic (GEN–HR) features only detected by more sensitive techniques such as SNP-microarray and MLPA.

Recent studies in B-ALL have uncovered sub-microscopic deletions involving genes such as *PAX5, IKZF1, CREBBP, JAK1/2, RB1, CBF1, CDKN2A* and *ETV6* [1,4,5,17,18,22–27]. These deletions contribute to poor outcomes observed for some patients with what are traditionally considered 'favourable' cytogenetic risk characteristics and have a poor prognostic effect that is independent of other clinical and laboratory risk factors [5,28]. Thus, in a uniformly treated non-high risk B-ALL cohort of patients enrolled on a preceding Australian trial, ANZCHOG Study 8, *IKZF1* microdeletions and *P2RY8-CRLF2* fusions predicted prognosis of patients with a greater impact on relapse free survival than *ETV6-RUNX1*, hyperdiploidy >50 or minimal residual disease (MRD) [29].

Deletions of *IKZF1* have been linked to a higher risk of relapse in several other cohorts [10,11,15,30–33]. Dutch patients classified as non-high risk in the ALL9 treatment protocol who carried an *IKZF1* deletion had a 12-fold greater risk of relapse than other non-high risk patients [34]. Further studies have shown when an *IKZF1* deletion is detected concurrently with a deletion in *PAX5 or CDKN2A/B* or the recurrent deletion in the PAR1 region resulting in a *CLRF2-P2RY8* fusion, and also in the absence of an *ERG* deletion, known as the *IKZF1*-plus group, there is an high risk of relapse [9,35]. This is a new GEN–HR feature of B-cell ALL, which is included in the overall risk stratification of patients on the latest AIEOP-BFM ALL 2017 trial (EudraCT Number: 2016-001935-12) [15].

Recent treatment protocols for ALL have included de-escalation for good risk disease as well as intensification of chemotherapy and sometimes bone marrow transplant for high risk disease. The identification of high risk ALL patients has relied heavily on early measures of response to therapy and has recently focus on identification of B-ALL cases with specific fusion genes that makes them amenable for treatment with tyrosine kinase inhibitors [36,37]. Based on recent evidence, detailed genomic evaluation at diagnosis could provide an equally vital contribution to predicting accurate risk prediction at initial diagnosis.

In this study, we define the molecular architecture, using SNP-microarray, in a clinically defined high-risk B-ALL patient cohort, enrolled in a prospective clinical trial with MRD based treatment response assessment. We describe the additional complexity and challenges observed using SNP-microarray in the classification and genomic risk stratification for B-ALL at diagnosis.

Materials and method

Patients

The study cohort comprised of 33 B-ALL high risk patients enrolled onto the Australian arm of the AIEOP-BFM ALL 2009 trial (EudraCT Number: 2007-004270-43) from December 2011 to December 2016. A total of 42 Australian patients were stratified as high risk B-ALL, but 9 were excluded from the study because of insufficient DNA for research (4), low blast counts (3), poor DNA quality (1) or the patient was not treated as high risk (1).The diagnosis of B-cell ALL was established by standard morphology and flow-cytometric criteria; all patients were BCR-ABL1 negative and were classified as high-risk as per trial protocol. The criteria for high risk included: prednisone poor-response (PPR), flow cytometric MRD ≥10% or higher in bone marrow on day 15, >5% blasts in bone marrow on day 33 (non-remission), PCR-MRD $>4\times10^{-4}$ at day 33 and positive at day 79 (MRD Slow early responder); PCR-MRD $>5\times10^{-4}$ at day 79 (MRD high risk); positive for the *KMT2A-AFF1* (t(4;11)(q21;q23)) fusion or hypodiploidy (<45 chromosomes) (Table 1, column A) at diagnosis. Any of these criteria was sufficient to qualify for high risk, with some patients having more than one high risk indicator. The patients were treated at 3 hospitals -Sydney Children's Hospital, Children's Hospital Westmead or Women and Children's Hospital Adelaide

Conventional cytogenetics and FISH analysis

Conventional G-banded metaphase analysis and FISH analysis were performed at participating AIEOP-BFM ALL 2009 trial Australian laboratories as per individual standard laboratory procedure.

