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Prognostic impact of copy number alterations in pediatric B-cell precursor acute lymphoblastic leukemia: A collaborative data from two major oncology centers of North India

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Abstract

In current study, copy number alteration (CNA) status and CNA risk profiles of $IKZF1^{\text{plus}}$, UK-ALL CNA risk groups and MRplus score, were evaluated for clinical and prognostic impact in a cohort of 493 B-ALL cases diagnosed and treated under ICICLE trial at two major oncology centres of Northern India. Overall CNA frequency was 59% with 60% of cases showing 2-loci deletion. $CDKN2A/B$ deletion was the most common CNA (36.3%), while $IKZF1$ deletion and $IKZF1^{\text{plus}}$ profile were noted in 19.5% and 13.4% of cases respectively. $IKZF1$ deletions and other CNA risk profiles were all significantly associated with poor/high risk clinical and genetic profile parameters ($p$-values $<0.001$). In addition, the 3-year OS, EFS was significantly poor with high RR of 38.6%, 46.5% and 35.2% for $IKZF1$ deletions, $IKZF1^{\text{plus}}$ profile and UK-ALL CNA –IR+PR risk group respectively ($p$-values $<0.001$). Integrated evaluation of UK-ALL CNA risk profile with ICICLE trial risk stratification groups also revealed a worse OS, EFS and RR of 63.3%, 43.2% and 35.2% for combined ICICLE...
groups with CNA-IR+PR profile compared to CNA-GR profile (81.3%, 65.0% and 21.0% ; p-<0.001). Hence, routine CNA testing in our setting is must to identify SR and IR cases likely to benefit from high risk treatment.

**Keywords:** B-ALL, Pediatric, MLPA, Prognostic, *IKZF1*

**Introduction**

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy. With the present multi-agent chemotherapy protocols, a cure rate of 85-90% has been achieved in developed countries due to combination of protocol refinement, better risk stratification and availability of enhanced supportive care. Though, survival rates have improved vastly in LMICs and other developing nations including India, treatment related mortalities (11-25%) and relapse rates (15-41%) have remained high.

Approximately 60 % of pediatric ALL cases have been shown to harbour copy number alterations (CNAs) in at least one of the important loci related with cell differentiation, cell cycle control and apoptosis related genes that drive leukaemogenesis and contribute to relapse. These genetic abnormalities have an influence on the treatment outcome and have been incorporated into integrated risk scoring systems widely in European trials based upon CNA categorization into good risk and intermediate/poor risk groups (Table 1 definition). In a recent study by Stanulla et al, *IKZF1* profile has been defined and shown to be associated with worse minimal residual disease (MRD), poor prednisolone response (PPR) and high cumulative incidence of relapse (Table 1 definition). Studies from our group have also shown variable risk outcomes of CNAs including role of MRplus scoring in Ph-negative pediatric B cell ALL (B-ALL) to better stratify treatment outcomes. In the ongoing ICICLE multi-centric collaborative treatment trial which involves major centers in our country, integrated MRD and primary genetic risk stratification of cases is routinely performed. The focus in future trials is shifting towards development of better risk stratification and
prediction scores. With this as aim we sought to comprehensively evaluate CNA data in relation to their clinical and prognostic impact. To achieve this goal, we initiated a multi-centric collaborative effort involving two major pediatric B-ALL treatment centers in India.

**Materials and Methods**

**Cohort enrolment**

Patients aged 1-18 years from two major Indian medical institutes AIIMS, New Delhi (center 1) and PGIMER, Chandigarh (center 2) with B-ALL during 2014-2021 were treated and followed up uniformly as per the ICICLE treatment protocol (Clinical Trials Registry-India number, CTRI/2015/12/006,434) (Supplementary Figure 1). Risk stratification was performed upfront based on NCI criteria, prednisolone response at day 8 (poor prednisolone response or PPR if absolute blast count in peripheral blood >1 x 10⁹/L) and primary genetic event. Patients were evaluated for bone marrow remission and flow based end induction (day 28/day35) MRD. The final risk stratification at end of induction was based on bone marrow remission status and MRD as- ICICLE Standard risk (SR), ICICLE Intermediate-risk (IR); ICICLE High-risk (Figure 1 and Supplementary Figure 1).

