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Genetic alterations and MRD refine risk assessment within KMT2A-rearranged B-cell precursor ALL in adult: a GRAALL study

Short title:
Genomics and MRD in KMT2A-r adult BCP-ALL

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Data sharing statement :
For original data, please contact emmanuelle.clappier@aphp.fr
Key points

- In adult KMT2A-r BCP-ALL, TP53 and IKZF1 alterations are associated with very poor outcome
- KMT2A genomic fusion should be the preferred MRD marker over IG/TR to assess early treatment response and predict long-term outcome

Abstract

KMT2A-rearranged (KMT2A-r) B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is widely recognized as a high-risk leukemia in both children and adults. However, there is a paucity of data on adults treated in recent protocols and the optimal treatment strategy for these patients is still a matter of debate. In this study, we set out to refine the prognosis of adult KMT2A-r BCP-ALL treated with modern chemotherapy regimen and investigate the prognostic impact of co-mutations and minimal residual disease (MRD). Of 1091 adult patients with Philadelphia-negative BCP-ALL enrolled in three consecutive GRAALL trials, 141 (12.9%) had KMT2A-r, with 5-year cumulative incidence of relapse (CIR) and overall survival (OS) rates of 40.7% and 53.3%, respectively. Molecular profiling highlighted a low mutational burden in this subtype, reminiscent of infant BCP-ALL. However, the presence of TP53 and/or IKZF1 alterations defined a subset of patients with significantly poorer CIR (69.3% vs 36.2%, p=0.001) and OS (28.1% vs 60.7%, p=0.006). We next analyzed the prognostic implication of MRD measured after induction and first consolidation, using both immunoglobulin (IG)/T-cell receptor (TR) rearrangements and KMT2A genomic fusion as markers. In approximately one third of patients, IG/TR rearrangements were absent or displayed clonal evolution during the disease course, compromising MRD monitoring. By contrast, KMT2A-based MRD was highly reliable and was strongly associated with outcome, with early good responders having an excellent outcome (3-year CIR 7.1% and OS 92.9%). Altogether, our study reveals striking heterogeneity in outcomes within adults with KMT2A-r BCP-ALL and provides new biomarkers to guide risk-based therapeutic stratification.
Introduction

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) harboring a rearrangement of \textit{KMT2A} (\textit{KMT2A}-r), formerly \textit{MLL}, represents a distinct entity accounting for 75% of infant, 2-5% of childhood non-infant, and 5-10% of adult BCP-ALL\textsuperscript{1–5}. Historically, all these patients have experienced poor outcomes, leading to the inclusion of \textit{KMT2A}-r as a high-risk criterion in all risk-adapted treatment protocols.\textsuperscript{6–9} The high prevalence and poor prognosis of \textit{KMT2A}-r in infants has generated considerable efforts to improve the clinical management of this leukemia.\textsuperscript{10–12} By contrast, there is a paucity of studies specifically addressing this BCP-ALL patients’ subgroup within adults, and the optimal treatment strategy for these patients is still a matter of debate.\textsuperscript{6–9} Similarly, the biology of \textit{KMT2A}-r BCP-ALL has been extensively studied as an infant leukemia.\textsuperscript{13–19} In this context, it was proposed that specific properties of the cell-of-origin at the early developmental stage may confer prominent susceptibility to leukemogenesis induced by \textit{KMT2A}-r.\textsuperscript{14,20} Moreover, the landscape of genomic alterations in infant \textit{KMT2A}-r leukemia has been shown to be exceptionally limited, in agreement with a one-hit model of oncogenesis.\textsuperscript{19,21,22} Noteworthy, the presence of additional genetic alterations and their possible role in leukemogenesis and prognosis have not been investigated in adult \textit{KMT2A}-r ALL.

Over the past two decades, the use of more intensive regimens in young adults has yielded significant improvements in outcomes, challenging the conventional risk stratification criteria.\textsuperscript{23–28} Like previously in children, the assessment of minimal residual disease (MRD) at early stages of treatment has allowed for the refinement of risk groups within adult BCP-ALL.\textsuperscript{24,25,28–30} Interestingly, studies in childhood BCP-ALL using large datasets have demonstrated that distinct genetic subtypes are typically associated with different response kinetics, suggesting that MRD results should be interpreted in the context of subtype-specific patterns.\textsuperscript{31} While MRD has proven to have prognostic value within infant \textit{KMT2A}-r BCP-ALL,\textsuperscript{32,33} no study has investigated the significance of MRD in the setting of adult \textit{KMT2A}-r BCP-ALL, likely owing to limited patient numbers in each study. Thus, \textit{KMT2A}-r BCP-ALL is usually regarded as a high-risk leukemia regardless of other risk criteria.

Considering the current outcomes of BCP-ALL in young adults, we hypothesized that additional criteria may allow to refine the prognosis of \textit{KMT2A}-r BCP-ALL. In this study, we
conducted a comprehensive analysis of a large cohort of patients treated in three consecutive trials from the Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL). Through analyses of genetic co-mutations and MRD, we could identify distinct subsets of patients having markedly heterogeneous clinical outcomes. Overall, our study highlights the significant heterogeneity in clinical outcomes within this population and provides criteria for further treatment stratification.