Cytogenomic risk evaluation

This study cohort consists of patients categorised as high-risk by the AIEOP-BFM ALL 2009 trial criteria (EudraCT Number: 2007-

Table 1
Cytogenomic risk stratification of B-ALL as adapted from the AIEOP-BFM ALL 2009 protocol (EudraCT Number: 2007-004270-43) and the AIEOP-BFM ALL 2017 protocol (EudraCT Number: 2016-001935-12).

AIEOP-BFM ALL 2009 protocol	AIEOP-BFM ALL 2017 protocol
Good-risk • ETV6-RUNX1 fusion (t(12;21))	Good-risk • ETV6-RUNX1 fusion (t(12;21)) • Hyperdiploid (51–65 chromosomes. No additional abnormalities)
Intermediate-risk All others not classified as good-risk or poor-risk	Intermediate-risk All others not classified as good-risk or poor-risk
High-risk • Hypodiploidy (<45 chromosomes) • KMT2A-AFF1 fusion • BCR-ABL1 fusion[a]	High-risk • Hypodiploidy (<45 chromosomes) • KMT2A-AFF1 fusion • BCR-ABL1 fusion • IKZF1-plus deletion[b] • TCF3-HLF fusion

[a] BCR-ABL1 fusions are a high-risk cytogenetic feature, however patients were treated on an independent study arm with targeted therapy. The BCR-ABL1 positive patients were excluded from both studies.
[b] A deletion of IKZF1 with one or more co-deletions of CDKN2A, CDKN2B, PAX5 or the fusion-forming deletion of CLRF2-P2RY8 when found in the absence of an ERG deletion.

Table 2
FISH probes applied at diagnosis for genomic changes across 33 B-ALL patients.

FISH Probe	Performed		Positive	
	n	%	n	%
ETV6-RUNX1	25	75.8	1	3.0
BCR-ABL1*	28	84.8	0	0.0
KMT2A rearrangements	29	87.9	3	9.1
Hyperdiploidy (Chr 4,10,17)	10	30.3	0	0.0
CLRF2 rearrangements	3	9.1	0	0.0
TCF3 rearrangements	10	30.3	0	0.0
CDKN2A deletion	1	3.0	0	0.0
IGH rearrangements	1	3.0	0	0.0
RUNX1 (iAMP21)	25	75.8	1	3.0

* BCR-ABL1 positive patients were excluded from this trial.

004270-43), which includes the cytogenomic risk stratification outlined in Table 1A. However, for the purpose of this study we applied the cytogenomic risk stratification as per Table 1B. This stratification is based on the AIEOP-BFM ALL 2017 protocol (EudraCT Number: 2016-001935-12).

We recognise the detection of balanced translocations and fusion genes is a limitation of SNP-microarray. Hence, a combination of SNP-microarray and any FISH probe used to identify a translocation or fusion gene was used to establish a genomic risk for each patient (Tables 2 and 3). This was compared to results obtained from conventional methods - any FISH result combined with G-banded metaphase analysis results.

SNP-microarray

SNP-microarray was performed on DNA extracted from whole bone marrow collected at diagnosis for 31/33 B-cell ALL patients. DNA was extracted from whole peripheral blood collected at diagnosis for two patients. A total of 12/33 samples were assessed using the CytoScan® HD [Affymetrix] platform and the Chromosome analysis suite (ChAS) [Affymetrix] software. The calling algorithm for a loss was set at 25 markers across the genome, 20 markers within cancer genes, 50 markers or 50 kb across the genome and 20 markers within cancer genes for a gain. Loss of heterozygosity was set at ≥3Mb genome wide. Eighteen samples were assessed using the Infinium™ CytoSNP-850 K v1.1 [Illumina] platform and results were viewed and curated using the BlueFuse Multi v4.4 [Illumina] software suite. Algorithm calling was set at 10 markers for a copy number change and ≥3Mb genome-wide for loss of heterozygosity. Three samples were assessed by both SNP-microarray platforms. All data was aligned to reference genome hg19.

