Regular Article

CLINICAL TRIALS AND OBSERVATIONS

A novel integrated cytogenetic and genomic classification refines risk stratification in pediatric acute lymphoblastic leukemia

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Key Points

- Integrating cytogenetic and genomic data in pediatric ALL reveals 2 subgroups with different outcomes independent of other risk factors.
- A total of 75% of children on UKALL2003 had a good-risk genetic profile, which predicted an EFS and OS of 94% and 97% at 5 years.

Recent genomic studies have provided a refined genetic map of acute lymphoblastic leukemia (ALL) and increased the number of potential prognostic markers. Therefore, we integrated copy-number alteration data from the 8 most commonly deleted genes, subordinately, with established chromosomal abnormalities to derive a 2-tier genetic classification. The classification was developed using 809 ALL97/99 patients and validated using 742 United Kingdom (UK)ALL2003 patients. Good-risk (GR) genetic features included *ETV6-RUNX1*, high hyperdiploidy, normal copy-number status for all 8 genes, isolated deletions affecting *ETV6/PAX5/BTG1*, and *ETV6* deletions with a single additional deletion of *BTG1/PAX5/CDKN2A/B*. All other genetic features were classified as poor risk (PR). Three-quarters of UKALL2003 patients had a GR genetic profile and a significantly improved event-free survival (EFS) (94%) compared with patients with a PR genetic profile (79%). This difference was driven by a lower relapse rate (4% vs 17%), was seen across all patient subgroups, and was independent of other risk factors. Even genetic GR patients with minimal residual disease (>0.01%) at day 29 had an EFS in excess of 90%. In conclusion, the integration of genomic and cytogenetic data defines 2 subgroups with distinct responses to treatment and identifies a large subset of children suitable for treatment deintensification. (*Blood*. 2014; 124(9):1434-1444)

Introduction

Somatic genetic alterations that initiate and drive carcinogenesis are the hallmarks of cancer. Genomic profiling has revolutionized our understanding of cancer and refined the classification of patients into clinically relevant subgroups.[1] This is exemplified in pediatric B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) by significant chromosomal abnormalities that provide important diagnostic and prognostic information that is routinely used in risk stratification for treatment. Patients harboring *ETV6-RUNX1* or high hyperdiploidy (51-65 chromosomes) typically have a favorable outcome, while those with t(9;22)(q34;q11.2)/*BCR-ABL1*, *MLL* rearrangements, near haploidy (<30 chromosomes), low hypodiploidy (30-39 chromosomes), intrachromosomal amplification of chromosome 21 (iAMP21), or t(17;19)(q23;p13) have an inferior outcome when treated with standard therapy.[2] Modern treatment protocols stratify patients on factors such as genetics, age, white cell count (WCC), and treatment response. High-risk (HR) patients receive more intensive chemotherapy, stem cell transplantation,

or targeted therapy, whereas low-risk (LR) patients are treated less intensively.

Genomic technologies have identified a plethora of novel copy-number alterations (CNAs) and sequence mutations that typically affect genes involved in lymphoid differentiation, proliferation, cell cycle, and transcription.[3] In contrast to chromosomal abnormalities, which are commonly initiating events, these CNAs are usually cooperating aberrations that correlate with specific cytogenetic subtypes.[4] Several studies have reported a poor outcome for patients with these aberrations, especially *IKZF1* deletions[5-8] and *P2RY8-CRLF2* fusion,[9-12] but a consensus view of how best to incorporate the results into current stratification algorithms has failed to emerge. The major complicating factor is the frequency with which these so-called poor-risk (PR) secondary abnormalities coexist with each other and with other HR genetic subtypes, such as iAMP21, *BCR-ABL1*, and, particularly, the *BCR-ABL1*–like (or Philadelphia chromosome–like) subtype.[4,13] The *BCR-ABL1*–like subtype is defined

There is an Inside *Blood* Commentary on this article in this issue.

Figure 1. Development of a risk index based on CNAs. The copy-number status of 8 genes/regions was assessed in a cohort of 864 patients treated on ALL97/99 by MLPA using the P335 kit. Patients were classified according to the copy-number status (deleted/not deleted) of all 8 genes/regions. A total of 67 unique combinations were observed (see supplemental Table 2, available on the *Blood* Web site). Patients with each unique combination were classified into risk groups according to the algorithm shown below (A). For combinations observed in ≥10 patients, a Cox regression model was used to estimate the risk of an event and patients were assigned to risk groups (good-risk [GR], PR, intermediate risk [IR] 1 [INT-1], or IR 2 [INT-2]) based on the magnitude of the hazard ratio (HR) and the size of the *P* value (p). All combinations observed in <10 patients were assigned to the indeterminate (IND) risk group. The event-free survival (EFS) of the 5 risk groups is shown in the Kaplan-Meier graph (B).
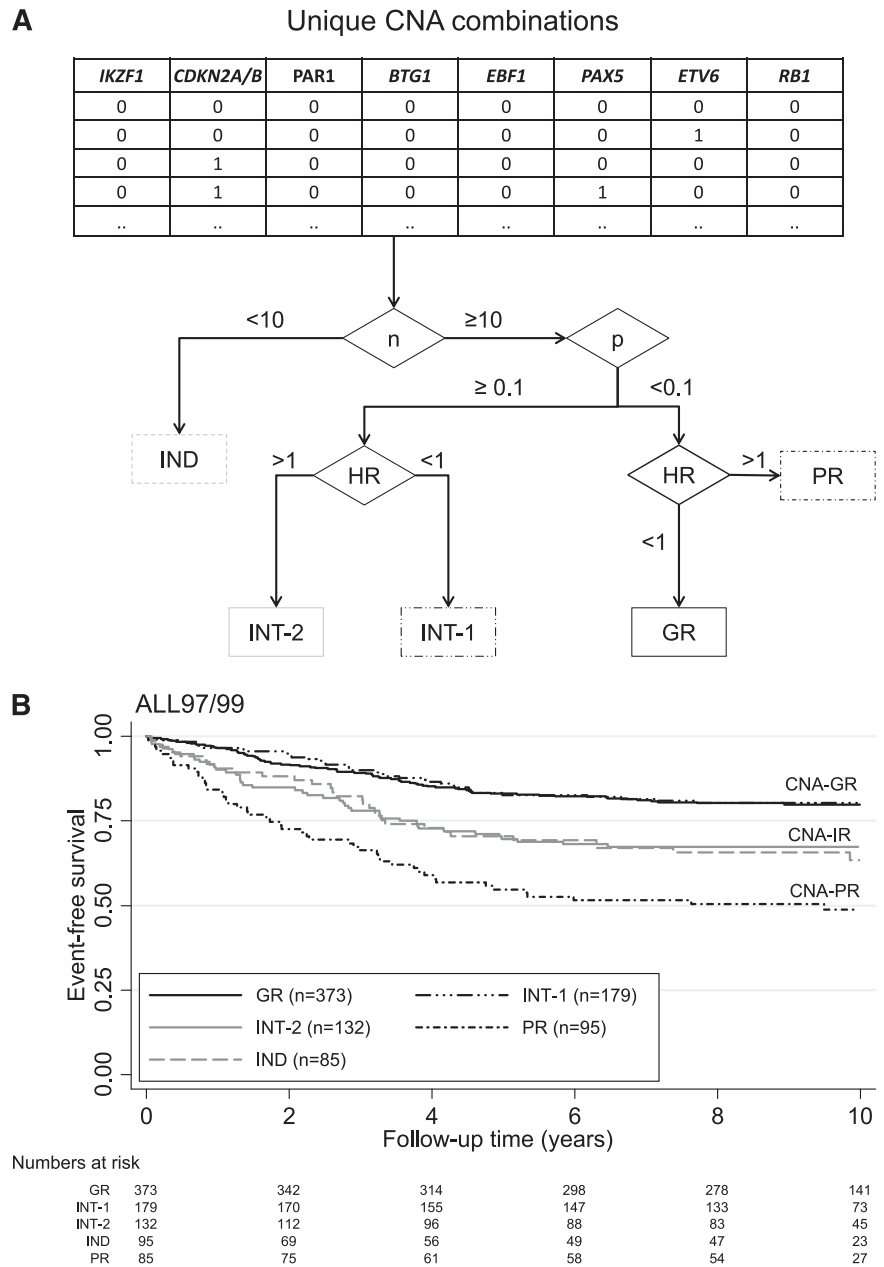
by a gene expression signature that is similar to that observed for *BCR-ABL1* cases but occurs in patients lacking this gene fusion. *BCR-ABL1*–like is mutually exclusive of established chromosomal abnormalities but is enriched for *IKZF1* deletions, *CDKN2A*/B deletions, *JAK2* mutations, and *CRLF2* rearrangements.[4,13] However, to date, only 2 groups have published expression data on this subtype; the Dutch Childhood Oncology Group[14,15] described 2 unselected cohorts of patients, whereas the US Children's Oncology Group reported on an HR cohort of patients.[13] Although both groups reported inferior outcomes for their *BCR-ABL1*–like patients, the key drivers of relapse have yet to be elucidated. Moreover, as it is unclear if the 2 profiles identify the same subset of patients, other groups cannot reliably adopt this approach for risk stratification. Therefore, there remains an urgent clinical need to optimally integrate genomic data into an informative classification system.

