Copy number alterations in B-cell development genes, drug resistance, and clinical outcome in pediatric B-cell precursor acute lymphoblastic leukemia

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Pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is associated with a high frequency of copy number alterations (CNAs) in \(\text{IKZF1} \), \(\text{EBF1} \), \(\text{PAX5} \), \(\text{CDKN2A/B} \), \(\text{RB1} \), \(\text{BTG1} \), \(\text{ETV6} \), and/or the PAR1 region (henceforth: B-cell development genes). We aimed to gain insight in the association between CNAs in these genes, clinical outcome parameters, and cellular drug resistance. 71% of newly diagnosed pediatric BCP-ALL cases harbored one or more CNAs in these B-cell development genes. The distribution and clinical relevance of these CNAs was highly subtype-dependent. In the DCOG-ALL10 cohort, only loss of \(\text{IKZF1} \) associated as single marker with unfavorable outcome parameters and cellular drug resistance. Prednisolone resistance was observed in \(\text{IKZF1} \)-deleted primary high hyperdiploid cells (~1500-fold), while thiopurine resistance was detected in \(\text{IKZF1} \)-deleted primary \(\text{BCR-ABL1} \)-like and non-\(\text{BCR-ABL1} \)-like B-other cells (~2.7-fold). The previously described risk stratification classifiers, i.e. \(\text{IKZF1} \) plus and integrated cytogenetic and CNA classification, both predicted unfavorable outcome in the DCOG-ALL10 cohort, and associated with \(\text{ex vivo} \) drug cellular resistance to thiopurines, or L-asparaginase and thiopurines, respectively. This resistance could be attributed to overrepresentation of \(\text{BCR-ABL1} \)-like cases in these risk groups. Taken together, our data indicate that the prognostic value of CNAs in B-cell development genes is linked to subtype-related drug responses.

Acute lymphoblastic leukemia (ALL) is the most common cancer diagnosed in children. The introduction of risk-adjusted treatment protocols has significantly improved survival rates, which nowadays is approaching 90% survival\(^1\)-\(^3\). Outcome of B-cell precursor ALL (BCP-ALL) differs by genetic subtype, i.e. \(\text{ETV6-RUNX1} \), high hyperdiploid, and \(\text{TCF3-PBX1} \) cases have favorable prognosis, whereas \(\text{BCR-ABL1} \) and \(\text{KMT2A} \)-rearranged BCP-ALL is associated with an unfavorable treatment outcome\(^3\). Approximately 25% of the patients has a genetically unclassified disease, which is defined as ‘B-other’. This heterogeneous group can be subdivided in \(\text{BCR-ABL1} \)-like patients and non-\(\text{BCR-ABL1} \)-like B-other patients\(^4,5\). Within the \(\text{BCR-ABL1} \)-like subtype intrachromosomal amplification of chromosome 21, dicentric chromosome (9;20), and kinase activating lesions are reported\(^4,9\). In non-\(\text{BCR-ABL1} \)-like B-other cases chromosomal translocations involving \(\text{DUX4} \), \(\text{ZNF384} \), and \(\text{MEF2D} \) were identified\(^10,12\). In addition to the major classifying abnormalities, secondary aberrations have been observed, including copy number alterations (CNAs) in genes involved in B-cell development (e.g. \(\text{IKZF1} \), \(\text{EBF1} \), \(\text{PAX5} \), \(\text{ETV6} \)), cell cycle and proliferation (e.g. \(\text{CDKN2A} \), \(\text{CDKN2B} \), \(\text{RB1} \), \(\text{BTG1} \)), and cytokine receptors (e.g. \(\text{CRLF2} \))\(^4,5,9,13-18\). Interestingly, some of these genetic lesions (e.g. \(\text{IKZF1} \)) were shown to predict clinical
outcome.\textsuperscript{5,14,17} The Dutch Childhood Oncology Group (DCOG) implemented IKZF1 status as risk factor in the ongoing DCOG-ALL11 protocol. In addition, risk stratification strategies were designed by integration of CNA profiles and genetic subtypes\textsuperscript{16–20}.

Cellular drug resistance is an important cause of relapse. Ex vivo drug resistance at diagnosis is associated with high risk of early treatment failures\textsuperscript{21–23}. In addition, BCP-ALL cells at relapse are more resistant towards glucocorticoids, L-asparaginase, anthracyclines, and thiopurines\textsuperscript{24}. IKZF1 deletions are reported to mediate resistance towards glucocorticoids, but the relationship between remaining CNAs and cellular drug resistance is yet unknown\textsuperscript{25–27}. Therefore, we performed an explorative study, which aimed to gain insight in associations between CNAs, cellular drug resistance, and clinical outcome.