**Diagnostic genetic testing and retrospective screening for copy number alterations (CNA)**

Diagnostic (2-3ml) peripheral blood (>60% blasts) and or bone marrow (0.5ml) EDTA samples from patients were evaluated for CNA by Multiplex Ligation Dependent Probe Amplification (MLPA) assay using probe-sets of P335-ALL-IKZF1 and P-202 IKZF1-ERG. MLPA was performed as per standard protocol standardized and published earlier 9–11,13. The deletions in the loci of following 9 genes were scored as deleted or non-deleted: CDKN2A/B, IKZF1, RB1, EBF1, ERG, PAX5, PAR1, BTG1 and ETV6. In all cases, MLPA data was normalized with control samples to calculate relative copy number. Dosage quotient (DQ) values between 0.75-1.3 were considered normal copy number of 2, while any value above or below this threshold was scored as gain or loss and values below 0.25 were considered as biallelic loss (copy number 0). For CDKN2A/B, deletion of either locus was
considered as deleted. For \( PAX5 \) deletions, intragenic amplifications were scored along with deletions as both are functionally similar\(^{15}\).

The cohort enrolment at center 1 was consecutive but enrolment from center 2 was biased since cases with recurrent cytogenetic abnormalities and aneuploidies were excluded for MLPA analysis in initial one year of enrolment. Initially, a total of 535 cases were shortlisted, of which 493 patients, that received treatment were further analyzed (Figure 1 CONSORT Flow chart). Cases enrolled at center 1 were analyzed by conventional cytogenetics, multiplex RT-PCR, and/or fluorescent in situ hybridization (FISH) for recurrent genetic translocations and aneuploidies including \( BCR::ABL1 \), \( KMT2A \) rearrangements, \( TCF3::PBX1 \) and \( ETV6::RUNX1 \). At center 2, cases that were enrolled after 2018 had RT-PCR and FISH data with additional centromere probes. The primary genetic data evaluation was done as per following groups:- Good-risk cytogenetics group (\( ETV6::RUNX1 \) and high hyperdiploidy), Intermediate-risk cytogenetics group (cases with either negative RT-PCR/FISH/Ploidy results or other genetic abnormality like \( TCF3::PBX1 \) and \( P2RY8::CRLF2 \) fusion) and High-risk cytogenetics group (\( KMT2A-r \), \( BCR::ABL1 \), hypodiploidy (<45 chromosomes), \( t(17;19) \) (q22;p13) and \( iAMP21 \)).

In addition, center 2 also tested a limited number of B-other samples (n=32) on targeted RNA-NGS Ion Ampliseq panel on Ion Torrent S5 (110 translocations related with B-ALL; mean coverage 500x) and in 7 of the cases noted high risk cytogenetic abnormality (\( MEF2D::BCL9 \), \( n=1 \); \( ABL1 \) kinase fusions, \( n=2 \); \( KMT2A-r \), \( n=2 \); \( BCR::ABL1 \), \( n=2 \)). However, since results were available post induction, final treatment based risk stratification continued as per initial categorization.

**CNA risk-score definitions**

The various CNA risk scoring systems being evaluated in the study trial have been defined in the Table 1 and include the \( IKZF1^{\text{plus}} \) profile, UK-ALL CNA risk groups of CNA-GR (good risk) and CNA-IR+PR\(^7\) and the MRplus scoring that integrates CNA risk group scores (M0 and M1) along
with \(IKZF1^{+}\) (1-present; 0-absent) profile to derive three categories of MRplus0, MRplus1 and MRplus2. In addition, we evaluated a combined Final risk stratification system integrating CNA risk group scoring with ICICLE treatment trial risk stratification groups (Table 1).