In this study, we have adopted a novel approach to overcome this problem by integrating CNA data from 8 key genes/loci with

established cytogenetic risk groups to generate a novel overarching genetic risk classification of pediatric BCP-ALL. We have validated this new classification using an independent patient cohort treated on a modern protocol and demonstrated how it interacts with other risk factors.

Patients and methods

Patients were diagnosed with BCP-ALL by standard flow-cytometric criteria and were treated on Medical Research Council (MRC) ALL97/99 (1997–2002) or United Kingdom (UK)ALL2003 (2003–2011) as previously described.[16,17] Local ethical committee approval was obtained for ALL97 by individual treatment centers, whereas approval for UKALL2003 was obtained from the Scottish Multi-Centre Research Ethics Committee. Informed consent was given by parents and patients in accordance with the Declaration of Helsinki. In

Table 1. Demographic, clinical, genetic, and outcome features of MRC ALL97/99 and UKALL2003 patients with BCP-ALL stratified by genetic risk group

Variable/category	ALL97/99			UKALL2003		
	GEN-GR, n (%)	GEN-PR, n (%)	P	GEN-GR, n (%)	GEN-PR, n (%)	P
Total number of patients	579 (72)	230 (28)	—	561 (76)	181 (24)	—
Sex						
Female	255 (44)	100 (44)	1	262 (47)	78 (43)	.49
Male	324 (56)	130 (56)		299 (53)	103 (57)	
Age (y)						
1-9	501 (87)	150 (65)	<.001	465 (83)	103 (56)	<.001
10-14	63 (11)	66 (29)		65 (11)	48 (27)	
≥15	15 (3)	14 (6)		31 (5)	30 (17)	
WCC						
<50 × 10^9/L	498 (86)	142 (62)	<.001	486 (87)	129 (71)	<.001
>50 × 10^9/L	81 (14)	88 (38)		75 (13)	52 (29)	
NCI risk group*						
Standard	426 (74)	87 (38)	<.001	399 (71)	69 (37)	<.001
High	153 (26)	143 (62)		162 (29)	112 (63)	
Cytogenetic risk group†						
GR	469 (81)	0 (0)	<.001	453 (81)	0 (0)	<.001
IR	110 (19)	157 (70)		108 (19)	138 (70)	
HR	0 (0)	73 (30)		0 (0)	43 (30)	
MRD‡						
LR	—	—	—	244 (43)	58 (32)	<.001
HR	—	—	—	176 (31)	86 (48)	
IR	—	—	—	141 (25)	37 (20)	
Complete remission						
No	3 (1)	5 (2)	.05	2 (0)	0 (0)	1
Yes	576 (99)	225 (98)		559 (100)	181 (100)	
Event						
No	476 (82)	119 (52)	—	528 (94)	143 (79)	—
Yes	103 (18)	111 (48)		33 (6)	38 (21)	
Relapse						
No	490 (85)	133 (59)	—	540 (97)	153 (85)	—
Yes	86 (15)	92 (41)		19 (3)	28 (15)	
Dead						
No	529 (91)	147 (64)	—	544 (97)	150 (83)	—
Yes	50 (9)	83 (36)		17 (3)	31 (17)	

*NCI standard risk includes patients who at diagnosis were <10 years old and had a WCC <50 × 10^9/L; NCI HR includes all other cases.

†Cytogenetic GR (CYTO-GR) includes *ETV6-RUNX1* and high hyperdiploidy (51-65 chromosomes); cytogenetic PR (CYTO-PR) includes t(9;22)(q34;q11.2)/*BCR-ABL1*, *MLL* translocations, near haploidy (<30 chromosomes), low hypodiploidy (30-39 chromosomes), intrachromosomal amplification of chromosome 21 (iAMP21), or t(17;19)(q23;p13)/*HLF-TCF3*; cytogenetic IR (CYTO-IR) includes all other cases with abnormal or normal cytogenetics. Cytogenetics failed or was not performed for 55 ALL97/99 and 38 UKALL2003 patients.