**Results**

A pediatric BCP-ALL cohort of 515 newly diagnosed cases, representing all major ALL subtypes, was screened for CNAs in eight genes involved in transcription of lymphoid genes and the differentiation and proliferation of precursor B-cells (henceforth: B-cell development genes; Supplementary Fig. 1). In total, 71% of the pediatric BCP-ALL cases harbored one or more CNAs in these B-cell development genes (Fig. 1). The CNA frequency differed between genetic BCP-ALL subtypes. The percentage of patients with one of more CNAs was the highest in BCR-ABL1-like cases and the lowest in TCF3-PBX1 cases (Supplementary Fig. 2A).

**CNAs in B-cell transcription factors.** \textit{IKZF1}. Deletions of the transcription factor IKZF1 were detected in 20% of the BCP-ALL cases. This frequency differed between subtypes: IKZF1 deletions were enriched in BCR-ABL1 (65%) and BCR-ABL1-like (44%) cases, whereas deletions were low or absent in ETV6-RUNX1 (3%) and TCF3-PBX1 (0%), respectively (Fig. 1A; Supplementary Fig. 2B). In addition, 76% (78/102) of the cases with an IKZF1 deletion harbored CNAs in additional genes, which mainly involved PAX5 and CDKN2A/B (Fig. 2). This co-occurrence was subtype dependent: a strong association (OR ≥ 2, p < 0.001) was observed in BCR-ABL1, BCR-ABL1-like and B-other cases, whereas in high hyperdiploid cases IKZF1 deletions mainly occurred independent of CNAs in PAX5 and/or CDKN2A/B. Within the group of genetically unclassified patients, loss of IKZF1 was associated with dicentric chromosome (9;20) and tyrosine kinase fusion genes (Supplementary Table 1).

IKZF1-deleted cases more often showed high MRD levels (≥ 10\(^{-3}\)) after induction therapy (TP1; p = 0.013), and intermediate MRD levels (10\(^{-4}\) ≤ MRD < 10\(^{-3}\)) after the first consolidation course (TP2; p = 0.028), compared to IKZF1-wildtype cases (Fig. 3A). This association could be predominantly attributed to high MRD levels in BCR-ABL1-like and B-other cases, but was not observed in high hyperdiploid or ETV6-RUNX1 cases (Supplementary Figs 3–6). In addition, IKZF1-deleted cases more often suffered from a non-response or relapse compared to IKZF1-wildtype cases (5-year CIR: 30.4% versus 9.0%; p < 0.001; Fig. 4A,B), confirming previous findings\textsuperscript{3,4,17}. An IKZF1 deletion remained predictive for an unfavorable outcome in DCOG-ALL10 cases treated in the medium risk arm (Fig. 4B), indicating that the prognostic value of IKZF1 is independent of the early treatment response monitored by MRD.

As cellular drug resistance might underlie this poor outcome, we examined the ex vivo efficacy of chemotherapeutic agents that are commonly used during induction and consolidation therapy. Primary BCP-ALL cells harboring IKZF1 deletions were more resistant to prednisolone and thiopurines compared to IKZF1 wild-type cells (p < 0.05; Fig. 5A,B). Resistance against these agents was subtype dependent, as visualized in Fig. 5B: prednisolone resistance was predominantly observed in high hyperdiploid cells (~1500-fold, p = 0.009), whereas thiopurine resistance (6-thioguanine (1.6 fold, p = 0.011) and 6-mercaptopurine (1.7 fold, p < 0.001)) was mainly identified in IKZF1-deleted BCR-ABL1-like and B-other cells (Fig. 5B). Moreover, high hyperdiploid cells with a deletion of IKZF1 were more resistant to L-asparaginase (Supplementary Fig. 8A).