**Outcome assessment and Statistical analysis**

Treatment outcome parameters analyzed included event free survival (EFS) - defined as time from start of therapy to an event which included relapse or death or refractory disease-with censoring at last contact. Relapse free survival (RFS) or relapse rate (RR) - time period from onset of therapy to disease relapse for those achieving complete remission with censoring at death in remission or last contact. Overall survival (OS) - defined as time period from onset of therapy to death with censoring at last contact. In addition, treatment related mortality (TRM) was defined as death due to non-relapse related causes. Induction failure was defined as per post-induction bone marrow criteria of more than 5% blasts. Cases continuing to be in non-remission status post re-induction therapy were labeled as having refractory disease or treatment failure. A very early relapse was defined as relapse before 18 months post CR, early relapse as between 18-36 months and late relapse as relapse occurring after 36 months post CR.

Continuous variables are represented as mean/median (range) and categorical variables as ratio/proportion for whole cohort as well as patient sub-categories. Chi-square test is performed between different clinical, hematological and treatment outcome parameters and patient sub-groups and isolated CNAs as well as CNA risk groups. Survival curves (EFS, RR, OS) and survival rates for overall cohort along with effect different ICICLE risk groups and isolated CNAs, CNA risk group and profiles are calculated using Kaplan Meier methods and log-rank tests. A p-value of <0.05 is considered as significant. All statistical analysis have been performed using SPSS v26.0.

**Results**

*Patient demographics & cytogenetic profile*
A total of 493 pediatric and adolescent patients (age range 1-18 years) with B-ALL being treated at either center 1 (n=317) or center 2 (n=176) under ICICLE protocol were included in the study. The median age of the cohort was 5 years with male to female ratio of 1.9:1 (Table 2). The WBC range was between 0.3-980 x 10^9/L. MRD data was available for 444 patients and 111 (25%) had a positive MRD of ≥0.01% post induction. In addition, 61/493 (12.4%) patients had induction failure. According to ICICLE risk criterion- 119 (24.1%) patients belonged to ICICLE-SR, 159 (32.3%) ICICLE-IR and 215 (43.6%) ICICLE-HR category. Eighty-three (16.8%) patients had Good-risk cytogenetics, 363 (73.6%) Intermediate-risk and 47 (9.5%) High-risk. The detailed baseline characteristics of the whole cohort (n=493) as well as center wise breakdown is highlighted in Table 2.

**ICICLE risk and Primary genetic risk group correlation data**

We examined the correlation of ICICLE risk categories with different clinical and outcome parameters (Supplementary Table 1). The ICICLE intermediate risk group had 46% (73/159) cases in the poor prognostic age group of 10-18 years and 83.6% (133/159) in NCI high risk group compared to 29% (62/215) and 60.5% (130/215) respectively in ICICLE high risk group. Amongst the outcome factors, 56% (69/123) of all relapses occurred in the ICICLE high risk group compared to 22% each in intermediate and standard ICICLE risk groups (p<0.001).

In primary genetic sub-groups analysis (Supplementary Table 2), high risk cytogenetic cases had older age, high WBC, higher induction failure, MRD positivity and death rate as compared to those with good risk cytogenetics (p<0.01). In addition, nearly 43% (20/47) of all high risk cytogenetics cases had a relapse compared to around 23% in each intermediate and standard risk cytogenetic groups (p=0.013).

**Copy number alteration frequency and correlation data with clinical variables and risk stratification groups**
Out of 493 cases, 291 (59%) harboured a CNA in at least one of the nine loci tested. Of these, 114 (39%) had single loci deletions, followed by 89 (30.5%), 58 (19.9%) and 15 (5.1%) cases with two, three and four loci deletion respectively (Figure 2a). Rare (8/291; 2.7%) cases had five or more loci involved.

The most frequent deletion noted in the study group (N=493) was CDKN2A/B (36.3%) followed by PAX5 (24.7%) and IKZF1 (19.5%). Deletional frequency in BTG1, EBF1 and RB1 was 7.7%, 4.1% and 7.7%, respectively. Data for ERG deletions was available for 449 cases and 14 cases (3.1%) were found to harbour ERG deletion. IKZF1plus profile was identified in 13.4% (n=66) of cases. CNAs classified, as per Moorman et al13 (UKALL-CNA), revealed 259 (52.5%) cases in CNA-GR and 234 (47.5%) in CNA-IR+PR group. As per MRplus scoring system, 259 (52.5%) cases had MRplus0 score, 168 (34.1%) MRplus1 and 66 (13.4%) MRplus 2 score.