**Patients and Methods**

**Patients and treatment**

Between 2003 and 2020, a total of 1,163 patients aged 15-59 years with newly diagnosed Philadelphia-negative (Ph-neg) BCP-ALL were treated in the GRAALL-2003 (n=149), -2005 (n=525) and -2014 (n=489) trials (ClinicalTrials.gov numbers: NCT00222027, NCT00327678, NCT02617004, respectively). The present study included 1,091 of these patients with a known status for 11q23/KMT2A rearrangement, of whom 141 (12.9%) were positive. All patients or their relatives provided informed consent according to the Declaration of Helsinki, and the study was approved by the local ethics committee. The three GRAALL protocols used similar intensive pediatric-inspired chemotherapy regimens. In the GRAALL-2003 and 2005 protocols, all patients with 11q23/KMT2A rearrangement were eligible to allogeneic hematopoietic stem cell transplantation (HSCT). In the GRAALL-2014, chemotherapy intensity was reduced in patients aged 45 years or more, and HSCT indication was restricted to patients with suboptimal early IG/TR MRD response, regardless of 11q23/KMT2A status. In addition, a nested phase 2 sub-study introduced blinatumomab in consolidation for higher-risk patients, including those with a KMT2A-r BCP-ALL (n=16 patients).

**Genetic characterization**

The presence of a 11q23/KMT2A rearrangement was determined at the diagnosis in local centers through cytogenetic analysis, including karyotype and KMT2A break-apart fluorescence in situ hybridization. In 97 KMT2A-r cases with available material (n=7/21, 34/64, and 56/56 patients in GRAALL-2003, -2005, and -2014 trials, respectively), molecular
analyses were performed centrally. Briefly, mononuclear cells were isolated from bone marrow or blood specimens through Ficoll centrifugation, quantitation of leukemia cells percentage was performed by flow cytometry and extractions of DNA and RNA were performed using standard procedures. Targeted screening for recurrent KMT2A fusion transcripts was conducted using reverse transcription quantitative PCR or reverse transcription mixed ligation probe assay. Screening for additional alterations was performed through targeted DNA sequencing of a custom panel consisting of 189 known or putative target genes in BCP-ALL, as previously described. Data processing and visualization were performed using R software v4.2.3 and ggplot2 v3.4.1.

MRD assessment

MRD evaluation was performed in the national reference laboratories using quantitative PCR of clono-specific immunoglobulin and/or T-cell receptor genes (IG/TR) rearrangements, according to EuroMRD guidelines. For KMT2A-r BCP-ALL treated in the GRAALL-2014 trial, the KMT2A breakpoint genomic sequence was retrospectively assessed as an additional target for MRD evaluation. The cutoff for MRD positivity was set at $10^{-4}$.

Statistical analysis

Complete remission (CR) was defined by <5% blasts in the bone marrow. Disease-free survival (DFS) was calculated as the time from the first CR to relapse, death in CR, and censored at the last follow-up for patients without events. Overall survival (OS) was defined as the time from diagnosis to death. Kaplan-Meier estimators were used for DFS and OS. Cumulative incidence of relapse (CIR) was defined as the time from CR to first relapse considering death in CR as a competing event. A cause-specific cumulative incidence function was used to estimate CIR. For OS and DFS, univariate analyses of covariate effects were performed using a Cox model. Fisher’s exact and Mann–Whitney tests were employed for clinical, biological, and mutational analyses. The role of HSCT in KMT2A-r BCP-ALL was evaluated using four-month landmark intention-to-treat analyses for CIR, DFS and OS from CR, excluding patients who died or relapsed before the first four months following CR achievement. Statistical analyses were performed with STATA/SE software (Version 17.0, StataCorp LLC, College Station, Texas, USA). All tests were two-sided, and a p-value <0.05 was considered statistically significant.
Data Sharing Statement

For original data, please contact emmanuelle.clappier@aphp.fr

Results

Baseline characteristics and early response to treatment of KMT2A-r BCP-ALL

The baseline characteristics of the 141 patients with KMT2A-r BCP-ALL compared to other Ph-negative BCP-ALL are presented in Table 1. Patients with KMT2A-r BCP-ALL were significantly older (median age 42.0 vs 36.8, p=0.02) and had a higher proportion of females (male/female ratio 0.78 vs 1.3, p=0.005). They also presented significantly higher white blood cell (WBC) counts at diagnosis (median 94.4 vs 6.3 G/L, p<0.0001) and predominantly a CD10-negative pro-B immunophenotype (74.8 vs 14.3%, p<0.0001). The most common KMT2A rearrangement was the translocation t(4;11)(q21;q23) resulting in the KMT2A::AFF1 fusion, identified in 121 out of 141 (86%) patients, while the t(11;19)(q23;p13.3)/KMT2A::MLLT1 fusion was identified in 12 patients (8.5%). The only significant difference in presenting features between KMT2A::AFF1 and non-AFF1 KMT2A-r BCP-ALL was a lower WBC count in the latter (median 116 vs 14.7 G/L, p=0.0002). Early response to corticosteroids, as evaluated by peripheral blood (PB) blast clearance on day 8 of the prephase, was similar between KMT2A-r and other BCP-ALL. Surprisingly, KMT2A-r BCP-ALL patients had a lower rate of poor bone marrow (BM) blast clearance on day 15 (26.6 vs 42.0%, p=0.001). No difference was observed between the two groups regarding the complete remission (CR) rate, and only 2 out of 131 (1.5%) KMT2A-r patients required a second induction phase to achieve CR, as compared to 3.9% for others. Therefore, while KMT2A-r patients display baseline features historically associated with higher risk (i.e older age, higher WBC, pro-B phenotype), they do not show evidence for early treatment resistance.