**ETV6.** Deletions of the transcription factor ETV6 were detected in all BCP-ALL subtypes, but were especially enriched in ETV6-RUNX1 cases (71%; Fig. 1D). After induction therapy (TP1), ETV6-deleted cases more often...
showed low ($<10^{-4}$) MRD levels compared to ETV6-wildtype cases (Fig. 3D; OR = 2.6, p = 0.02). However, this association was subtype dependent: in BCR-ABL1-like and B-other cases an adverse association between ETV6 deletions and MRD levels was observed (Supplementary Fig. 3). Prognosis of cases with loss of ETV6 was not different compared to ETV6-wildtype cases (Fig. 4). ETV6-deleted cells appeared to be more sensitive
to prednisolone (~3.2 fold, p = 0.046), but more resistant to vincristine (~1.8 fold, p < 0.01) and daunorubicin (~1.9 fold, p = 0.028). Remarkably, loss of the wildtype ETV6 allele in ETV6-RUNX1-positive cells associated with a high sensitivity to vincristine instead of resistance (p = 0.013, Fig. 5D), suggesting that associations of vincristine resistance differ between genetic subtypes of ALL. Moreover, deletion of ETV6 was associated with L-asparaginase resistance in high hyperdiploid cells and high 6-thioguanine sensitivity in ETV6-RUNX1 cells (Supplementary Fig. 8C).

CNAs in cell cycle and proliferation genes. CDKN2A/B. Deletions of the cell cycle regulators CDKN2A and/or CDKN2B were often observed (33%) in the pediatric BCP-ALL cohort (Fig. 1E). Similar to PAX5, the deletions in CDKN2A/B were found in all BCP-ALL subtypes, but were especially enriched in BCR-ABL1 (65%, OR = 3.95, p = 0.003), BCR-ABL1-like (54%, OR = 2.88, p < 0.001), and B-other cases (OR = 1.67, p = 0.026). No association with clinical outcome parameters or cellular drug resistance was observed (Figs 3–5).

RB1. The cell cycle regulator RB1 was deleted in a minority (~7%) of the BCP-ALL cases (Fig. 1F) and deletions were detected in all BCP-ALL subtypes. Within the DCOG-ALL10 cohort, RB1-deleted cases showed a trend towards a poor event free survival (5-year EFS: 68.2% versus 86.7%, p = 0.057), which was caused by an unfavorable response in the medium risk (MR) treatment group (5-year EFS: 46.9% versus 88.3%, p = 0.003; Supplementary Fig. 7A). No association with MRD levels or cellular resistance to the tested drug panel was observed (Fig. 5).

BTG1. The anti-proliferative gene BTG1 was deleted in a minority (~8%) of the BCP-ALL cases. No deletions were detected in KMT2A-rearranged or TCF3-PBX1 cases, whereas the highest frequency was observed in BCR-ABL1 (30%) and ETV6-RUNX1 (16%) cases (Fig. 1G). Four out of five BTG1-deleted BCR-ABL1-like and B-other cases also harbored an IKZF1 deletion. These four cases all experienced an event and only the patient with wildtype IKZF1 remained in remission (Supplementary Fig. 7B). This finding underlines an earlier report, in which a cooperative effect of BTG1 and IKZF1 lesions in leukemogenesis was observed27.

CNAs in cytokine receptors. PAR1. Deletions in the pseudoautosomal region 1 (PAR1) were the least prevalent (~4%) in this pediatric BCP-ALL cohort. CNAs in this region indicate presence of interstitial deletions or a translocation, which both induce overexpression of CRLF229. Deletions of the PAR1 region were detected in BCR-ABL1-like (11%), B-other (10%), and high hyperdiploid cases (2%), but not in remaining BCP-ALL subtypes (Fig. 1H). Unfortunately, power was lacking to reliably study the association between deletions in the PAR1 region, MRD levels, clinical prognosis, and cellular drug resistance.
Taken together, with the exception of loss of the \( \text{IKZF1} \) gene, none of the CNAs in the remaining B-cell development genes strongly associates with clinical outcome and cellular drug resistance as single marker. Our results show that the clinical value of CNAs in B-cell development genes is highly context dependent and differs between the diverse oncogenic drivers of pediatric BCP-ALL.