To further investigate the correlation of IKZF1 deletion and other CNA risk profiles with clinical and risk group stratification variables, chi-square test was performed (Table 3). Significant correlation (p<0.05) of IKZF1 deletion, IKZF1plus profile, CNA-IR+PR and MRplus2 score with poor prognostic age group 10-18 years, high WBC >50 x 10^9/l, high NCI risk, induction failure and MRD≥0.01% was observed. On assessment of other CNAs (Supplementary Table 3), CDKN2A/B deletion was more common in cases with older age group (p=0.001), high WBC (p=0.003), NCI-HR (p<0.001), and ICICLE-IR/HR (p=0.009) and primary genetic-IR/HR (p<0.001) cases. RB1 and PAR1 deletions were statistically more common in cases with induction failure (p<0.02 and <0.001). Further, PAX5 deletions were statistically common in older age group and NCI-HR (p<0.001). ERG deletions were seen primarily in ICICLE-SR/IR cases and associated with CR status (p<0.01).

Further we analyzed the proportion of cases harbouring a particular CNA in different ICICLE risk groups (Figure 2b). A statistically significant CNA burden in ICICLE-HR group (295 CNA events) compared to SR (84 CNA events) and IR (184 CNA events) groups (p<0.001) was observed.
IKZF1, PAX5, BTG1 and RB1 deletions were seen to increase in proportion from ICICLE-SR to ICICLE-HR group while ERG deletions were nearly absent from the ICICLE-HR group. In addition, IKZF1 deletion, IKZF1plus, CNA risk and MRplus profiles were noted to be significantly more common in ICICLE-HR group (Supplementary Table 4; p<0.001). The Figure 3a shows correlation matrix highlighting co-occurrence of various CNAs in whole cohort and reveals that PAX5 deletions are usually seen along with CDKN2A/2B deletions, while deletion of PAR1 region is rarely seen with IKZF1 or BTG1 deletions and never with ETV6 deletion.

The primary genetic risk groups were also analyzed for presence of CNAs (Figure 3b and Supplementary Table 5). IKZF1 deletion was noted in 29/47 (61.7%) of high-risk primary genetic group (p<0.001). Further 24/29 (82.7%) of these IKZF1 deletion cases had IKZF1plus profile (p<0.001). In addition, 76.5% (36/47) of cases with high-risk cytogenetics had CNA-IR+PR profile and 51.5% (24/47) MRplus2 score. Figure 3b shows that IKZF1, CDKN2A/2B and PAX5 deletions increased in proportion from primary genetic good risk to high risk cytogenetic groups while ERG deletions were not seen in high risk cytogenetics group.

**Outcome analysis of whole cohort, ICICLE risk stratification groups, CNAs and integrated proposed risk stratification categories**

Kaplan Meyer survival analysis (OS, EFS, RR) and log-rank test have been performed for the whole cohort, ICICLE and primary genetic risk groups, IKZF1 deletion and IKZF1plus, CNA and MRplus profiles. In addition, survival outcome analysis of the CNAs i.e. IKZF1 deletion, IKZF1plus and CNA risk profiles have been studied with MRD and the three ICICLE risk groups (SR, IR & HR). The median follow up for cases in the cohort was 41 months and hence 3-year survival analysis data with 95% CI is presented (Tables 4&5 and Figures 3a-f) (Supplementary Table 6 and Supplementary Figures 2-8).
The events considered for survival analysis were refractory disease (n=7), relapse (n=123) and deaths (n=131). Amongst those who relapsed (n=123), 36.5% (n=45) cases had very early relapse, 37.3 % (n=46) early and 24.2% (n=30) late relapse. Death due to non-relapse reasons was 22.9% (113/493), with 38 (33.6) induction deaths and 75 (66.3%) non-induction phase treatment deaths.

Eighteen cases (15.9 %) had late death post treatment completion at a median time of 42 months. Induction failure was noted in 61 (12.4%) cases. Majority of these 70.5% (43/61) were NCI high risk as per age and WBC and 18 were female and 43 male. Besides induction failure a sub-set of patients also showed refractory disease (n=7) despite re-induction therapy.