MRD

MRD (minimal residual disease) was evaluated for trial patients by real-time quantitative polymerase chain reaction (PCR) analysis of immunoglobulin and T-cell receptor gene rearrangements as previously described [38,39].

Ethics

Approval was given for both the conduct of the AIEOP-BFM ALL 2009 trial in Australia (HREC/09/CHW/158) and for improving risk stratification techniques for patients treated for acute lymphoblastic leukaemia (HREC Reference:(LNR/13/SCHN/367)) by the Sydney Children's Hospitals Network Human Research Ethics Committee. Informed consent was given by parents and patients as per the Declaration of Helsinki.

Results

Patient characteristics

All of the patients in this study were B-ALL cases enrolled in Australia on the international AIEOP-BFM ALL 2009 trial and stratified into the high-risk treatment group. A total of 33 high-risk B-ALL patients were investigated for genomic changes, of which 58% were male. The majority of patients (55%) were aged nine years or younger and 15% were 15 years or older at diagnosis. Peripheral blood studies showed 79% of patients had a white cell count (WCC) of $<50 \times 10^9$/L. Just over half (51%) of the patients were categorised into a high risk by the NCI Rome criteria. To date, 21% of patients have relapsed and 15% have died (Table 4).

Cytogenomic risk classification

Cytogenomic evaluation of each patient was performed at diagnosis using a combination of G-banded metaphase analysis and FISH used to detect specific fusion genes or translocations identified only 2/33 (6%) cases with a GEN–HR feature - both cases KMT2A-AFF1 fusion positive. For the other patients, 6/33 (18%) cases had GEN-GR features and 25/33 (76%) cases had intermediate genomic risk (GEN-IR) (Table 3; *supplementary Table 1*).

In contrast, the combination of SNP-microarray and FISH probes 4/33 (12.1%) identified an additional 3 patients with GEN–HR features giving a total of 5/33 (15%) classified as GEN–HR, while 3/33 (9%) remained GEN-GR and 25/33 (76%) were classified in the GEN-IR category (Table 3). The new GEN–HR cases identified by SNP-microarray included one near-haploid case; one hypodiploid case and a case with the IKZF1-Plus deletion pattern – i.e. carrying a deletion of *IKZF1, CDKN2A* and *CDKN2B (supplementary Table 1)*.

Of those patients identified as GEN–HR a near-haploid genome was observed for patient A30 showing copy neutral loss of heterozygosity (cnLOH) for all chromosomes except for chromosome 18 and 21, which was not detectable in their G-banded metaphase analysis or FISH analysis. Patient A19 exhibited a hypodiploid genome consisting of 37 chromosomes by SNP-microarray. The G-banded metaphase analysis of this patient was normal, and the analysis FISH indicated loss of *ETV6*, chromosome 4 and 17, however was not conclusive of hypodiploidy. The final patient in the GEN–HR group, A33, was also upgraded from a GEN-IR category. Initial results from the G-banded metaphase analysis and FISH analysis showed an atypical hyperdiploid genome with an interstitial deletion of 7p, encompassing the *IKZF1* gene. The SNP-microarray analysis revealed a genome consisting of 54 chromosomes and a deletion of *IKZF1, CDKN2A* and *CDKN2B*, which is consistent with an IKZF1-Plus positive genome and a GEN–HR.