**Figure 3.** The association between CNAs and MRD levels after induction therapy and the first consolidation course in newly diagnosed BCP-ALL. MRD levels of DCOG-ALL treated BCP-ALL cases (all risk groups) after induction (TP1; \( n = 183 \)) and first consolidation course (TP2; \( n = 183 \)). The percentage of cases with high (\( \geq 10^{-3} \)), medium (\( 10^{-4} \leq \text{MRD} < 10^{-3} \)), and undetectable MRD levels (< \( 10^{-4} \)) is depicted per CNA. The Fisher’s Exact test was applied to study associations between CNAs and MRD levels. **\( p \leq 0.01 \), *\( p \leq 0.05 \). del = deletion.
Figure 4. Prognostic value of CNAs in DCOG-ALL10 treated cases. (A) The association between CNAs in all risk groups and cumulative incidence of relapse (CIR) and event-free-survival (EFS) was examined. BCP-ALL patients (n = 210) were treated according to DCOG-ALL10 protocol. CIR was estimated using a competing risk model. Relapse and non-response were considered as event, and death as competing event. To test equality of the CIRs, the Gray’s test was applied. Non-response, relapse, and death were considered as events for EFS. EFS rates were determined using Cox regression, and compared using the Wald test. For reliable test results, groups should contain at least 5 cases. (B) CIR and EFS curves of cases without or with an IKZF1 deletion. Curves contain either all risk groups, or the medium risk arm only.

**Risk stratification classifiers.** In recent studies, IKZF1\textsuperscript{+} and integrated cytogenetic and CNA classification were shown to be prognostic classifiers. In the DCOG-ALL10 cohort 12 of the 210 cases were classified as IKZF1\textsuperscript{+}. The prognosis of IKZF1\textsuperscript{+} cases was unfavorable compared to cases with wildtype IKZF1 (Supplementary Fig. 9). Strikingly, no prednisolone resistance was observed in IKZF1\textsuperscript{+} cells, which could be explained by underrepresentation of high hyperdiploid cases in this group (n = 1, Fig. 5A). However, IKZF1\textsuperscript{+} cases did show ex vivo resistance to 6-thioguanine and 6-mercaptopurine, mainly caused by the high proportion of BCR-ABL1-like and B-other cases in this group.

Integration of cytogenetic and CNA data as reported by Moorman et al.\textsuperscript{18} identified cases with a genetic good and poor risk. Cases that were classified as poor risk showed an unfavorable 5-years EFS and CIR compared good risk cases, as shown in Supplementary Fig. 10A. These genetically poor risk cases showed high MRD levels after induction therapy and the first block of consolidation therapy, indicating a poor response to drugs that are used during these treatment phases (Supplementary Fig. 10B). Indeed, ex vivo cellular drug response data showed resistance of poor risk cells to L-asparaginase, 6-thioguanine, and 6-mercaptopurine (Fig. 5A, Supplementary Fig. 10C). Enrichment of BCR-ABL1-like cases could attribute to the thiopurine and L-asparaginase resistance observed in the poor risk group\textsuperscript{4}.

**Discussion**

BCP-ALL cases harbor various genetic aberrations in genes involved in lymphoid maturation, cell cycle regulators, tumor suppressors, and tyrosine kinases. We performed an explorative study to gain insight in the association between CNAs in B-cell development genes, MRD levels, long-term prognosis, and cellular drug resistance. Interestingly, the distribution and clinical relevance of these CNAs was subtype-dependent. A high
Figure 5. The association between CNAs and the ex vivo cellular drug response. (A) Leukemic cells were incubated for four days with a concentration range of prednisolone (µg/ml), vincristine (µg/ml), L-asparaginase (IU/ml), daunorubicin (µg/ml), 6-mercaptopurine (µg/ml), and 6-thioguanine (µg/ml), after which cell viability was measured using an MTT assay. The Mann-Whitney U test was applied to compare LC50-values. No association is depicted in grey, resistance in blue (p < 0.05, fold induction (FI) > 1), sensitive in green (p < 0.05, FI < 1), and not determined in white. The number of cases that were tested for prednisolone is depicted, and represent the maximum number of cases. For reliable test results, groups should contain at least 5 cases (groups ≤ 5 are depicted as ND). Results of single CNAs are depicted for all risk groups and for the three subtypes of BCP-ALL (grey, ETV6-RUNX1-like/B-other cells, high hyperdiploid cells). The association between CNAs and the cellular drug response is depicted in grey, resistance in blue (p < 0.05, fold induction (FI) > 1), sensitive in green (p < 0.05, FI < 1), and not determined in white. The number of cases that were tested for prednisolone is depicted, and represent the maximum number of cases. For reliable test results, groups should contain at least 5 cases (groups ≤ 5 are depicted as ND). Results of single CNAs are depicted for all risk groups and for the three subtypes of BCP-ALL (grey, ETV6-RUNX1-like/B-other cells, high hyperdiploid cells). The red line represent the median LC50 value in the each group. (B) LC50 values for prednisolone (µg/ml) of cases without or with IKZF1 deletion. Columns include all BCP-ALL subtypes (grey), BCR-ABL1-like/B-other cells (blue), and high hyperdiploid cells (green). The red line represent the median LC50 value in the each group. (C) LC50 values for prednisolone (µg/ml) of cases without or with PAX5 CNA. Columns include all BCP-ALL subtypes (grey), BCR-ABL1-like/B-other cells (blue), and high hyperdiploid cells (green), and ETV6-RUNX1 cells (yellow). The red line represent the median LC50 value in the each group. (D) LC50 values for prednisolone (µg/ml) of cases without or with ET6 CNA. Columns include all BCP-ALL subtypes (grey), BCR-ABL1-like/B-other cells (blue), and high hyperdiploid cells (green), and ETV6-RUNX1 cells (yellow). The red line represent the median LC50 value in the each group.
for prednisolone (µg/ml), vincristine (µg/ml), and daunorubicin (µg/ml) of primary leukemic cells without or with ETV6 deletion. Columns include all BCP-ALL subtypes (grey), BCR-ABL1-like/B-other cells (blue), high hyperdiploid cells (green), and ETV6-RUNX1 cells (yellow). The red line represent the median LC50 value in the each group. *p < 0.05; **p < 0.01; Mann-Whitney U test.