The 3-year OS, EFS and RR for the cohort is 73.1%, 54.8% and 26.6% respectively. Both ICICLE-HR and primary genetic high risk groups had a statistically poor EFS and high RR. Amongst CNAs, \textit{IKZF1} deletion and \textit{IKZF1} plus profile had significantly poor OS, EFS and RR at 57.2%, 37.3%, 38.6% and 53.0%, 30.0%, 46.5% respectively compared to patients without those deletions/profiles ($p<0.05$) (\textbf{Table 5 and Figure 3a-b}). In addition, the OS, EFS and RR of the CNA-IR+PR and MRplus2 score was significantly poor ($p<0.001$) (\textbf{Table 5 and Figures 3c-d}).

The relapse rate also worsened significantly in cases with MRD positivity ($p<0.001$) when combined with UKALL-CNA risk profile, from 29.9% (only MRD) to 41.7% (MRD and CNA-IR+PR). A similar effect on relapse rate were noted when MRD was combined with \textit{IKZF1} deletion- 26.1% to 46.7% ($p<0.002$) and \textit{IKZF1} plus profile- 31.3% to 55.6% ($p<0.001$) (Supplementary Table 6).

Survival and outcome analysis for \textit{IKZF1} deletions and the \textit{IKZF1} plus and UKALL-CNA risk profiles was also performed in different ICICLE risk groups as these treatment based risk stratification groups are already integrated with MRD, NCI and primary genetic data (\textbf{Table 5}). In ICICLE-SR, though the number of \textit{IKZF1} deletion and \textit{IKZF1} plus profile cases were low, the OS and EFS was significantly poor in cases with \textit{IKZF1} deletion, CNA-IR+PR and MRplus2 profiles. In ICICLE-IR and ICICLE-HR, \textit{IKZF1} plus profile and UKALL-CNA profile (Groups 4 and 6) had clear prognostic
impact with significantly worse EFS and high RR. *IKZF1* deletions had statistically poor EFS and high RR in ICICLE-IR group but was borderline significant in ICICLE-HR group. On combining the proposed integrated ICICLE and UKALL-CNA groups with respect to CNA-GR or CNA-IR+PR status, (ICICLE+CNA-GR vs ICICLE + CNA-IR+PR), significantly improved OS, EFS and lower RR is noted for CNA-GR compared to CNA-IR+PR profile (p<0.01) (Table 4 and Figure 3e-f).

**Discussion**

The present study was initiated to comprehensively investigate and analyze the prognostic role of CNAs in pediatric B-ALL cases being treated under a uniform ICICLE treatment protocol. A total of 493 pediatric B-ALL cases were evaluated for nine important loci for CNAs and correlated with clinical and treatment outcome parameters.

The study is limited by restricted primary genetic analysis in B-ALL cases to primarily RT-PCR and or conventional cytogenetics/FISH data. Only a limited number of cases especially from center 2 were evaluated with centromere probes on FISH, flow based DNA ploidy and targeted RNA based NGS panel evaluation. Hence, we focused on categorization of primary genetic abnormalities as good risk, intermediate risk and high risk. However, despite this limitation, the study data on CNA is important to highlight since CNAs have been shown to be independent prognostic factors in many different trials 14–18.

The 3-year OS, EFS and RR of the overall cohort was 73.1%, 54.8% and 26.6% respectively. Studies from our subcontinent as reviewed by Arora et al19 show improved OS and EFS over the past decade or so to 60-80% and >50% respectively.

Majority of the cases (43.6%; 215/493) belonged to ICICLE-HR category and only 32.2% and 24.1% to IR and SR. The overall survival of ICICLE treatment trial risk stratification groups was not statistically significant but RR was significantly worse for ICICLE-HR group compared to ICICLE-SR & IR groups (33.9% vs. 20.75%; p-0.002).
The overall frequency of a CNA, in either of the 9 loci tested in our cohort, was 59% with 60% (170/284) showing 2 or more loci deletion. CDKN2A/B deletion was the most frequent CNA identified (36.3%), but prognostically, the most significant CNA in our cohort was IKZF1 deletion (n=96; 19.5%). The frequency of IKZF1 deletion in the current study population was consistent with previously published reports (15-26%)\textsuperscript{17,18,20}. We noted that IKZF1 deletion was concentrated in the high risk groups (genetic poor risk and ICICLE high risk) consistent with literature data\textsuperscript{7,21–23}.