Outcome according to baseline and early response risk criteria

The median follow-up was 4.3 years. The 5-year CIR, DFS and OS rates for KMT2A-r patients were 40.7% (95% CI 32.7-49.9%), 50.3% (95% CI 41.1-58.8%) and 53.3% (95% CI 44.5-61.4%), as compared to 33.7% (95% CI 30.3-37.2%, p=0.02), 55.5% (95% CI 51.7-59.0, p=0.07) and
59.9% (95% CI 56.2-63.3%, p=0.02), respectively, for other patients (Table 1, Figure 1A-B). A higher percentage of patients undergoing HSCT in first remission was observed in KMT2A-r BCP-ALL patients (48.9% vs 30.3%, p<0.0001). With a median time from CR to HSCT of 3.8 months (95%CI [3.2-4.2]) for these patients, we performed a four-month landmark intention-to-transplant analysis to evaluate the impact of HSCT in first CR. We did not evidence improvement in CIR, DFS or OS (Supplementary Table 1). These intermediate survival rates of KMT2A-r patients prompted us to search for prognostic criteria within the KMT2A-r group. Strikingly, age, sex, central nervous system (CNS) infiltration, pro-B phenotype and WBC (either as continuous or with different cutoff values) were not found to be associated with outcomes (Supplementary Table 1). No significant impact of the KMT2A-r partner gene, whether AFF1 or non-AFF1 genes could be demonstrated (Figure 1C-D), although this may be related to the low number of non-AFF1 cases. Finally, poor responses to corticosteroids on day 8 or to chemotherapy on day 15 did not serve as predictors for poorer survival. Therefore, historical risk criteria could not predict distinct clinical outcomes within KMT2A-r BCP-ALL.

**Landscape of additional genetic alterations**

We then characterized the spectrum of additional genetic alterations in KMT2A-r BCP-ALL patients, through targeted DNA sequencing of a comprehensive panel of genes (Figure 2A and complete list of mutations in Supplementary Table 2). Overall, after excluding KMT2A partial gains or losses directly resulting from 11q23 unbalanced rearrangements, 32 (33%) of patients had no detectable alterations, 30 (31%) had a single altered gene, 18 (19%) had two altered genes and 17 (17%) had three or more altered genes. The most frequent genetic alterations were activating mutations in the NRAS and KRAS genes, detected in 20% and 19% of patients, respectively. Mutations in the FLT3 gene were observed in 11 (11%) patients, encompassing internal tandem duplications and point mutations in the tyrosine kinase and juxta-membrane domains. Collectively, mutations affecting the receptor tyrosine kinase (RTK)-RAS signaling pathways were present in 37 (38%) of these patients. Notably, multiple mutations in signaling factors were often identified within the same case, with low variant allele frequencies (VAF) (median 6%, range 2-47%) contrasting with high leukemic infiltration (median, 89%), indicating subclonality. TP53 mutations were detected in 14 (14%) of patients, with half of them also exhibiting copy-number losses related to 17p abnormalities.
Only one patient displayed a high VAF consistent with TP53 biallelic alteration in the major clone, while in most cases alterations were likely subclonal. CDKN2A alterations were found in 14 (14%) of patients, including biallelic deletions (n=8), monoallelic deletions (n=3) and truncating mutations (n=3). IKZF1 deletions were observed in 8 (8%) of patients, all of which were related to gross abnormalities of chromosome 7, predominantly isochromosome 7q. No PAX5 deletions were found, but a unique PAX5 mutation (p.S133R) was identified in three patients. It is noteworthy that no KMT2A-r cases met the definition of IKZF1Plus ALL.

Recurrent mutations were also identified in TBL1XR1 (n=4 cases), TCF3 (n=3 cases) and TET2 (n=3 cases). No other gene alterations were found in more than two cases. Alterations affecting factors involved in epigenetic modifications were collectively observed in 11 (11%) of the patients. To summarize, adult KMT2A-r BCP-ALL exhibit a specific pattern of additional genetic alterations being infrequent, subclonal, and primarily targeting the RTK-RAS pathway and the TP53, CDKN2A and IKZF1 tumor suppressor genes.