frequency of CNAs in these B-cell development genes was found in the poor prognostic subtypes BCR-ABL1, BCR-ABL1-like, and B-other. Cooperative lesions may favor the aggressive phenotype of a leukemia, such as exemplified by the synergistic effect between loss of IKZF1 and the BCR-ABL1 fusion gene in leukemogenesis 28, and the antagonizing effect of IKZF1 deletions in the response to imatinib 31. In contrast, the prognosis of ETV6-RUNX1, DUX4-rearranged, and ERG-deleted BCP-ALL is probably not affected by IKZF1 deletions, but numbers with IKZF1 deletions in these subtypes are low 11,12,15,29,32,33,37. These observations indicate that the genetic context influences the functional effect of CNAs in B-cell development genes. The importance of the genetic context is exemplified by the fact that isolated deletions of BTG1 do not affect cellular drug resistance or the prognosis of BCP-ALL cases, whereas all four patients with concomitant loss of BTG1 and IKZF1 experienced an event. Moreover, combined loss BTG1 and IKZF1 was shown to enhance glucocorticoid resistance 22. In contrast to BTG1-IKZF1 synergy, we observed that CNAs in PAX5 may counteract the effect of an IKZF1 deletion on prednisolone resistance. Various combinations of cooperative lesions may therefore have different effects on the pathobiology of B-cell precursor ALL cells.

In the present study we observed an association between deletion of IKZF1 and prednisolone resistance, especially in high hyperdiploid cells. In correspondence, a direct association has been demonstrated between IKZF1 deletion and glucocorticoid-induced cell death 23,34. IKZF1 functions as a metabolic gatekeeper and consequently loss of IKZF1 results in increased intracellular ATP and glucose levels 35. Interestingly, we previously observed a direct relation between an increased glycolytic rate and prednisolone resistance in primary BCP-ALL cells 25,26. In these leukemic cells, inhibition of glycolysis restored the efficacy of prednisolone 36. Hence, inhibition of glycolysis might also be a potential treatment strategy to re-sensitize IKZF1-deleted cells to prednisolone and should be explored in more detail in future studies, also in the context of BTG1 and PAX5.

In contrast to high hyperdiploid cells, deletion of IKZF1 was not linked to prednisolone resistance in primary BCR-ABL1-like and B-other ALL cells, suggesting that additional factors (e.g. differentiation stage, other oncogenic drivers) are important for the functional effect of a deletion of the IKZF1 gene in these type of cells. Instead of prednisolone resistance, we observed thiopurines resistance in these BCR-ABL1-like and B-other ALL cells. Thiopurine resistance might be caused by deficiencies in the DNA mismatch repair system and indeed DNA repair genes were reported to be downregulated in IKZF1-deleted cells 37,38. Interestingly, this characteristic might offer opportunities to target these leukemic cells via the DNA mismatch repair apparatus, e.g. by PARP inhibitors, and warrants further studies.