‡MRD was evaluated by real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements as defined by the European MRD Study Group. Patients were classified by MRD at day 29 (end of induction) as HR (> 0.01%), LR (< 0.01%) or indeterminate (no sample, test failed). Patients treated on ALL97/99 were not evaluated by MRD.

UKALL2003, minimal residual disease (MRD) was evaluated by real-time quantitative polymerase chain reaction (PCR) analysis of immunoglobulin and T-cell receptor gene rearrangements as defined by the European MRD Study Group.[18] Patients were classified into 1 of 3 groups based on their MRD results: (1) LR (patients with undetectable MRD at the end of induction [day 29] or patients with low-level MRD [<0.01%] at day 29 and undetectable MRD at the start of interim maintenance); (2) HR (patients with MRD >0.01% at day 29); or (3) indeterminate group (patients with no MRD results due to no or poor sample and patients with persistent low-level disease [<0.01% MRD] before the start of interim maintenance).[16] Full details of the treatment regimens have been published.[16,17] Briefly, in ALL99 and

UKALL2003, patients were assigned to regimen A or B based on whether they were National Cancer Institute (NCI) standard (<10 years old and WCC <50 × 10^9/L) or HR (≥10 years old or WCC >50 × 10^9/L), respectively. Regimen A comprised a 3-drug induction (vincristine, steroids, and asparaginase) followed by consolidation (daily mercaptopurine and weekly intrathecal methotrexate), central nervous system–directed therapy, interim maintenance (daily mercaptopurine, weekly methotrexate, monthly vincristine, and steroid pulses), delayed intensification (asparaginase, vincristine, dexamethasone, and doxorubicin), and continuing therapy (oral mercaptopurine and methotrexate, monthly vincristine and steroid pulses, and intrathecal methotrexate every 3 months). Regimen B patients additionally received

Table 2. Outcome features of MRC ALL97/99 and UKALL2003 patients with BCP-ALL stratified by genetic risk group

Outcome measure	ALL97/99 10-y rate (99% CI)			UKALL2003 5-y rate (99% CI)		
	GEN-GR	GEN-PR	P	GEN-GR	GEN-PR	P
EFS	82% (78-86)	51% (42-59)	<.001	94% (90-96)	79% (69-85)	<.001
Relapse risk	15% (12-20)	44% (35-53)	<.001	4% (2-7)	17% (10-26)	<.001
OS	91% (88-94)	65% (56-73)	<.001	97% (94-98)	83% (73-89)	<.001

CI, confidence interval; OS, overall survival.

Table 3. Outcome features of MRC ALL97/99 and UKALL2003 patients with BCP-ALL

|	HR (99% CI), *P* value	
Outcome measure	ALL97/99	UKALL2003
EFS	3.40 (2.60-4.45), <.001	3.98 (2.50-6.35), <.001
Relapse risk	3.45 (2.57-4.63), <.001	5.29 (2.96-9.48), <.001
OS	4.96 (3.49-7.05), <.001	6.09 (3.37-11.01), <.001

daunorubicin during induction and Berlin-Frankfurt-Münster consolidation (4 weeks of cyclophosphamide and cytarabine). Regimen C patients received an additional 4 doses of vincristine and 2 doses of PEGylated asparaginase during Berlin-Frankfurt-Münster consolidation. Furthermore, regimen C patients received escalating doses of intravenous methotrexate without

folinic acid rescue and vincristine and PEGylated asparaginase as interim maintenance (Capizzi maintainance).

Cytogenetic and fluorescence in situ hybridization testing was performed on pretreatment bone marrow samples by member laboratories of the UK Cancer Cytogenetics Group or centrally by the Leukaemia Research Cytogenetics Group, and results were reported using established nomenclature and definitions.[2] Multiplex ligation-dependent probe amplification (MLPA) was performed on DNA extracted either directly from pretreatment bone marrow samples or from fixed cell suspensions. The tested cohorts were representative in terms of sex, age, and outcome but were marginally enriched for patients with higher WCC and HR cytogenetics (supplemental Table 1). The SALSA MLPA kit P335 (MRC Holland, Amsterdam, The Netherlands), which included probes for *IKZF1*, *CDKN2A/B*, *PAX5*, *EBF1*, *ETV6*, *BTG1*, *RB1*, and PAR1 (*CSF2RA/IL3RA/CRLF2*), was used to identify CNAs in
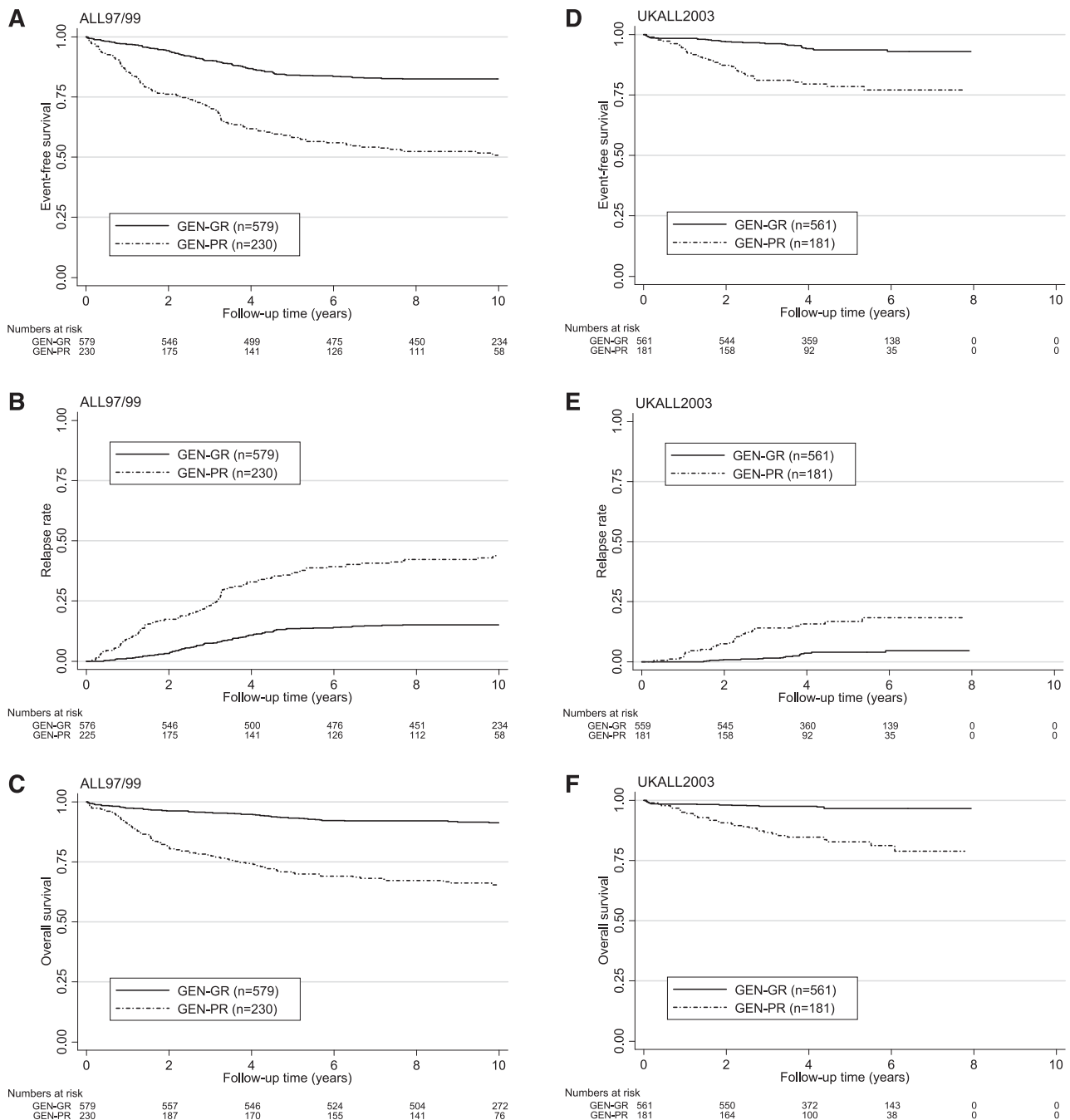
Figure 2. Kaplan-Meier survival graphs for 809 ALL97/99 and 742 UKALL2003 patients classified according to genetic risk group. EFS (A,D), relapse risk (B,E), and OS (C,F).