**Prognosis of additional genetic alterations**

Next, we assessed the impact of recurrent alterations on CIR, DFS and OS (Figure 2B-C, Supplementary Table 3). Mutations in RTK-RAS pathway genes and CDKN2A deletions did not show any significant association with clinical outcomes. In contrast, TP53 alterations were associated with significantly worse CIR (SHR=2.95, 95%CI [1.39-6.27]; p=0.005), DFS (HR=2.20, 95%CI [1.06-4.58]; p=0.034) and OS (HR 2.67 95%CI [1.31-5.45]; p=0.007) rates (Figure 2B-C and Supplementary Figure 1). Of the 14 patients with TP53 alterations, ten succumbed to their disease within two years from diagnosis, either due to primary resistance (n=1) or early relapse (n=9), indicating an exceptionally aggressive disease course. Interestingly, analysis of relapse samples in 6 of these patients demonstrated an expansion of the TP53-mutant clone under treatment and additional alteration of the second allele in 3 cases (Supplementary Figure 2). IKZF1 deletions also appeared to be linked to a higher CIR (SHR=3.03 95%CI [1.25-7.34]; p=0.01), with a smaller number of patients (n=8) limiting the statistical significance on DFS (HR=2.31, 95%CI [0.99-5.57]; p=0.053) and OS (HR=2.01, 95%CI [0.85-4.80]; p=0.11). These results thus suggest that additional alterations in KMT2A-r BCP-ALL confer disease heterogeneity which translates in heterogeneous responses to treatment.

Overall, out of the total 97 patients, twenty (20.6%) had either TP53 or IKZF1 alterations (thereafter named “Onco+” status), forming a subgroup of patients who experienced
significantly poorer outcomes (CIR, 69.3% vs 36.2%, SHR=3.06, 95%CI [1.55-6.04]; p=0.001, DFS, 30.7% vs 49.8%, HR=2.18, 95%CI [1.14-4.14]; p=0.02 and OS, 28.1% vs 60.7%, HR=2.47, 95%CI [1.30-4.69]; p=0.006) (Figure 2D-E).

Prognostic significance of IG/TR MRD

We next evaluated the prognostic value of MRD within KMT2A-r BCP-ALL, as measured by quantification of clonal rearrangements of immunoglobulin or T-cell receptor genes (IG/TR) at the end of induction (MRD1) and after first consolidation (MRD2). With a cutoff value of \(10^{-4}\), 35 out of 78 (44.2%) patients had positive MRD1 and 20 out of 74 (27.0%) patients had positive MRD2. Both MRD1 and MRD2 positivity were significantly associated with poorer outcomes (Figure 3 and Supplementary Table 4). Hence, MRD evaluation in KMT2A-r BCP-ALL reveals heterogeneous responses to treatment that are associated with long-term outcomes, suggesting that MRD evaluation may provide additional value in refining risk assessment for adult KMT2A-r BCP-ALL.

Comparison between IG/TR and KMT2A-based MRD

Studies in infant KMT2A-r ALL have shown that they usually have few IG/TR rearrangements, and these are frequently immature and oligoclonal, most likely due to the rapid transformation of an immature B-lineage progenitor cell. As a consequence, these rearrangements are not always present in the entire leukemic clone nor stable during the disease course, resulting in false-negative or underestimated MRD values when used as MRD markers. To circumvent this issue, the breakpoint genomic sequence of KMT2A-r (gKMT2A) is used in infants as the preferred MRD marker. We questioned whether adult KMT2A-r ALL patients would display a similar pattern of IG/TR rearrangements, leading to similar limitations. We thus conducted a retrospective analysis in 56 patients with available diagnostic material, in order to compare IG/TR and gKMT2A markers (Figure 4A). Firstly, in 8 out of 56 (14.3%) patients, no clonal IG/TR rearrangement could be detected. In comparison, this situation was seen in only 14 out of 433 (3%) non-KMT2A-r BCP-ALL in the GRAALL-2014 cohort. Conversely, a specific and sensitive qPCR assay for gKMT2A was applicable to virtually all patients. A comparison of MRD1 and/or MRD2 results was feasible for 39 patients with leftover follow-up samples. While most of the results demonstrated high concordance, 13 results (7 MRD1 and 6 MRD2) in 9 out of 39 (23.1%) patients exhibited
discrepancies regarding the critical cutoff of $10^{-4}$. These discrepancies were attributed to IG/TR markers being undetectable or at significantly lower levels as compared to gKMT2A (Figure 4B). Moreover, analysis of IG/TR rearrangements at relapse showed in some cases a complete modification of IG/TR clonality, that is, the absence of initial IG/TR clonal sequences and the emergence of new ones (Figure 4C). Altogether, these findings show in adult KMT2A-r BCP-ALL that IG/TR rearrangements do not confidently allow tracking of the whole leukemic clone and suggest that gKMT2A-based MRD may predict outcome more accurately.

We thus evaluated the prognostic significance of MRD using gKMT2A as compared to IG/TR markers. The median follow-up of KMT2A-r patients enrolled in the GRAALL-2014 was 3.3 years. As expected, patients with discordant MRD, i.e. gKMT2A positive and IG/TR negative, behaved similarly to those with positive MRD on both markers, with significantly higher CIR and lower OS (Figure 4D-E), in agreement with false-negativity of IG/TR-based MRD. Considering all the 41 patients with gKMT2A MRD data regardless of IG/TR MRD, 27 (66%) had positive MRD1 and 17 (41%) had positive MRD2. Both MRD1 and MRD2 had a strong impact on CIR, DFS and OS, and the superiority of gKMT2A over IG/TR was further confirmed by C-Harrell’s concordance test (Figure 4F-G, Supplementary Figure 3 and Supplementary Table 4). Moreover, gKMT2A MRD1 allowed to identify a subgroup of ‘true’ good-responder patients having low CIR and good OS rates (3-y CIR 7.1% vs 61.7%, p=0.02 and OS 92.9% vs 31.9%, p=0.03). Noteworthy, only one of these good-responder patients underwent HSCT, owing to GRAALL-2014 eligibility criteria solely based on MRD response. Altogether, our results demonstrate that MRD measurement using the gKMT2A marker allows us to evidence heterogeneous responses to treatment in adult KMT2A-r BCP-ALL, which are dramatically associated with long-term outcomes.