A highly significant correlation of IKZF1 deletion (n=96) was noted with high TLC (p<0.001), NCI high risk (p<0.001), induction failure (p<0.001), ICICLE-HR (p<0.001) and with primary genetic high risk group (p<0.001). Further, when RR was analyzed independently with genetic variables, the RR of IKZF1 deletion cases was noted to be high (53.8%) compared to wild-type status (27.5%). The association of IKZF1 deletion with poor overall survival outcome has been reported by many studies from adult and pediatric BCP-ALL cohorts \textsuperscript{7,21,22,24–28} except for the ones with UKALL14 and UKALL60+ adults’ cohort \textsuperscript{29–31}. A number of studies have also identified the IKZF1\textsuperscript{plus} profile to be an independent stronger molecular stratification marker in pediatric population\textsuperscript{6,19}. In our study too, when IKZF1 deletion were further categorized as IKZF1\textsuperscript{plus} profile (n=66), similar associations were observed with different clinical parameters and with relapse (p-0.02), events (p-0.0001) and death (p-0.025). In addition, IKZF1 deletion and IKZF1\textsuperscript{plus} profile were also noted to be independent poor prognostic markers compared to MRD, which is currently the single most important prognostic factor in treatment of ALL \textsuperscript{32–35}. A highly significant correlation was observed with IKZF1 deletion and MRD positivity in our cohort (p<0.0001). Further, the relapse rate were higher in IKZF1 deletion and plus profile cases with positive MRD and increased dramatically from 26.1% (MRD alone) to 46.7% (p-0.002) and 31.3% to 55.6% (p<0.0001) respectively, as noted in other trials\textsuperscript{27,36}.

We also classified CNAs as per Moorman et al CNA risk classification (UKALL-CNA) into CNA-GR and CNA-IR+PR group and also scored the CNA risk group with IKZF1\textsuperscript{plus} profile as per our
published MRplus scoring system. The CNA-GR cases had significantly better OS, EFS and RFS and this remained unchanged despite MRD positivity suggesting CNA-GR profile to be an independent good prognostic marker in pediatric B-ALL. MRplus scoring too can be used as a risk stratification strategy as a high score of 2 helped identify a sub-set of CNA-IR+PR cases that had $IKZF1^{\text{plus}}$ profile, which clearly showed poor outcome and high relapse rate.

Finally, we also evaluated and proposed an integrated UK-ALL CNA profile and ICICLE risk group categorization. The outcome analysis, revealed that Group-2 and 4 (i.e. ICICLE SR and IR with CNA-IR+PR status) behaved similar to group 6 (ICICLE HR + CNA-IR+PR) with poor OS, EFS and RR and this was statistically different from other groups 1,3 and 5 with good-risk CNAs. Overall, in ICICLE-SR, few cases (7/119;6%) had $IKZF1$ deletion and $IKZF1^{\text{plus}}$ profile (3/119;2.5%) and none relapsed; but 28.5% cases (34/119) had CNA-IR+PR profile, of which around 50% (16/34) had an event with 31% relapsed (5/16). In ICICLE-IR group, $IKZF1$ deletion as well as $IKZF1^{\text{plus}}$ and UKALL-CNA profile were clearly prognostic with twice the number of cases having relapse compared with cases without deletion and plus profile respectively (p<0.001). The outcome analysis of combined groups 1,3,5 with groups 2,4,6 also clearly demarcated the prognostic impact of CNAs with CNA-IR+PR profile behaving as an independent poor prognostic marker irrespective of SR, IR or HR ICICLE stratification status. This provides strong evidence that CNA testing needs to be incorporated in our prospective enrolment strategy to identify subset of SR/IR cases that would benefit from high risk treatment during consolidation phase.