A

B
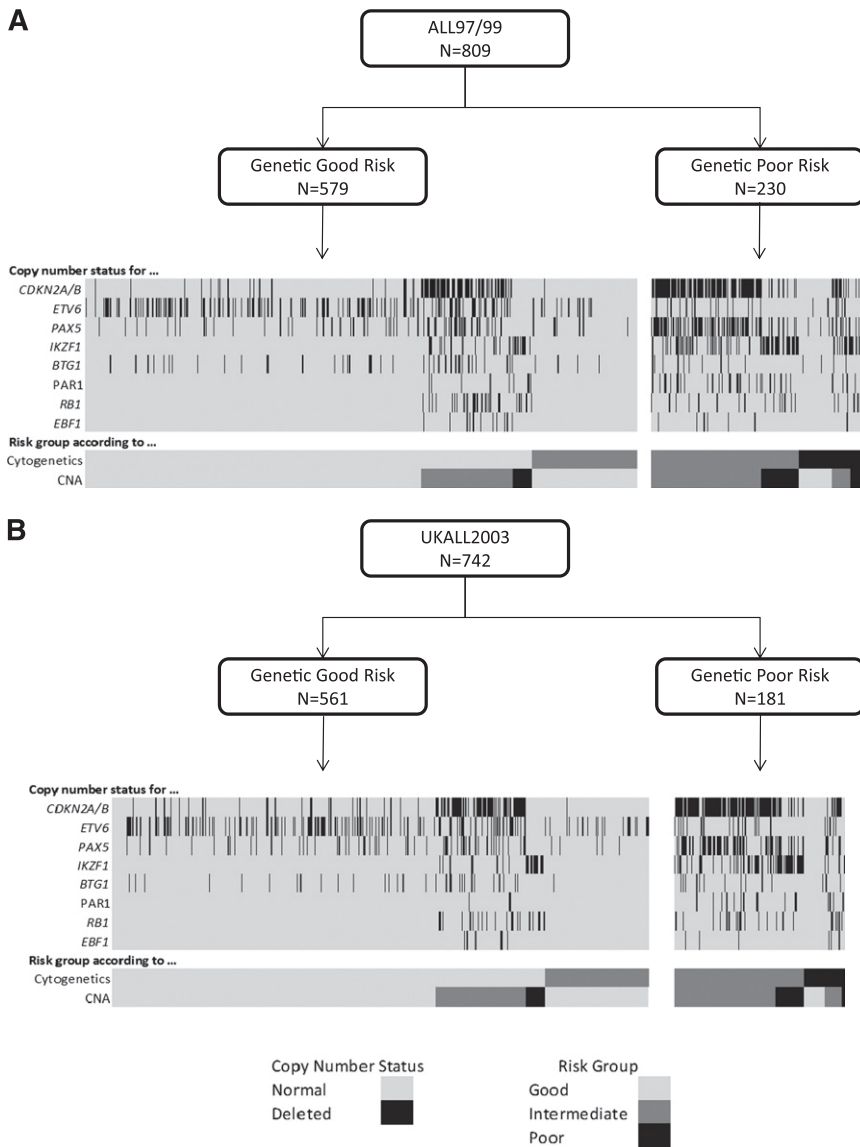
Figure 3. Cytogenetic and CNA landscape of novel genetic risk groups. Diagrams depicting the relationship between genetic risk, cytogenetic risk, CNA risk groups, and the copy-number status of 8 key genes/regions for patients treated on ALL97/99 (A) and UKALL2003 (B).

these genes.[4] Previous studies have demonstrated that MLPA can accurately detect deletions in all these genes, which are present in more than 20% to 30% of cells.[4,19] A detailed description and breakdown of each CNA and the correlation with specific chromosomal abnormalities for all the patients in these 2 cohorts has been published.[4]