Prognostic value of the combination of TP53/IKZF1 status and KMT2A-based MRD

Finally, we aimed to further improve the prognostic stratification of adults with KMT2A-r BCP-ALL by combining oncogenetics and gKMT2A MRD1 (Figure 5). No TP53 or IKZF1 alterations were observed within the good responders (i.e. no Onco+/MRD-). By contrast, the presence of TP53 or IKZF1 alterations within poor responders distinguished a subgroup of patients having significantly worse CIR (SHR=3.04, 95%CI [1.12-8.28]; p=0.03), DFS (HR=2.77, 95%CI [1.05-7.34]; p=0.04) and OS (HR=5.28, 95%CI [1.77-15.8]; p=0.003).
Bivariate analysis confirmed the strong impact of oncogenetics, with less significance for MRD in this model because all the patients with TP53/IKZF1 alterations were poor responders (Supplementary Table 5). Thus, the combination of oncogenetics and MRD response allowed to identify three subgroups of patients, i.e. Onco-/MRD-, Onco-/MRD+ and Onco+/MRD+, having markedly distinct 3-y CIR (7.1%, 47.6%, and 88.9% respectively), DFS (92.9%, 41.9% and 11.1%, respectively) and OS rates (92.9%, 60.0%, and 11.1%, respectively).

Discussion

This study represents the most comprehensive study of a large cohort of adults with KMT2A-r BCP-ALL, who were treated with modern intensive protocols. As previously reported, KMT2A-r was associated with typical high-risk presenting features (older age, high WBC counts, pro-B phenotype), but this did not translate into a higher risk for failure to achieve complete remission after induction course. Moreover, survival rates of patients with KMT2A-r BCP-ALL (DFS 50.3% and OS 53.3%) were only moderately lower than that of other BCP-ALL patients in our cohort. These results are in sharp contrast with other studies which reported 5-year overall survival rates for similar KMT2A-r cohorts ranging from 20% to 35%.6–9 Noteworthy, the proportion of KMT2A-r patients undergoing HSCT in first CR in the present study was not different from other cohorts and no benefit of HSCT could be observed for those patients, suggesting that the GRAALL pediatric-inspired chemotherapy effectively contributed to outcome improvement. These results prompted us to investigate disease or patient heterogeneity within this subtype, in order to recognize patients at high risk and those who may achieve long-term remission. Strikingly, no classical risk factors, namely age, WBC, pro-B phenotype and early blast clearance in blood and bone marrow were associated with outcome. While studies in children reported a better outcome associated with KMT2A-r involving non-AFF1 partners,38 we were unable to demonstrate this in our adult cohort and this may need larger cohorts. We therefore analyzed other characteristics which could serve as prognostic markers for risk stratification, namely co-mutations and MRD.

First, we hypothesized that the presence of co-mutations may affect prognosis. Hence, there are many instances in other leukemia subtypes where co-mutations detected on top of the
major driver lesion can act as modifiers of subtype-associated prognosis.\textsuperscript{39–42} By performing genomic characterization of a large cohort of adults with \textit{KMT2A}-r BCP-ALL, we could depict its specific pattern of co-mutations. Strikingly, we observed an exceptionally low mutational burden, with a median of one altered gene per patient. While we used a targeted approach on a large panel of genes involved in BCP-ALL, the actual number of alterations might be slightly higher. In addition, the most frequent mutations involved the RTK-RAS pathway and most mutations were present in minor clones. Altogether, this genomic pattern is very similar to that previously observed in infant \textit{KMT2A}-r.\textsuperscript{19,22} Interestingly, while it has been suggested that the low mutational burden and short latency of infant \textit{KMT2A}-r ALL may be related to a more permissive state of the fetal target cell of transformation, our results in adults argue for the ability of \textit{KMT2A}-r to induce overt leukemia without the need of cooperating events, regardless of developmental stage. This is also consistent with the short latency of therapy-induced \textit{KMT2A}-r leukemia.\textsuperscript{43} Another important finding of our study is the presence of \textit{TP53} alterations in a significant fraction of \textit{KMT2A}-r adult BCP-ALL, while it has not been observed in children. Moreover, such alterations, although often present in minor clones, dramatically worsened the prognosis of patients. \textit{IKZF1} deletions were also observed and they tended to confer poorer prognosis. Thus, the acquisition of \textit{TP53} and \textit{IKZF1} alterations in \textit{KMT2A}-r leukemia cells may create intra-clonal and inter-individual disease heterogeneity with consequences on response to treatment and outcome. Importantly, the subset of patients with \textit{TP53} or \textit{IKZF1} alterations had a particularly aggressive course, with most relapses occurring on-therapy within 6 months and being resistant to further treatments. These results call for the implementation of rapid screening for these alterations at \textit{KMT2A}-r BCP-ALL diagnosis, in order to propose early investigational therapeutic interventions to those patients at very high risk of resistant disease.