Using the combination of SNP-microarray and fusion FISH, four cases exhibited genomic changes associated with a GEN-GR (Table 3). Patient A3 had an *ETV6-RUNX1* fusion, which was detected by FISH. Interestingly, an atypical signal pattern was observed indicating a loss of one fusion signal. This was consistent with the SNP-microarray findings, which revealed a 10.2Mb loss of 12p that included exons 6–8 of *ETV6*. This was adjacent to a region of cnLOH that included exons 1–5 of *ETV6*. The loss/LOH pattern indicated a breakpoint within intron 5 of *ETV6*. Additionally, there was a region of gain at 21q22.1 to 21q22.12 that included exons 2–9 of *RUNX1*, which is also consistent with a breakpoint within intron 1. These breakpoints are typical for the formation of the ETV6-RUNX1 fusion gene showing breakpoints within *ETV6*(intron 5) and *RUNX1*(intron 1) [40]. However, additional complexity around the breakpoint, including cnLOH of *ETV6*(e1-e5), deletion of *ETV6*(e6-e8) and gain of *RUNX1*(e2-e9), was uncovered by SNP-microarray. While the *ETV6-RUNX1* fusion observed by FISH was not confirmed to exist in a mutagenic orientation located on the der(21), the SNP-

Table 3
Genomic risk categorisation of 33 B-ALL patients, all high risk on the trial, using a combination of G-banded metaphase analysis and FISH or SNP-microarray and FISH used to detect fusions. .

Genomic Risk Category	G-banded metaphase analysis + Any FISH		SNP + Fusion FISH	
High (GEN–HR)	Cases	%	Cases	%
Total	2	6.1	5	15.1
KMT2A-AFF1 fusion	2	6.1	2	6.1
Hypodiploidy/near haploidy (<44chr)	0	0.0	2	6.1
IKZF1-PLUS	0	0.0	1	3.0
Intermediate (GEN-IR)	Cases	%	Cases	%
Total	25	75.8	24	72.7
IKZF1 deletion	1*	3.0	8	24.2
IKZF1 deletion plus ERG deletion	0	0.0	1	3.0
iAMP21	1	3.0	1	3.0
Atypical Hyperdiploid (51–65 chr with additional abnormalities)	2	6.1	6	18.2
Other	15	45.5	9	27.3
Normal	7	21.2	0	0.0
Good (GEN-GR)	Cases	%	Cases	%
Total	6	18.2	3	9.1
ETV6-RUNX1 fusion	1	3.0	1	3.0
High-hyperdiploidy (55–65 chr and no additional abnormalities)	5	15.1	2	6.1

* This patient was also categorised as atypical hyperdiploid.

Table 4
Demographic, clinical and outcome features of the Australian AIEOP-BFM 2009 study patients with High Risk B-ALL.

Variable/Category		*n* (%)
Total number of patients		33
Sex		
	Male	19 (58)
	Female	14 (42)
Age (y)		
	0–9	18 (55)
	10–15	15 (45)
WCC		
	<50×10×9/L	26 (79)
	>50×10×9/L	7 (21)
NCI Rome risk		
	Standard	16 (49)
	High	17 (51)
PCR MRD Risk (D33, D79)		
	No MRD	1(3)
	Standard	4 (12)
	Medium	9 (27)
Slow early responder		7 (21)
	High	12 (36)
Flow MRD risk		
	D15 <10%	14 (42)
	D15>10%	19 (58)
First Remission (CR1)		
	Yes	25 (76)
	No	8 (24)
Relapse		
	No	26 (79)
	Yes	7 (21)
Death		
	No	28 (85)
	Yes	5 (15)

NCI = National Cancer Institute. WCC = white cell count.
βSER slow early responder as defined by the AIEOP-BFM 2009 study.
φMRD was evaluated by real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements as defined by the AIEOP-BFM 2009 study.

microarray results did. Furthermore, the breakpoints were not unusual in nature [40]. In this patient, there were five additional changes observed by SNP-microarray including the three aforementioned. In addition, the two remaining changes were a 25 kb deletion at 15q22.31, which includes the *RASL12* gene and a 1.9Mb deletion at 21q21. All aberrant regions, with the exception of the loss of *RASL12,* have been previously observed in conjunction with an *ETV6-RUNX1* fusion.

Three cases exhibited a hyperdiploid genome with no additional abnormalities detected. Only one of these three hyperdiploid cases was identified by G-banded metaphase analysis and FISH.