Patients were classified into 3 mutually exclusive cytogenetic risk groups[2] based on the clonal presence of the following chromosomal abnormalities: CYTO-GR (*ETV6-RUNX1*, high hyperdiploidy), CYTO-HR [*BCR-ABL1*, *MLL* rearrangements, near haploidy, low hypodiploidy, iAMP21 or t(17;19)], or cytogenetic CYTO-IR (all other cases with abnormal or normal cytogenetics). The results of the MLPA analysis were used to generate a CNA profile for each case, whereby each of the 8 genes/loci was coded as deleted or not deleted. For *CDKN2A/B*, deletion of either *CDKN2A* or *CDKN2B* was sufficient for the locus to be classified as deleted. For *PAX5*, intragenic amplifications were coded with the deletions as they are predicted to be functionally equivalent.[20] A deletion in the PAR1 region of chromosome X or Y, del(X)(p22.33p22.33) / del(Y)(p11.32p11.32), results in the loss of the *CSF2RA* and *IL3RA* probes but retention of the *CRLF2* probe on the MLPA P335 kit.[21,22] Therefore, PAR1 deletion is usually synonymous with *PR2Y8-CRLF2* fusion.[4,22] However, a PAR1 deletion can also arise from an unbalanced t(X;14)(p22;q32) or t(Y;14)(p11;q32) translocation, which results in *IGH-CRLF2* fusion.[21] Like *P2RY8-CRLF2*, this translocation results in overexpression of *CRLF2*. As this
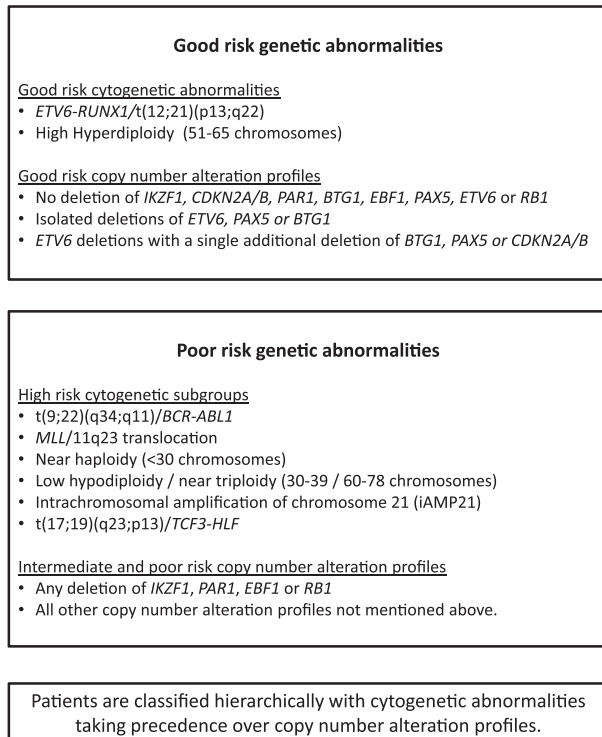
scenario of translocation plus deletion is rare and essentially produces the same result, for the purposes of this paper, we have considered PAR1 deletion synonymous with *P2RY8-CRLF2*.

Survival analysis considered 3 end points: EFS, defined as time to relapse, second tumor, or death, censoring at last contact; relapse rate (RR), defined as time to relapse for those achieving a complete remission, censoring at death in remission or last contact; and OS, defined as time to death, censoring at last contact. Survival rates were calculated and compared using Kaplan-Meier methods, log-rank tests, and Cox regression models (univariate and multivariate analyses). Other comparisons were performed using the χ^2 or Fisher's exact test. Due to the investigative nature of this analysis, all tests were conducted at the 1% significance level. All analyses were performed using Intercooled Stata 13.0 (Stata Corporation).

Results

Frequency and prognostic value of individual gene deletions

CNAs were determined by MLPA in representative cohorts of patients treated on ALL97/99 (n = 864) or UKALL2003 (n = 780) (supplemental Table 1). The most prevalent CNAs were *CDNK2A/B* and

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Good risk genetic abnormalities

<u>Good risk cytogenetic abnormalities</u>
- *ETV6-RUNX1*/t(12;21)(p13;q22)
- High Hyperdiploidy (51-65 chromosomes)

<u>Good risk copy number alteration profiles</u>
- No deletion of *IKZF1, CDKN2A/B, PAR1, BTG1, EBF1, PAX5, ETV6* or *RB1*
- Isolated deletions of *ETV6, PAX5* or *BTG1*
- *ETV6* deletions with a single additional deletion of *BTG1, PAX5* or *CDKN2A/B*

Poor risk genetic abnormalities

<u>High risk cytogenetic subgroups</u>
- t(9;22)(q34;q11)/*BCR-ABL1*
- *MLL*/11q23 translocation
- Near haploidy (<30 chromosomes)
- Low hypodiploidy / near triploidy (30-39 / 60-78 chromosomes)
- Intrachromosomal amplification of chromosome 21 (iAMP21)
- t(17;19)(q23;p13)/*TCF3-HLF*

<u>Intermediate and poor risk copy number alteration profiles</u>
- Any deletion of *IKZF1, PAR1, EBF1* or *RB1*
- All other copy number alteration profiles not mentioned above.

Patients are classified hierarchically with cytogenetic abnormalities taking precedence over copy number alteration profiles.

Figure 4. Definition of novel genetic risk groups for pediatric BCP-ALL.

ETV6 deletions occurring in ~20% to 25% cases, whereas ~15% cases harbored deletions of *IKZF1* or *PAX5* (supplemental Table2). The remaining CNAs were rarer and each observed in <10% cases. There was considerable heterogeneity with respect to the number of CNAs per case and distribution by cytogenetic risk group. No CNAs were observed in 43% cases, whereas 30%, 18%, and 10% of cases had 1, 2, or ≥3 CNAs. Individual assessment of the CNAs revealed that most deletions were associated with a poor outcome in both trials (supplemental Figure 1), the exception being *ETV6* deletions, which are associated with the GR abnormality *ETV6-RUNX1*. These findings indicate that all 8 genes contribute to patient outcome. Furthermore, they were unevenly distributed across the 3 cytogenetic risk groups that are known to predict outcome.[2] Therefore, we opted to integrate the CNA data into our existing cytogenetic classification in order to generate an overarching classification.

Development of a novel integrated genetic classification using the ALL97/99 cohort

Using the copy-number status (deleted vs not deleted) for each of the 8 genes/loci covered by the MLPA P335 kit, a total of 67 unique CNA profiles were observed among 864 ALL97/99 patients (supplemental Table 3). We assessed each unique CNA combination in turn as described in Figure 1. CNA combinations that were present in <10 patients were classified into the indeterminate group. The prognostic relevance of each of the remaining CNA combinations was assessed, and combinations were allocated to the GR, IR 1, IR 2, or PR groups depending on the HR for an event and *P* value derived from a Cox regression model. These groups were then combined into 3 risk groups: CNA-GR, CNA-IR, and CNA-PR (Figure 1). These 3 CNA risk groups comprised 61%, 29%, and 10% of cases and were associated with different EFS at 10 years: 80% (99% CI 75-84), 67% (59-74), and 49% (35-62) (supplemental Table 4 and supplemental Figure 2A-C). Next, we cross-tabulated these 3 groups (CNA-GR, CNA-IR, and

CNA-PR) with the 3 cytogenetic risk groups (CYTO-GR, CYTO-IR, and CYTO-HR). Therefore, 809 patients with both cytogenetic and MLPA data were classified into 9 mutually exclusive subgroups. Four subgroups showed EFS, RR, and OS rates >75%, <25%, and >80% at 5 years, respectively (supplemental Figure 3A-C). These subgroups comprised all CYTO-GR patients and CYTO-IR/CNA-GR patients and were henceforth combined to create a good-risk genetic group (GEN-GR) (supplemental Figure 3D). The remaining 5 subgroups showed inferior survival rates and were henceforth combined together to create a PR genetic group (GEN-PR) (supplemental Figure 3D). These newly defined genetic risk groups (Figures 3 and 4) comprised 72% (GEN-GR) and 28% (GEN-PR) of patients and were associated with distinct demographic, clinical, and outcome characteristic rates (Tables 1-3 and Figure 2A-C).