The second part of this study investigated the relevance of MRD level assessment in adults with \textit{KMT2A}-r BCP-ALL. MRD has been shown to be the most important prognostic parameter in ALL and is widely used in contemporary protocols for risk stratification and treatment adaptation. However, it has never been examined within defined genetic subtypes in adults. Virtually all adult ALL protocols consider \textit{KMT2A}-r BCP-ALL at very high-risk with HSCT eligibility, regardless of MRD.\textsuperscript{44} While this is reasonable when survival rates barely reach 30%, our improved results in the GRAALL protocols warranted further risk
stratification. Moreover, studies in infant and non-infant children ALL showed that MRD assessment can unveil heterogeneity in treatment response within KMT2A-r BCP-ALL and identify distinct subsets of patients with different outcomes.\textsuperscript{32,38,45} In our present study, we showed that MRD had indeed a strong prognostic significance. We then investigated the value of using the genomic KMT2A rearrangement sequence as an MRD marker, as compared to IG/TR. As previously shown in infants,\textsuperscript{32,45} we report that IG/TR rearrangements are frequently absent or subclonal in adult KMT2A-r, in agreement with malignant transformation of an early lymphoid precursor preceding IGH complete rearrangement.\textsuperscript{46} Consequently, we demonstrated the superiority of gKMT2A-based MRD over IG/TR in evaluating response and predicting treatment outcome. Of note, while flow-based MRD may be considered an alternative approach, it should be noted that it can also prove defective owing to phenotypic heterogeneity and plasticity of KMT2A-r, especially in the context of B-cell directed therapies favoring loss of B-cell antigens.\textsuperscript{15,47}

By combining oncogenetics and gKMT2A-based MRD, we highlighted three subgroups of patients with markedly distinct outcomes. While our study was conducted with a focus group of patients and would require validation in larger and prospective cohorts, these results pave the way for future risk-adapted treatment stratification of adult KMT2A-r BCP-ALL. Remarkably, we could identify good responder patients having excellent outcomes, without undergoing HSCT. Whether HSCT benefits adult patients with KMT2A-r has been a longstanding matter of debate with limited data available in the literature.\textsuperscript{48} While pediatric protocols have reduced indications of HSCT based on additional prognostic criteria,\textsuperscript{11,12} virtually all adult protocols still rely on HSCT for KMT2A-r patients.\textsuperscript{44} However, the benefit of HSCT was considered in a context where poor results were obtained with respective suboptimal chemotherapy regimens.\textsuperscript{6–9} Our results suggest that intensive pediatric-inspired ALL regimens are able to cure at least a subset of patients. We recognize that these findings were obtained from a group of young adults who underwent intensive treatment. Consequently, the relevance of our results may not be directly transferrable to other contexts, such as those involving older patients or implementing new treatment strategies. For patients who are not eligible to intensive chemotherapy and/or those who have a suboptimal response to chemotherapy, additional treatment strategies, including B-cell-directed immunotherapies\textsuperscript{49} or menin inhibitors,\textsuperscript{50} are urgently needed.
Acknowledgments

The authors thank the patients and all the GRAALL investigators and biologists who contributed samples and data for this study.

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Authorship Contributions


Conflict-of-interest disclosure

The authors declare no relevant competing financial interests with the present study.

References


Table 1: Characteristics, Treatment Response, and Outcomes of the Study Cohort

<table>
<thead>
<tr>
<th></th>
<th>All Ph-neg BCP-ALL patients (n=1091)</th>
<th>KMT2A-r BCP-ALL patients (n=141)</th>
<th>KMT2A-r BCP-ALL patients (n=141)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (%)</td>
<td>141 (12.9%)</td>
<td>950 (87.1%)</td>
<td>121 (85.8%)</td>
<td>20 (14.2%)</td>
</tr>
<tr>
<td>Patient-related characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>42.0 (18.4-59.4)</td>
<td>36.8 (15.2-59.9)</td>
<td>41.7 (18.4-59.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Sex, M/F (ratio)</td>
<td>62/79 (0.78)</td>
<td>542/408 (1.3)</td>
<td>53/68 (0.8)</td>
<td>0.005</td>
</tr>
<tr>
<td>Disease-related characteristics</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>WBC, G/L (range)</td>
<td>94.4 (1-712)</td>
<td>6.3 (0-396)</td>
<td>116 (2-712)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CNS involvement (%)</td>
<td>10/140 (7.1)</td>
<td>60/944 (6.4)</td>
<td>8/120 (6.7)</td>
<td>0.71</td>
</tr>
<tr>
<td>EGIL classification</td>
<td>119 (100)</td>
<td>805 (100)</td>
<td>103 (100)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pro-B (I)</td>
<td>89 (74.8)</td>
<td>115 (14.3)</td>
<td>78 (75.7)</td>
<td>11 (68.8)</td>
</tr>
<tr>
<td>Common (II)</td>
<td>4 (3.4)</td>
<td>538 (66.9)</td>
<td>3 (2.9)</td>
<td>1 (6.2)</td>
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<tr>
<td>Pre-B (III)</td>
<td>25 (21.0)</td>
<td>138 (17.1)</td>
<td>21 (20.4)</td>
<td>4 (25.0)</td>
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<tr>
<td>Mature (IV)</td>
<td>1 (0.8)</td>
<td>14 (1.7)</td>
<td>1 (1.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Response-related characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor early PB blast clearance</td>
<td>22/141 (15.6)</td>
<td>151/945 (16.0)</td>
<td>19/121 (15.7)</td>
<td>0.99</td>
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<tr>
<td>Poor early BM blast clearance</td>
<td>34/128 (26.6)</td>
<td>376/895 (42.0)</td>
<td>31/110 (28.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Late CR (achieved after induction 2)</td>
<td>2/131 (1.5)</td>
<td>34/875 (3.9)</td>
<td>1/112 (0.89)</td>
<td>0.22</td>
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<tr>
<td>CR (after induction 1 or 2)</td>
<td>131/141 (92.9)</td>
<td>875/950 (92.1)</td>
<td>112/121 (92.6)</td>
<td>0.87</td>
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<tr>
<td>Post-remission treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSCT in CR1</td>
<td>64/131 (48.9)</td>
<td>265/875 (30.3)</td>
<td>57/112 (50.9)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Post-remission outcome</td>
<td></td>
<td></td>
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<tr>
<td>5-year CIR, % (95% CI)</td>
<td>40.7 (32.7-49.9)</td>
<td>33.7 (30.3-37.2)</td>
<td>43.0 (34.2-52.9)</td>
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<tr>
<td>5-year DFS, % (95% CI)</td>
<td>50.3 (41.1-58.8)</td>
<td>55.5 (51.7-59.0)</td>
<td>49.3 (39.4-58.5)</td>
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<tr>
<td>5-year OS, % (95% CI)</td>
<td>53.3 (44.5-61.4)</td>
<td>59.9 (56.2-63.3)</td>
<td>51.4 (41.9-60.2)</td>
<td>0.02</td>
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</table>