High frequency of additional aberrations in 'hyperdiploid' genomes

There were only 10/33 (30.3%) patients investigated for ploidy status using FISH probes for chromosomes 4, 10 and 17 (Table 2) and none were identified as hyperdiploid. Where G-banded metaphase analysis was performed, it identified 7/33 (21.2%) hyperdiploid genomes with 2 of those carrying additional aberrations; i(17q) was identified in one patient and a deletion of 7p in the other *(supplementary Table 2).*

Of the 33 patients investigated by SNP-microarray, 9 (27.3%) exhibited a hyperdiploid genome with a chromosome complement ranging from 51 to 59 (*supplementary Table 1*). Additional aberrations were detected in seven of the 9 patients (77.8%) of which four included copy neutral loss of heterozygosity (cnLOH) *(supplementary Table 1).* Gains of chromosomes 4 and 10 were observed in three patients with hyperdiploidy, of which one exhibited no additional non-aneuploid changes.

A total of four additional regions of gain, three regions of loss and five regions of LOH were observed in patient A7, indicating a much more complex genome than the aneuploid changes originally detected by the G-banded metaphase analysis and FISH combination. Two additional regions of loss and two regions of LOH were detected in patient A11 by SNP-microarray and an additional six regions of loss, two of which were biallelic, were detected in patient A31. Patient A6 exhibited completely different aberrations by G-banded metaphase analysis when compared to SNP-microarray. One cell out of 20 analysed by G-banded metaphase analysis showed a hyperdiploid genome with 58 chromosomes including gains of chromosomes 4, 10 and 17; however, SNP-microarray de-

Table 5
IKZF1 and IKZF1^{PLUS} deletions.

	HR B-cell ALL	IKZF1 Del	IKZF1^{PLUS}
Stanulla, M., et al. (MLPA)	114	36 **(32%)**	11 **(10%)**
Australian AIEOP-BFM 2009 (SNP-microarray)	33	10 **(30%)**	1 **(3%)**

tected cnLOH of whole chromosome 18 in approximately 95% of cells and no evidence of the hyperdiploid cell observed by G-banded metaphase analysis.

Patients A24 and A29 remained in the GEN-GR category despite the investigation by SNP-microarray, which detected a hyperdiploid genome with 51 and 55 chromosomes, respectively, and no additional detectable genomic aberrations. However, the gained chromosomes observed for patient A24 did not fit a 'typical' B-ALL hyperdiploid pattern (+*X*,+8,+14,+21,+21).

IKZF1 deletions

No FISH studies were specifically performed to detect a deletion of the *IKZF1* gene across the 33 B-ALL patients. G-banded metaphase analysis detected a deletion in one (3%) patient with del(7)(p?11.2p15) *(supplementary Table 2)*. An *IKZF1* deletion was identified in 10 (30%) patients by SNP-microarray. Half were intragenic deletions extending from exon 4 to exon 7, which ranged from 47.4 kb to 52.2 kb in size. The remaining five cases exhibited a whole gene deletion ranging from 609 kb to 58Mb in size *(supplementary Table 1)*. The samples with *IKZF1* exon 4–7 deletions (except for A25) were independently confirmed by RQ-PCR analysis [41].

Investigation of IKZF1-plus patients was only performed by SNP-microarray. The IKZF1-plus category of B-ALL was defined as per Stanulla et al. [15] (Table 1B2). This methodology identified one patient who, in addition to having an *IKZF1* deletion, had a concurrent deletion of both *CDKN2A* and *CDKN2B* and one patient had a concurrent deletion of *ERG* by SNP-microarray. There were no patients with concurrent deletions of IKZF1 and either *PAX5* or the PAR1 region *(supplementary Table 1)*.

Discussion

Our SNP-microarray analysis of a high-risk B-ALL patient cohort from a prospective clinical trial, identified one or more genomic aberrations in all 33 patients. SNP-microarray found genomic aberrations not seen by FISH or karyotyping in 97% (32/33) of patients clearly demonstrating the superiority of this approach for the identification of genome wide changes. Moreover, the application of SNP-microarray increased the number of patients classified with a GEN–HR from 6.1% (2/33) to 15.1% (5/33), while the number of patients with GEN-IR features remained unchanged.