Independent validation of the novel genetic classification using the UKALL2003 cohort

We validated this novel genetic classification on an independent cohort of 742 UKALL2003 patients with both successful MLPA and cytogenetic analysis. A total of 19 new CNA profiles were observed in the validation cohort, each in single patients, and were assigned to the CNA-indeterminate group (supplemental Table 3). The majority of these new profiles (n = 15) comprised ≥3 deletions and matched the other CNA profiles classified in the GEN-PR group (Figures 3 and 4). The validation cohort did not include *BCR-ABL1* or Down syndrome patients (supplemental Table 1), but sensitivity analysis of the test cohort (excluding these patients) did not alter the spectrum, incidence, or classification of the CNA profiles. The distribution of GEN-GR/GEN-PR patients in the validation cohort was slightly, but not significantly, different from the test cohort (76%/24% vs 72%/28%, *P* = .08). The slight decrease in the proportion of GEN-PR patients in the validation cohort is likely due to exclusion of *BCR-ABL1* and Down syndrome patients, because *BCR-ABL1* patients are classified as CYTO-HR and both are associated with *IKZF1* deletions. The new genetic risk groups were significantly associated with age, WCC, MRD, and outcome in the validation cohort (Tables 1-3 and Figure 2D-F).

Clinical relevance of the novel genetic classification in UKALL2003

In UKALL2003, patients classified in the GEN-PR had an inferior outcome across all relevant patient subgroups with no evidence of heterogeneity in relation to other risk factors (Figure 4). MRD was associated with a worse outcome in both the GEN-GR and GEN-PR groups, but the effect of treatment regimen was less clear (Table 4). In UKALL2003, patients who were MRD-HR without other HR factors (eg, CYTO-HR or slow early response [SER]) were randomized to remain on regimen A/B or transfer to regimen C. Among GEN-GR/MRD-HR patients, there was no evidence that those patients who remained on regimen A/B, and therefore received less intensive therapy, had an inferior outcome (Table 4 and supplemental Figure 5A-C). Only 45 GEN-PR/MRD-HR patients were not also CYTO-HR or SER. However, among these patients, there was some evidence of a benefit for treatment with regimen C (Table 4 and supplemental Figure 5D-F), in particular a better OS rate, although not significant. Multivariate analysis incorporating genetic risk, MRD, age, WCC, and treatment arm revealed that only genetic risk and MRD are consistent independent predictors of outcome (Table 5).

Integrating cytogenetic and CNA data resulted in reclassification of CYTO-IR patients according to their CNA profile (Figure 4). CYTO-IR/CNA-GR patients (group A) had a superior outcome compared with CYTO-IR/CNA-IR and CYTO-IR/CNA-PR patients

Table 4. Effect of MRD and treatment on the outcome of UKALL2003 patients with BCP ALL stratified by genetic risk group

Genetic risk group	Number (%) of patients	5-y rate (%) or HR (99% CI), *P* value		
		EFS	Relapse risk	OS
GR				
Total	561 (100)	94 (90-96)	4 (2-7)	97 (94-98)
MRD risk group*				
Low	244 (43)	97 (91-99)	2 (0-7)	99 (95-100)
High	176 (31)	91 (83-96)	7 (3-15)	95 (88-98)
HR vs LR		3.45 (1.34-8.9), .01	5.49 (1.55-19.48), .008	4.77 (0.99-22.97), .05
Indeterminate	141 (25)	91(82-96)	4 (1-12)	94 (86-98)
Treatment regimen				
Regimen A	350 (62)	94 (90-97)	3 (1-8)	98 (95-99)
Regimen B	113 (20)	93 (83-97)	4 (1-15)	94 (82-98)
Regimen C	98 (17)	91 (80-96)	6 (2-17)	94 (83-98)
Regimen A vs B		0.78 (0.33-1.89), .6	0.73 (0.22-2.37), .6	0.32 (0.10-0.99), .05
Regimen C vs B		1.45 (0.54-3.90), .5	1.68 (0.47-5.97), .4	0.94 (0.29-3.09), .9
MRD HR†				
Regimen A/B	92 (62)	90 (78-96)	8 (3-20)	95 (83-99)
Regimen C	56 (38)	94 (77-99)	4 (1-23)	94 (75-99)
Regimen C vs A/B		0.75 (0.23-2.49), .6	0.73 (0.18-2.93), .7	1.19 (0.27-5.31), .8
PR				
MRD risk group*	181 (100)	79 (69-85)	17 (10-26)	83 (73-89)
LR	58 (32)	94 (76-99)	6 (1-24)	92 (69-98)
HR	86 (48)	68 (52-80)	26 (15-42)	77 (62-87)
HR vs LR		5.21 (1.82-14.95), .002	3.94 (1.34-11.59), .01	5.36 (1.6-17.98), .007
IR	37 (20)	78 (54-91)	15 (5-40)	81 (57-92)
Treatment regimen				
Regimen A	46 (25)	83 (61-94)	15 (5-38)	84 (60-94)
Regimen B	59 (33)	86 (70-94)	7 (2-24)	86 (68-94)
Regimen C	76 (42)	69 (51-81)	26 (15-45)	79 (64-89)
Regimen A vs B		1.29 (0.48-3.44), .6	2.30 (0.67-7.86), .2	0.96 (0.33-2.76), .9
Regimen C vs B		2.30 (1.02-5.18), .04	3.69 (1.24-10.97), .02	1.74 (0.75-4.03) .2
MRD HR‡				
Regimen A/B	25 (41)	68 (38-86)	22 (7-54)	71 (41-88)
Regimen C	20 (59)	67 (29-87)	33 (13-71)	81 (38-96)
Regimen C vs A/B		0.80 (0.28-2.32), .7	1.29 (0.39-4.23), .7	0.78 (0.30-2.03), .3

*MRD was evaluated by real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements as defined by the European MRD Study Group. Patients were classified by MRD at day 29 (end of induction) as HR (>0.01%), LR (<0.01%), or indeterminate (no sample, test failed).

†Patients (n = 28) who were allocated regimen C on the basis of an SER defined by percentage of blasts in the bone marrow at day 8 (regimen B) or day 15 (regimen A) were excluded.

‡Patients who were allocated regimen C on the basis of an SER (n = 18) or CYTO-HR (n = 23) were excluded.