Besides IKZF1, deletion of RB1 was predictive for a poor outcome in the MR-risk group of the DCOG-ALL10 cohort. RB1 deletions are known to be enriched in poor prognostic iAMP21 and hypodiploid cases, which might explain the unfavorable outcome of RB1-deleted cases 11,39,46. However, the unfavorable outcome could not be explained by or cellular resistance against induction therapy drugs.

Recently, two independent studies showed that integration of genetic aberrations improved the risk stratification of BCP-ALL in children 20,34. Both IKZF1 11,12,15,18,29 and integrated cytogenetic and CNA classification predicted poor outcome in the DCOG-ALL10 cohort, and associated with drug resistance to thiopurines, or L-asparaginase and thiopurines, respectively. The cellular drug resistance could be attributed to overrepresentation of BCR-ABL1-like cases in these risk groups 4. Taken together, our results suggest that the prognostic value of CNAs in B-cell development genes is linked to subtype-related drug resistance.

In the current study, we restricted our analyses to CNAs in eight genes that are recurrently deleted in pediatric BCP-ALL. However, additional genetic aberrations may be of importance for prognosis and cellular drug resistance and should be explored in future research. Moreover, as we performed an explorative study, it is of importance to confirm the associations that are proposed in the present paper in independent studies.

In conclusion, results obtained in the present study revealed that, with the exception of an IKZF1 deletion, none of the remaining CNAs as single marker associated both with an unfavorable clinical prognosis and cellular drug resistance. Our results indicate that the biological and clinical importance of CNAs in B-cell development genes (and presumably also other aberrations) is highly context dependent and differs between the diverse oncogenic drivers of pediatric BCP-ALL. Functional studies that focus on potential causes of cellular drug resistance should therefore take the oncogenic driver and additional genetic aberrations into account.

Methods

Processing of primary patient material. Bone marrow and/or peripheral blood samples were obtained from children (1–18 years) with newly diagnosed ALL. Written informed consent was obtained from parents or guardians to use excess of diagnostic material for research purposes, as approved by the Medical Ethics Committee of the Erasmus Medical Center, The Netherlands. These studies were conducted in accordance with the Declaration of Helsinki. Mononuclear cells were isolated using Lymphoprep gradient separation and the leukemia blast percentage was determined microscopically by May-Grünewald Giemsa stained cytosin preparations, as described previously 31. Samples were enriched to over 90% purity of leukemic cells by depletion of non-leukemic cells using immunomagnetic beads. Primary leukemic cells were maintained in RPMI-1640 Dutch modification supplemented with 20% fetal calf serum (Integro), with 0.1% insulin-transferrin-sodium selenite (Sigma), 0.4 mM glutamine (Invitrogen), 0.25 µg/ml gentamycin (Gibco), 100 IU/ml penicillin (Gibco), 100 µg/ml streptomycin (Gibco), 0.125 µg/ml fungizone (Gibco).
The major cytogenetic subtypes, i.e. high hyperdiploid (>50 chromosomes), ETV6-RUNX1, TCF3-PBX1, KMT2A-rearranged, BCR-ABL1, BCR-ABL1-like, and B-other (negative for all before mentioned genomic lesions), were determined using fluorescent in situ hybridization and (RT-)PCR. The 110-probe set gene expression classifier was used to identify BCR-ABL1-like cases. Patients were treated according to the Dutch Childhood Oncology Group (DCOG)-ALL8, -ALL9, -ALL10, the EsPhALL protocol, or the COALL-06-97 and COALL-07-03 study protocols. Patient characteristics were provided by the central study centers of DCOG, The Hague, the Netherlands and COALL, Hamburg, Germany. PCR-detected MRD was evaluated according to the EuroMRD guidelines.