M, male; F, female; WBC, white blood cell count; CNS, central nervous system; EGIL, European Group for the Immunological Characterization of Leukemias; PB, peripheral blood; BM, bone marrow; CR, complete remission; HSCT, allogeneic hematopoietic stem cell transplantation; CIR, cumulative incidence of relapse; DFS, disease-free survival; OS, overall survival. Poor early PB blast clearance at day 8 is defined by ≥1G/L blast. Poor early BM blast clearance at day 15 is defined by ≥5% blasts.
Figure legends

Figure 1. Outcomes of all adult patients with Ph-negative BCP-ALL
CIR (A) and OS (B) in Ph-negative BCP-ALL adult patients according to KMT2A-rearrangement status, and CIR (C) and OS (D) according to KMT2A gene fusion partners, either AFF1 or others.

Figure 2. Landscape and prognostic significance of additional genetic alterations in adult KMT2A-r BCP-ALL
(A) Heatmap of recurrent gene alterations, including mutations and deletions, detected by targeted sequencing. Forests plots showing the hazard ratio for CIR (B) and OS (C) estimated by univariable Cox proportional-hazards model, for genes found altered in more than 5% of cases. CIR (D) and OS (E) in patients according to TP53 and IKZF1 alteration status.

Figure 3. Prognostic significance of IG/TR MRD
CIR and OS in patients at the end of induction (MRD1) (A-B), and after first consolidation (MRD2) (C-D). The cutoff for MRD positivity was set at 10^{-4}.

Figure 4. KMT2A genomic fusion is a more reliable MRD marker than IG/TR to assess treatment response
(A) Flowchart of the study design. (B) Comparison of the MRD levels between IG/TR and KMT2A genomic fusion (gKMT2A) measured at MRD1 and MRD2. (C) Illustrative cases showing discordant MRD results between gKMT2A and IG/TR markers CIR and OS in patients at the end of induction (MRD1) according to IG/TR and gKMT2A markers (D-E) and according to gKMT2A marker only (F-G), including 5 additional patients without IG/TR MRD due to the lack of clonal IG/TR rearrangement.

Figure 5. Combined genetics and MRD define three subsets of KMT2A-r BCP-ALL adult patients with distinct outcomes
CIR (A) and OS (B) in patients according to oncogenetics and MRD. Onco+ patients were defined as having at least one alteration in TP53 and/or IKZF1, Onco- as having no alteration in both genes. MRD is based on gKMT2A measured at the end of induction (MRD1).
Figure 1

(A) Cumulative Incidence of Relapse

(B) Overall Survival

(C) Cumulative Incidence of Relapse

(D) Overall Survival

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Other BCP-ALL</th>
<th>KMT2A-r BCP-ALL</th>
<th>Non-AFF1 KMT2A-r BCP-ALL</th>
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<tbody>
<tr>
<td>Number at risk</td>
<td>950</td>
<td>789</td>
<td>610</td>
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<tr>
<td>Time since diagnosis (years)</td>
<td>950</td>
<td>789</td>
<td>610</td>
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Table 2

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<tr>
<td>Number at risk</td>
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<td>75</td>
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<td>Time since diagnosis (years)</td>
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<td>105</td>
<td>75</td>
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Table 3

<table>
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<th>Non-AFF1 KMT2A::AFF1 BCP-ALL</th>
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<tbody>
<tr>
<td>Number at risk</td>
<td>950</td>
<td>789</td>
<td>610</td>
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<tr>
<td>Time since diagnosis (years)</td>
<td>950</td>
<td>789</td>
<td>610</td>
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Table 4