Of the five high risk patients who had GEN–HR factors identified by the combination of SNP-microarray and fusion FISH analysis, two carried a *KMT2A-AFF1* fusion and were also identified by G-banded metaphase analysis and FISH. The remaining three GEN–HR patients were upgraded from the GEN-IR group.

The original analysis by G-banded metaphase analysis and FISH identified 18.2% (6/33) of patients with GEN-GR, specifically one *ETV6-RUNX1* and 5 hyperdiploid cases. After evaluation using SNP-microarray instead of karyotyping, 3 hyperdiploid cases moved to GEN-IR due to identification of new features. It is interesting to consider why some GEN-GR patients are classified as high risk by their poor response to therapy, so we examined them for potential contributing factors identifiable by microarray. It is well recognised that the *ETV6-RUNX1* fusion is not sufficient to cause disease in itself, suggesting that additional genomic changes are required for the development of leukemic disease [42,43]. In the high risk ETV6-RUNX1 case (A3), the loss of *RASL12* loss may contribute to the slow early MRD response and subsequent trial stratification of this patient as high risk [43]. The two hyperdiploid patents who remained as GEN-GR were also atypical patients which may explain their resistance to early chemotherapy (high flow MRD at day 15 or slow early PCR-MRD response). Patient A24 was observed to have an extra chromosome 8, which is rarely reported in B-ALL, but is a common feature in myeloid malignancies [1,44–46]. There was also a notable absence of gains of chromosome 4, 10 and 17, which are usual features of GEN-GR high hyperdiploid ALL [21,45]. The other atient (A29) did include gains of chromosomes 4, 10 and 17; however, the +10 was observed in only a sub clonal population (p∼10%) of the cells when compared to the remaining aberrations (∼90%).The four remaining patients who would be classed as having GEN-GR features by conventional approaches based on hyperdiploidy (>50 chromosomes) categorisation, A6, A7, A11 and A31, were all upgraded to the GEN-IR category due to additional findings revealed by SNP-microarray. SNP array also revealed a much more complex genome for A7; several more losses for A31; and cnLOH for chromosome 18 for patient A6.

Previously published data suggests whole chromosome LOH (wLOH) is prevalent in 25–30% of hyperdiploid genomes [6,47,48]. We detected wLOH in 44.4% (4/9) of patients with hyperdiploidy, this enrichment may be due to the high-risk nature of this cohort. Since only 9 patients were found to have hyperdiploidy based solely on chromosome number, investigation in other high-risk cohorts may be worthwhile.

A previous microarray study performed on 74 hyperdiploid B-ALL genomes by Paulsson et al. [6] identified additional copy number changes >10Mb in size in 22% of patients and changes <10Mb in size in 60% of patients. In contrast we observed additional copy number changes in a higher number of cases with hyperdiploid genomes, 66.7% and 77.8%, respectively. The fact that unlike us, the previous study did not select patients who were stratified into high-risk but was largely based on their response to therapy may explain the differences in results between the two studies.

Despite the small study size of 33, the frequency of *IKZF1* deletions detected in this cohort by SNP-microarray is similar to those reported by Stanulla et al. [15] in a cohort of 114 high-risk B-ALL patients, using multiplex ligation-dependent probe amplification (MLPA) as a detection method (Table 5). However, the incidence of IKZF1-Plus patients differs from this study, showing a lower incidence rate. This may be attributed to the small study size or the difference in technique (MLPA v's SNP-microarray). Case A33, was identified as IKZF1-Plus by SNP-microarray and was originally classified as an atypical hyperdiploidy showing 54 chromosomes with an interstitial deletion of 7p. This case in particular highlights the importance of incorporating high-resolution whole genome techniques at diagnosis for patients with B-ALL.