**Multiplex Ligation-Dependent Probe Amplification.** To identify genomic lesions in IKZF1, CDKN2A, CDKN2B, ETV6, PAX5, RB1, BTG1, EBF1, and the PAR1 region (CSF2RA/IL3RA/CRLF2), the SALSA P335 ALL IKZF1 (a3) and the SALSA P202 Multiplex Ligation-dependent Probe Amplification (MLPA) assays (MRK-Holland, Amsterdam, Netherlands) were used as described previously. In short, DNA fragments with incorporated FAM nucleotides were generated using 125 ng of genomic DNA, according to the manufacturer’s protocol. To quantify the amplified fragments, an ABI-3130 genetic analyzer (Applied Biosystems, Carlsbad, CA) was used. The manufacturer’s control probes were used to normalize peak intensities, as well as a synthetic control reference generated from five normal DNA samples in the same MLPA run (normal copy number = 0.75 < peak ratio ≤ 2.0; deletions: peak ratio < 0.75; gain: peak ratio > 2.0). A deletion was defined by a peak ratio below 0.75 for at least one MLPA probe per gene. CDKN2A/B deletions included loss of either CDKN2A or CDKN2B. The effect of intragenic amplifications and/or deletions in PAX5 were analyzed within one group, as they were predicted to be functionally equivalent. Loss of the PAR1 region was defined by deletion of both IL3RA and CSF2RA probes while expression of the CRLF2 and SHOX-AREA probes was maintained. MLPA analyses were performed in 515 BCP-ALL cases, representing the major genetic subtypes in childhood ALL, i.e. 3.9% BCR-ABL1, 17.3% BCR-ABL1-like, 20.2% non-BCR-ABL1-like B-other, 28.7% ETV6-RUNX1, 24.5% high hyperdiploid, 1.6% KMT2A-rearranged, 3.9% TCF3-PBX1.

**Clinical characteristics and statistics.** To identify whether CNAs were underrepresented or enriched in a subtype, the Fisher’s exact test was applied using R software (version 3.2.1). Obtained odds ratios (ORs), 95% confidence interval, and p-values are reported. The Fisher’s exact test was also applied to compare minimal residual disease (MRD) levels after induction and first consolidation therapy between patients groups with CNAs and wildtype patients. Cumulative incidence of relapse (CIR) was estimated using a competing risk model and significance was determined using the Gray’s test. Relapse and non-response (counted as event at day 79) were considered as event, with death as competing event. Event-free survival (EFS) probabilities were estimated using cox regression and compared using the Wald test. Relapse, non-response, secondary malignancies and death were counted as events. Outcome analyses were performed in R (version 3.2.1), using the packages cmprsk version 2.2–746, mstate version 0.2.7 and survival version 2.38–4. Five-year EFS and CIR are reported. The DCOG-ALL10 trial is the most recently completed nationwide trial in which patients were risk-stratified by minimal residual disease (MRD) levels and for whom sufficient long-term follow-up data were available. Therefore, we restricted the analysis of associations between CNAs and clinical response parameters (MRD, clinical outcome) to this cohort. In addition, the genetic subtypes are represented with a distribution that is comparable to the general pediatric BCP-ALL population (excluding BCR-ABL1-positive cases since these patients are eligible for the EsPhALL protocol), i.e. 12.2% BCR-ABL1-like, 13.9% non-BCR-ABL1-like B-other, 33.5% ETV6-RUNX1, 32.7% high hyperdiploid, 2.0% KMT2A-rearranged, and 5.7% TCF3-PBX1 positive cases. The clinical characteristics of this cohort are displayed in Supplementary Table 2.

**Ex vivo drug resistance assays.** Ex vivo cytotoxicity of prednisolone, vincristine, L-asparaginase, daunorubicin, 6-mercaptopurine, and 6-thioguanine was evaluated using 3-(4,5-dimethylthiazolyl-2)-2,5-di-phenyltetrazolium bromide (MTT), as previously described. In brief, cells were exposed to a concentration range of chemotherapeutics (prednisolone: 0.008 to 250 μg/mL; vincristine: 0.05 to 50 μg/mL; L-asparaginase: 0.003 to 10 IU/mL; daunorubicin: 0.002 to 2 μg/mL; 6-mercaptopurine: 15.6 to 500 μg/mL; and 6-thioguanine: 1.56 to 500 μg/mL) in a 96 wells plate for four days at 37°C and 5% CO2. After four days of culture, samples were included if control wells harbored more than 70% leukemic cells and an optical density higher than 0.050 arbitrary units (adjusted for blank values). The concentration of drug lethal to 50% of the cells (LC50) was calculated. LC50-values were compared by the Mann-Whitney U test and adjusted for tied ranks if applicable.

**Data Availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions
E.M.P.S., A.H.Q. and J.M.B. analyzed and interpreted data. A.B. performed experiments. R.P., H.A.d.G.K., V.d.H., M.A.H. and G.E. provided clinical characteristics, clinical outcome data, and interpreted data. R.P. and MLdB conceptualized the study, and interpreted data. E.M.P.S. and M.L.d.B. drafted the manuscript. The manuscript was revised and approved by all authors.

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