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<th>Other BCP-ALL</th>
<th>KMT2A::AFF1 BCP-ALL</th>
<th>Non-AFF1 KMT2A::AFF1 BCP-ALL</th>
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<tbody>
<tr>
<td>Number at risk</td>
<td>121</td>
<td>88</td>
<td>61</td>
</tr>
<tr>
<td>Time since diagnosis (years)</td>
<td>121</td>
<td>88</td>
<td>61</td>
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Table 5

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<th>Other BCP-ALL</th>
<th>KMT2A-r BCP-ALL</th>
<th>Non-AFF1 KMT2A-r BCP-ALL</th>
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<tbody>
<tr>
<td>Number at risk</td>
<td>20</td>
<td>17</td>
<td>14</td>
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<tr>
<td>Time since diagnosis (years)</td>
<td>20</td>
<td>17</td>
<td>14</td>
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Table 6

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<th>Other BCP-ALL</th>
<th>KMT2A-r BCP-ALL</th>
<th>Non-AFF1 KMT2A-r BCP-ALL</th>
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<tbody>
<tr>
<td>Number at risk</td>
<td>20</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Time since diagnosis (years)</td>
<td>20</td>
<td>17</td>
<td>14</td>
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**Figure 2**

A. Genetic alterations in hematopoietic stem cells.

B. Cumulative incidence of relapse.

<table>
<thead>
<tr>
<th>Gene</th>
<th>n</th>
<th>HR</th>
<th>95% CI</th>
<th>p</th>
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<tr>
<td>KRAS</td>
<td>19</td>
<td>1.24</td>
<td>0.57-2.72</td>
<td>0.59</td>
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<td>NRAS</td>
<td>18</td>
<td>0.84</td>
<td>0.35-2.01</td>
<td>0.69</td>
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<tr>
<td>FLT3</td>
<td>11</td>
<td>1.09</td>
<td>0.39-3.09</td>
<td>0.87</td>
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<tr>
<td>TP53</td>
<td>14</td>
<td>2.95</td>
<td>1.39-6.27</td>
<td>0.005</td>
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<tr>
<td>CDKN2A</td>
<td>14</td>
<td>1.03</td>
<td>0.40-2.65</td>
<td>0.95</td>
</tr>
<tr>
<td>IKZF1</td>
<td>8</td>
<td>3.03</td>
<td>1.25-7.34</td>
<td>0.01</td>
</tr>
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</table>

C. Overall survival.

<table>
<thead>
<tr>
<th>Gene</th>
<th>n</th>
<th>HR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS</td>
<td>19</td>
<td>1.12</td>
<td>0.54-2.34</td>
<td>0.76</td>
</tr>
<tr>
<td>NRAS</td>
<td>18</td>
<td>0.78</td>
<td>0.35-1.75</td>
<td>0.54</td>
</tr>
<tr>
<td>FLT3</td>
<td>11</td>
<td>1.11</td>
<td>0.44-2.83</td>
<td>0.82</td>
</tr>
<tr>
<td>TP53</td>
<td>14</td>
<td>2.67</td>
<td>1.31-5.45</td>
<td>0.007</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>14</td>
<td>1.40</td>
<td>0.62-3.15</td>
<td>0.42</td>
</tr>
<tr>
<td>IKZF1</td>
<td>8</td>
<td>2.01</td>
<td>0.85-4.80</td>
<td>0.11</td>
</tr>
</tbody>
</table>

D. Cumulative incidence of relapse in TP53/IKZF1wt and TP53/IKZF1alt.

E. Overall survival in TP53/IKZF1wt and TP53/IKZF1alt.

p = 0.001

p = 0.006
Figure 3

A

B

C

D

Overall Survival

p=0.02

Overall Survival

p=0.001

Number at risk
IG/TR MRD1 neg 43
IG/TR MRD1 pos 35

Number at risk
IG/TR MRD2 neg 54
IG/TR MRD2 pos 20
**Figure 4**

**A**

GRAALL-2014 KMT2A-ε BCP-ALL patients n=56

Clonal IG/TR rearrangements
- Presence n=48 (86%)
- Absence n=8 (14%)

No residual DNA for retrospective analyses n=9 (16%)

**B**

Comparative study between IG/TR and gKMT2A MRD n=39
- Concordant MRD n=30 (77%)
- Discordant MRD n=9 (23%)

**C**

**D**

**E**

**F**

**G**
Figure 5

A

Cumulative Incidence of Relapse

Time since CR1 (years)

0.00 0.20 0.40 0.60 0.80 1.00

Onco-/MRD-

Onco-/MRD+

Onco+/MRD+

p=0.08

p=0.002

p=0.03

Number at risk

Time since diagnosis (years)

0.00 0.20 0.40 0.60 0.80 1.00

Onco-/MRD-

Onco-/MRD+

Onco+/MRD+

p=0.14

p=0.02

p=0.002

B

Overall Survival

Time since CR1 (years)

0.00 0.20 0.40 0.60 0.80 1.00

Onco-/MRD-

Onco-/MRD+

Onco+/MRD+

Number at risk

Onco-/MRD- 14 13 9 6 4 2

Onco-/MRD+ 18 16 13 6 3 0

Onco+/MRD+ 9 5 2 1 0 0

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