(group B) in both the test and validation cohorts (Tables 6-8 and supplemental Figure 4). Group B patients had a much higher incidence of *CDKN2A/B* (~70%), *PAX5* (~45%), and *IKZF1* (~40%)

Table 5. Multivariate Cox regression models for genetic risk based on 742 BCP patients treated on UKALL2003

	HR	99% CI	*P*
EFS			
Genetic risk group: poor vs good	3.29	1.49-7.27	<.001
Age (y)*	1.03	0.96-1.11	.2
WCC*	1.22	0.94-1.57	.04
MRD risk group: high vs low	4.05	1.61-10.13	<.001
Relapse risk			
Genetic risk group: poor vs good	3.68	1.47-9.20	<.001
Age (y)*	1.04	0.95-1.13	.2
WCC*	1.19	0.89-1.60	.1
MRD risk group: high vs low	4.46	1.50-13.22	<.001
OS			
Genetic risk group: poor vs good	4.96	1.68-14.61	<.001
Age (y)*	1.06	0.98-1.16	.04
WCC*	1.31	0.97-1.78	.02
MRD risk group: high vs low	4.66	1.31-16.54	.002

*Added to the model as continuous variables.

deletions compared with group A patients (1%, 5%, and 0%; all *P* < .001), who frequently (~80%) harbored none of the deletions studied (supplemental Table 5). In addition, the incidence of normal karyotypes and t(1;19)(q23;p13) was increased twofold compared with group B patients. Patients with a dicentric chromosome were almost always classified into group B. As most dicentric chromosomes involved 9p deletions, resulting in loss of *PAX5* and *CDKN2A/B*, this result was not surprising, as group B patients were enriched for both deletions. Although the frequency of *IGH* translocations among UKALL2003 group A and B patients was similar (8% vs 7%), there was a marked difference in their age profile (mean 7.4 vs 14.9 years, respectively; *P* = .0008).

Discussion

We have defined a novel genetic risk classification for pediatric BCP-ALL by integrating CNA and cytogenetic information. The classification defines 2 risk groups (good and poor) that have different outcomes across all subgroups independent of other risk factors. The genomic landscape of ALL is complex, heterogeneous, and not yet fully documented or understood. However, the treatment of childhood

Table 6. Demographic, clinical, genetic, and outcome features of MRC ALL97/99 and UKALL2003 patients with BCP-ALL and intermediate-risk cytogenetics stratified according to CNA risk profile

| | IR cytogenetic patients | | | | | |
| | ALL97/99 | | | UKALL2003 | | |
Variable/category	CNA-GR profile group A, n (%)	CNA-IR/PR profile group B, n (%)	P	CNA-GR profile group A, n (%)	CNA-IR/PR profile group B, n (%)	P
Total number of patients	110 (41)	157 (59)	—	108 (44)	138 (56)	—
Sex						
Female	55 (50)	66 (42)	.2	45 (41)	60 (44)	.7
Male	55 (50)	91 (58)		63 (59)	78 (56)	
Age (y)						
1-9	78 (71)	102 (65)	.5	71 (66)	78 (56)	.3
10-14	26 (23)	47 (30)		21 (19)	36 (26)	
≥15	6 (5)	8 (5)		16 (15)	24 (18)	
WCC						
<50 × 10^9/L	95 (86)	101 (64)	<.001	92 (85)	107 (77)	.1
>50 × 10^9/L	15 (14)	56 (36)		16 (15)	31 (23)	
NCI risk group*						
Standard	67 (61)	60 (38)	<.001	57 (53)	56 (40)	.05
High	43 (39)	97 (62)		51 (47)	82 (60)	
MRD†						
LR	—	—	—	36 (33)	55 (39)	.8
HR	—	—		43 (39)	59 (43)	
IR	—	—		29 (28)	24 (17)	
Complete remission						
No	0 (0)	5 (3)	.08	0 (0)	0 (0)	—
Yes	110 (100)	152 (97)		108 (100)	138 (100)	
Event						
No	85 (77)	91 (58)	—	104 (96)	114 (82)	—
Yes	25 (23)	66 (42)		4 (4)	24 (18)	
Relapse						
No	91 (83)	97 (64)	—	105 (97)	119 (86)	—
Yes	19 (17)	55 (36)		3 (3)	19 (14)	
Dead						
No	92 (84)	107 (68)	—	106 (98)	120 (87)	—
Yes	18 (16)	50 (32)		2 (2)	18 (13)	

*NCI standard risk includes patients who at diagnosis were <10 years old and had a WCC <50 × 10^9/L. NCI HR includes all other cases.

†MRD was evaluated by real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements as defined by the European MRD Study Group. Patients were classified by MRD at day 29 (end of induction) as HR (>0.01%), LR (<0.01%), or indeterminate (no sample, test failed). Patients treated on ALL97/99 were not evaluated by MRD.

ALL is highly effective, with cure rates exceeding 90%.[16] These 2 facts present a challenge for developing a genetic classification based on risk. We opted to take a pragmatic approach and develop a single genetic classification that could be readily adopted into routine clinical practice. Therefore, we focused on integrating CNA data on commonly deleted genes/loci with established chromosomal abnormalities. Analysis of the CNA data revealed a complex architecture consistent with previous studies; therefore, we grouped patients according to their CNA profile and integrated the subgroups with established cytogenetic risk groups to define a novel classification that was validated on an independent cohort.

There are strengths and limitations to our study compared with other attempts to use genomic data to develop or refine risk classifications. Firstly, we validated our classification on an independent cohort of patients treated on a contemporary protocol. Secondly, implementation of the classification into routine laboratory practice only requires the adoption of a simple, inexpensive, and widely available technique, namely MLPA, onto the existing background of gold-standard cytogenetic/molecular methods used for detecting significant chromosomal abnormalities (Figure 4). Although MLPA will not detect low-level subclones (<30%),[19] which are known to occur in ALL,[23] there is no evidence to suggest that they are relevant in predicting outcome. One potential limitation to our strategy is that not all 256 possible CNA combinations were observed in the test cohort. However, most of the new combinations identified in the validation cohort involved 3 or more CNAs and hence

Table 7. Outcome features of MRC ALL97/99 and UKALL2003 patients with BCP-ALL and IR cytogenetics stratified according to CNA risk profile

| | IR cytogenetic patients | | | | | |
| | ALL97/99 10-y rate (99% CI) | | | UKALL2003 5-y rate (99% CI) | | |
Outcome measures	CNA-GR profile group A	CNA-IR/PR profile group B	P	CNA-GR profile group A	CNA-IR/PR profile group B	P
EFS	78% (66-86)	57% (45-66)	<.001	96% (85-99)	82% (72-89)	<.001
Relapse risk	17% (10-29)	39% (29-51)	<.001	3% (1-14)	14% (8-25)	.001
OS	84% (72-91)	70% (59-79)	.001	98% (89-100)	87% (76-93)	.001