A recent study had also reported $BTG1$ as one of the prognostic markers in acute leukemia. However, in our cohort no promising correlation was observed for $BTG1$ deletion with any of the clinical variables. The most common CNA noted i.e. deletion of $CDKN2A/B$ did show significant correlation with a few variables however the significant association with relapse, as reported earlier by our group, could not be substantiated in this cohort. Similar observation had been noted in
UKALL14 study except for the trend for biallelic deletions of *CDKN2A/B* in *BCR-ABL1* where they found association with lower EFS and OS.

In conclusion, despite limited primary genetic analysis and slightly biased cohort enrolment with partial exclusion of recurrent good risk and high risk cytogenetic cases, our study provides strong evidence for CNAs as one of the important independent prognostic factors, in addition to MRD, for better risk stratification of pediatric B-ALL cases. The study data strongly suggests routine screening and prospective testing for CNAs in our ICICLE treatment trial. This coupled with extensive primary genetic analysis in our treatment trial can help generate better prospective data on status of *IKZF1* deletion and plus profile in Ph-Like, Ph-positive and Ph-negative groups. The study also proposes integration of CNA risk group status with ICICLE risk stratification categories to define novel subset of cases in both ICICLE-SR and IR group that might benefit from therapy escalation post induction.

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**Author Contributions:** SKG and PB- Designed and conceptualized the study and analyzed the data. MS and PB- Wrote the manuscript and performed MLPA analysis at centre-2; AVM- Mentored and guided the manuscript writing and data analysis as a subject expert; PHC, AT, DB, RJ, SP, SB- Clinicians involved with patient recruitment, enrolment in ICICLE trial, treatment and follow-up work at centres-1 and 2. RT, SG, DP & PS-Performed MLPA at centre 1 and 2 and analyzed MLPA
data. SS, RG & MSS- Primary Genetic and MRD analysis at centre 1 and 2, Manuscript review and editing.

Competing Interests:- No financial competing interests to declare.

Data Availability Statement:- All relevant raw data will be freely available to any researcher wishing to use it for non-commercial purposes.

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Table 1: Various genetic risk profile and proposed risk definitions used in the study trial

Table 2: Baseline characteristics of the cohort (N=493)

Table 3: Clinico-hematological and treatment outcome parameters in IKZF1 deletions and CNA risk profiles (n=493)

Table 4: Highlights 3-year outcome analysis of whole cohort, ICICLE and primary genetic risk groups, \textit{IKZF1} deletion, \textit{IKZF1}^{plus} profile, UKALL-CNA profile, MRplus score and interaction of combined ICICLE groups with CNA-Good risk and CNA-Intermediate/Poor risk profile

Table 5: Prognostic effect of \textit{IKZF1} deletion and other CNA profiles in the ICICLE standard risk group
**Figure legends**

**Figure 1:** CONSORT Flow diagram to highlight case enrolment and treatment trial risk stratification details

**Figure 2:**
(a) Pie chart to show frequency of CNA distribution as per single or multiple loci involvement
(b) Bar plot to show proportion of different CNAs in ICICLE –SR/IR/HR risk groups
(c) Correlation matrix to highlight the co-occurrence of various CNAs in whole cohort (n=493); The integers in the matrix represent the number of cases and the colour scale the Pearson correlation coefficient
(d) Bar plot to show proportion of different CNAs in Primary genetic risk groups

**Figure 3:**
(a) Relapse Rate (RR) of IKZF1 deletion (1) and non-deletional group (0) (n=493)
(b) Relapse Rate (RR) of $IKZF1^{plus}$ profile (1) and non-plus cases (0) (n=493)
(c) Relapse Rate (RR) of UKALL-CNA profile CNA-GR (1) and CNA-IR+PR (0) (n=493)
(d) Relapse Rate (RR) of MRplus scores: MRplus0 (0), MRplus1c(1) and MRplus2 (2) (n=493)
(e) Overall survival (OS) of combined
proposed risk stratification groups; Group 1, 3, 5 (1) and Group 2, 4, 6 (2) (n=493) (f) Relapse rate (RR) of combined proposed risk stratification groups; Group 1, 3, 5 (1) and Group 2, 4, 6 (2) (n=493)
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CONSORT Flow diagram to highlight case enrolment and treatment trial risk stratification details
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Supplementary Files

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- Supplementarydata1752022final.pdf