The implementation of SNP-microarray challenges the traditional tools used to classify patients into B-ALL cytogenetic risk groups. This study of high-risk patients has highlighted the additional complexity observed in the traditionally classified high hyperdiploid group, which is based on having 51–65 chromosomes with no additional aberrations. Interestingly, there are significantly more traditionally categorised 'hyperdiploid' patients (29%) in this high-risk cohort when compared to the AIEOP-BFM 2000 study (3.7%), which also used an MRD-based risk stratification system [3]. However, the AIEOP-BFM 2000 study used DNA indexing and

not traditional cytogenetics or SNP-microarray to assess for hyperdiploid status. DNA indexing would not detect small genomic gains or losses nor would it detect LOH, but it does give an overall DNA load for each cell. Although we have used different methods, after adjusting for the additional genomic changes observed by SNP-microarray in this cohort the total hyperdiploid genomes reduce to 6%, which is not significantly higher than the BFM study.

In summary, we present high-resolution whole genome profiles using SNP-microarray, of an Australasian cohort high-risk B-ALL, from a pivotal clinical trial. This study demonstrates the value of performing SNP-microarray investigations at diagnosis by unveiling the increased genomic complexity of patients with high-risk B-ALL. SNP-microarray at diagnosis was more accurate and efficient in detecting genomic changes that contribute to a genomic risk assessment than traditional cytogenetic techniques alone. In particular, it provides a consistent genome-wide analysis that obviates the heterogeneity in FISH panel probe selection for copy number investigations.

While this study represents a small cohort, it provides key insights into patients who have high-risk disease and require intensified treatment options. Our knowledge of the molecular consequences of small genomic alterations will expand with the wider use of SNP-microarray studies to reliably detect smaller genome-wide changes and loss of heterozygosity. The improved genomic risk stratification of patients at diagnosis enables the use of targeted or risk- based therapeutic interventions. SNP-microarray is increasingly being accepted as a diagnostics tool for haematological malignancies with incorporation of this technique into guidelines and prospective trials.

Funding and support

This work was supported by the Children's Cancer Institute and funded by NHMRC grant APP1009087, Norris; NHMRC grant APP1057746, Sutton; Tour de Cure Haber; Cancer Australia PdCCRs APP1128727, Sutton; Pathology North PTTF funds (PN-H 06/2016) and Hunter Cancer Research Alliance grants (HCRA 03/2016). AKE is supported by the clinical translational research fellowship (NSW HP/ CMN/ HNE LHD and HCRA, UON 2017–2020).

Declaration of Competing Interest

There is no conflict of interest for any of the authors.

CRediT authorship contribution statement

Nadine K Berry: Methodology, Conceptualization, Data curation, Formal analysis, Writing - review & editing. **Rodney J Scott:** Conceptualization, Writing - review & editing. **Rosemary Sutton:** Conceptualization, Writing - review & editing. **Tamara Law:** Writing - review & editing. **Toby N Trahair:** Writing - review & editing. **Luce Dalla-Pozza:** Writing - review & editing. **Petra Ritchie:** Writing - review & editing. **Draga Barbaric:** Writing - review & editing. **Anoop K Enjeti:** Conceptualization, Writing - review & editing.

Acknowledgements

The authors would like to thank the AIEOP-BFM ALL2009 international trial committee and chairmen Prof Martin Schrappe and Prof Valentino Conter for trial concept and organisation. We acknowledge and thank South Australia Pathology (Adelaide); Children's Hospital Westmead and South Eastern Area Laboratory Services (Randwick) for performing cytogenetic analyses and the Tumour bank and MRD staff at Children's Cancer Institute for processing samples and developing MRD tests for each patient. We'd also like to thank Oskar Haas's team at St. Anna Children's Cancer Research Institute (CCRI), Austria for passing on their invaluable SNP-microarray analysis skills and knowledge. Without their help this publication would not have been possible. We are also thankful to Alexandra Smith for her help with editing the manuscript for publication.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2020.01.051.

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