Table 8. Outcome features of MRC ALL97/99 and UKALL2003 patients with BCP-ALL and IR cytogenetics stratified according to CNA risk profile

Outcome measures	HR (99% CI), P	
	ALL97/99	UKALL2003
EFS	2.07 (1.31-3.28), .002	5.09 (1.76-14.66), .003
Relapse risk	2.30 (1.37-3.88), .002	5.45 (1.61-18.43), .006
OS	2.15 (1.25-3.69), .005	7.19 (1.67-31.01), .008

fitted well alongside other combinations in the GEN-PR group. In contrast, the CNA combinations included in the GEN-GR group were mostly simple, involving 0, 1, or, occasionally, 2 deletions. Almost all of these simple combinations have already been observed among the 1644 patients screened (supplemental Table 2). Although additional CNA combinations are possible, they will be rare and complex; thus, classifying them as detailed above is logical and consistent with the evidence. The fact that this classification relies on the use of a commercial MLPA kit to detect the 8 most prevalent CNAs has clear advantages in terms of confirming these findings and implementing the classification into a clinical setting. Although it means that other CNAs (eg, *ERG* deletions[24,25]) and sequence variants (eg, *RAS*, *JAK2*, and *TP53* mutations[26]) are not represented, it is known that many of these alterations correlate closely with either chromosomal abnormalities or CNAs already represented in the classification. For example, *RAS* mutations with near haploidy/high hyperdiploidy,[27,28] *TP53* mutations with low hypodiploidy,[28,29] and *JAK2* mutations with *P2RY8-CRLF2* (PAR1 deletions).[21,30] Therefore, it could be argued that this classification as it stands already accounts for much of this additional genetic heterogeneity. Nevertheless, further work will be necessary to determine whether the inclusion of such abnormalities can further refine this classification.

The prognostic impact of GR and HR cytogenetic abnormalities was not altered by their CNA profile. This finding implies that the primary genetic abnormality is more important in determining treatment response than subclonal secondary aberrations. This conclusion is both logical and consistent with studies that show evidence for the pleiotropic effect of *IKZF1* deletions.[7,12,24,25,31,] Therefore, the principal effect of integrating cytogenetic and CNA information has been to reclassify CYTO-IR patients according to their CNA profile. Although the demographic and clinical features of the 2 groups of patients were similar, their genetic and outcome profiles were distinct (Tables 6-8; supplemental Table 5; and supplemental Figure 4). Group B was enriched for *IKZF1* deletions, *CDKN2A/B* deletions, *P2RY8-CRLF2* (PAR1 deletions), dic(9;20), and older patients with *IGH* translocations, all of which have been associated with poor outcome.[5-12,22,31,32] Gene expression profiling studies,[13,14] which have sought to define novel HR subgroups, have defined the *BCR-ABL1*–like subtype, which has some similar features (poor outcome, enriched for *IKZF1* deletions, *CDKN2A/B* deletions, and *P2RY8-CRLF2*) and also accounts for 15% to 20% of BCP-ALL. Although the outcome of the US Children's Oncology Group *BCR-ABL1*–like subgroup was much worse (4-year EFS <25%), their analysis was based on an HR cohort.[13] In contrast, the outcome of the Dutch Childhood Oncology Group *BCR-ABL1*–like subgroup was very similar to that observed for our group B patients treated on ALL97/99 (5-year EFS, 56% vs 57%). Future collaborative studies will be required to determine the degree of overlap between existing and novel *BCR-ABL1*–like gene signatures and this novel genetic classification.

This genetic classification defines a large (75%) subset of patients who have an excellent outcome on UKALL2003 (5-year survival >95% and RR <5%). Further improvements in the treatment of childhood ALL cannot be measured in terms of RR and death toll. Instead, toxicity and long-term side effects are becoming the major issues.[33] Hence, the identification of a large group of children with an excellent outcome who could be considered for treatment reduction is a key finding of this study. Although GEN-GR patients who were MRD HR had an inferior outcome compared with MRD-negative patients, the EFS at 5 years remained >90%. Moreover, the effect of treatment escalation among these patients did not significantly improve outcome. Our data indicate that current treatment is curative for the vast majority of GEN-GR patients, and future treatment strategies for these patients should focus on deintensification, compliance, and targeted therapies.

A quarter of patients were classified as PR based on genetics, including those with HR cytogenetic entities and those with an adverse CNA profile (group B). Although survival of UKALL2003 GEN-PR patients was significantly inferior to GEN-GR patients, it reached >80% at 5 years. GEN-PR patients with HR cytogenetics (~30% GEN-PR patients) are allocated to regimen C, and, as an example, iAMP21 patients have been shown to clearly benefit from such an intervention.[34] The remaining GEN-PR patients (~70%) were classified on the basis of their CNA profile (group B), accounting for ~20% of all BCP-ALL patients. All MRD HR patients on the new UK trial (UKALL2011) will be allocated to regimen C. These data (Table 4) have shown that GEN-PR patients in the MRD LR group have a LR of relapse and good survival. Thus, there is no clear evidence to support further treatment intensification for the remaining group B patients. Instead, the focus should be on identifying the key driver(s) of relapse and potential therapeutic targets within this subgroup. Gene fusions that result in tyrosine kinase activation provide excellent examples of therapeutic targets that are known to be present in some patients with *BCR-ABL1*–like ALL. Recent research has identified a complex network of such translocation involving *ABL1*, *ABL2*, *PDGFRB*, and *CSF1R* that are experimentally sensitive to tyrosine kinase inhibitors.[35] Although these gene fusions are rare (~1%), 2 recent case reports of patients with refractory ALL and *EBF1-PDGFRB* fusion responded well to imatinib, demonstrating the utility of using tyrosine kinase inhibitors in the clinical setting.[36,37] Further work is required to determine the full spectrum of such tyrosine kinase–activating fusions, their prognostic relevance, and how they interface with this novel genetic classification.

In conclusion, we have integrated cytogenetic and CNA data into a single genetic classification that can be used to refine patient treatment according to more detailed genetic description of their leukemia. GEN-GR patients have an excellent outcome with modern therapy, and future treatment approaches should focus on achieving the same results with reduced therapy. Although, GEN-PR patients have an inferior outcome compared with GEN-GR patients, their survival still exceeds 80%. As many of these patients are already receiving intensive treatment, further genetic research is required to identify patients curable with current drugs and those requiring alternative therapies.

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