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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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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 *KMT2A* (*KMT2A-r*), formerly *MLL*, represents a distinct entity accounting for 75% of infant, 2-5% of childhood non-infant, and 5-10% of adult BCP-ALL¹⁻⁵. Historically, all these patients have experienced poor outcomes, leading to the inclusion of *KMT2A-r* as a high-risk criterion in all risk-adapted treatment protocols.⁶⁻⁹ The high prevalence and poor prognosis of *KMT2A-r* in infants has generated considerable efforts to improve the clinical management of this leukemia.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.⁶⁻⁹ Similarly, the biology of *KMT2A-r* BCP-ALL has been extensively studied as an infant leukemia.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 *KMT2A-r*.^{14,20} Moreover, the landscape of genomic alterations in infant *KMT2A-r* leukemia has been shown to be exceptionally limited, in agreement with a one-hit model of oncogenesis.^{19,21,22} Noteworthy, the presence of additional genetic alterations and their possible role in leukemogenesis and prognosis have not been investigated in adult *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.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.^{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.³¹ While MRD has proven to have prognostic value within infant *KMT2A-r* BCP-ALL,^{32,33} no study has investigated the significance of MRD in the setting of adult *KMT2A-r* BCP-ALL, likely owing to limited patient numbers in each study. Thus, *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 *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).^{23,26} 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.³⁴ 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.³⁵ 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.³⁶ 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.³² 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 $t(11;19)(q23;p13.3)/KMT2A::MLL1$ 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 *IKZF1*^{plus} ALL.³⁷ 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.³² 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.^{39–42} By performing genomic characterization of a large cohort of adults with *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 *KMT2A-r*.^{19,22} Interestingly, while it has been suggested that the low mutational burden and short latency of infant *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 *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 *KMT2A-r* leukemia.⁴³ Another important finding of our study is the presence of *TP53* alterations in a significant fraction of *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. *IKZF1* deletions were also observed and they tended to confer poorer prognosis. Thus, the acquisition of *TP53* and *IKZF1* alterations in *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 *TP53* or *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 *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 *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 *KMT2A-r* BCP-ALL at very high-risk with HSCT eligibility, regardless of MRD.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.^{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,^{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.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.^{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.⁴⁸ While pediatric protocols have reduced indications of HSCT based on additional prognostic criteria,^{11,12} virtually all adult protocols still rely on HSCT for *KMT2A*-r patients.⁴⁴ However, the benefit of HSCT was considered in a context where poor results were obtained with respective suboptimal chemotherapy regimens.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 immunotherapies⁴⁹ or menin inhibitors,⁵⁰ are urgently needed.

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Authorship Contributions

R.K., H.D, N.B. and E.C. conceived and designed the research and oversaw the project; C.P., F.P., E.R., N.G., E.D., B.W.S., A.T-B., M.B., M.E-B., S.B., M.B., A.B., N.S., M-P.G-H., Y.C., C.G., J.S., T.L., M.H., F.H., N.B. and E.C. provided study materials or patients; R.K., H.B., C.P., L.L., M.P., N.G., E.D., B.W.S., A.C-E., C.M., R.M. and E.C. performed molecular analyses; R.K., H.B., V.L., N.B. and E.C. collected and assembled data; R.K. and N.B. performed statistical analysis; R.K., H.B., N.B. and E.C. analyzed and interpreted data; and R.K., H.B., J.S., H.D., N.B. and E.C. wrote the manuscript. All authors approved the final version of the manuscript.

Conflict-of-interest disclosure

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

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

	All Ph-neg BCP-ALL patients (n=1091)			KMT2A-r BCP-ALL patients (n=141)		
	KMT2A-r BCP-ALL	Other BCP-ALL	P value	KMT2A::AFF1	Other KMT2A-r	P value
Number of patients (%)	141 (12.9%)	950 (87.1%)		121 (85.8%)	20 (14.2%)	
Patient-related characteristics						
Median age, years (range)	42.0 (18.4-59.4)	36.8 (15.2-59.9)	0.02	41.7 (18.4-59.4)	46.2 (21.1-58)	0.24
Sex, M/F (ratio)	62/79 (0.78)	542/408 (1.3)	0.005	53/68 (0.8)	9/11 (0.8)	0.99
Disease-related characteristics						
WBC, G/L (range)	94.4 (1-712)	6.3 (0-396)	<0.0001	116 (2-712)	14.7 (1-216)	0.0002
CNS involvement (%)	10/140 (7.1)	60/944 (6.4)	0.71	8/120 (6.7)	2/20 (10.0)	0.64
EGIL classification	119 (100)	805 (100)	<0.0001	103 (100)	16 (100)	0.53
Pro-B (I)	89 (74.8)	115 (14.3)		78 (75.7)	11 (68.8)	
Common (II)	4 (3.4)	538 (66.9)		3 (2.9)	1 (6.2)	
Pre-B (III)	25 (21.0)	138 (17.1)		21 (20.4)	4 (25.0)	
Mature (IV)	1 (0.8)	14 (1.7)		1 (1.0)	0 (0.0)	
Response-related characteristics						
Poor early PB blast clearance	22/141 (15.6)	151/945 (16.0)	0.99	19/121 (15.7)	3/20 (15.0)	0.99
Poor early BM blast clearance	34/128 (26.6)	376/895 (42.0)	0.001	31/110 (28.2)	3/18 (16.7)	0.40
Late CR (achieved after induction 2)	2/131 (1.5)	34/875 (3.9)	0.22	1/112 (0.89)	1/19 (5.3)	0.27
CR (after induction 1 or 2)	131/141 (92.9)	875/950 (92.1)	0.87	112/121 (92.6)	19/20 (95.0)	0.99
Post-remission treatment						
HSCT in CR1	64/131 (48.9)	265/875 (30.3)	<0.0001	57/112 (50.9)	7/19 (36.8)	0.32
Post-remission outcome						
5-year CIR, % (95% CI)	40.7 (32.7-49.9)	33.7 (30.3-37.2)	0.02	43.0 (34.2-52.9)	27.6 (12.4-54.5)	0.18
5-year DFS, % (95% CI)	50.3 (41.1-58.8)	55.5 (51.7-59.0)	0.07	49.3 (39.4-58.5)	56.5 (31.2-75.5)	0.36
5-year OS, % (95% CI)	53.3 (44.5-61.4)	59.9 (56.2-63.3)	0.02	51.4 (41.9-60.2)	64.6 (39.7-81.3)	0.21

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 $\geq 1\text{G/L}$ blast. Poor early BM blast clearance at day 15 is defined by $\geq 5\%$ 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. Forest 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

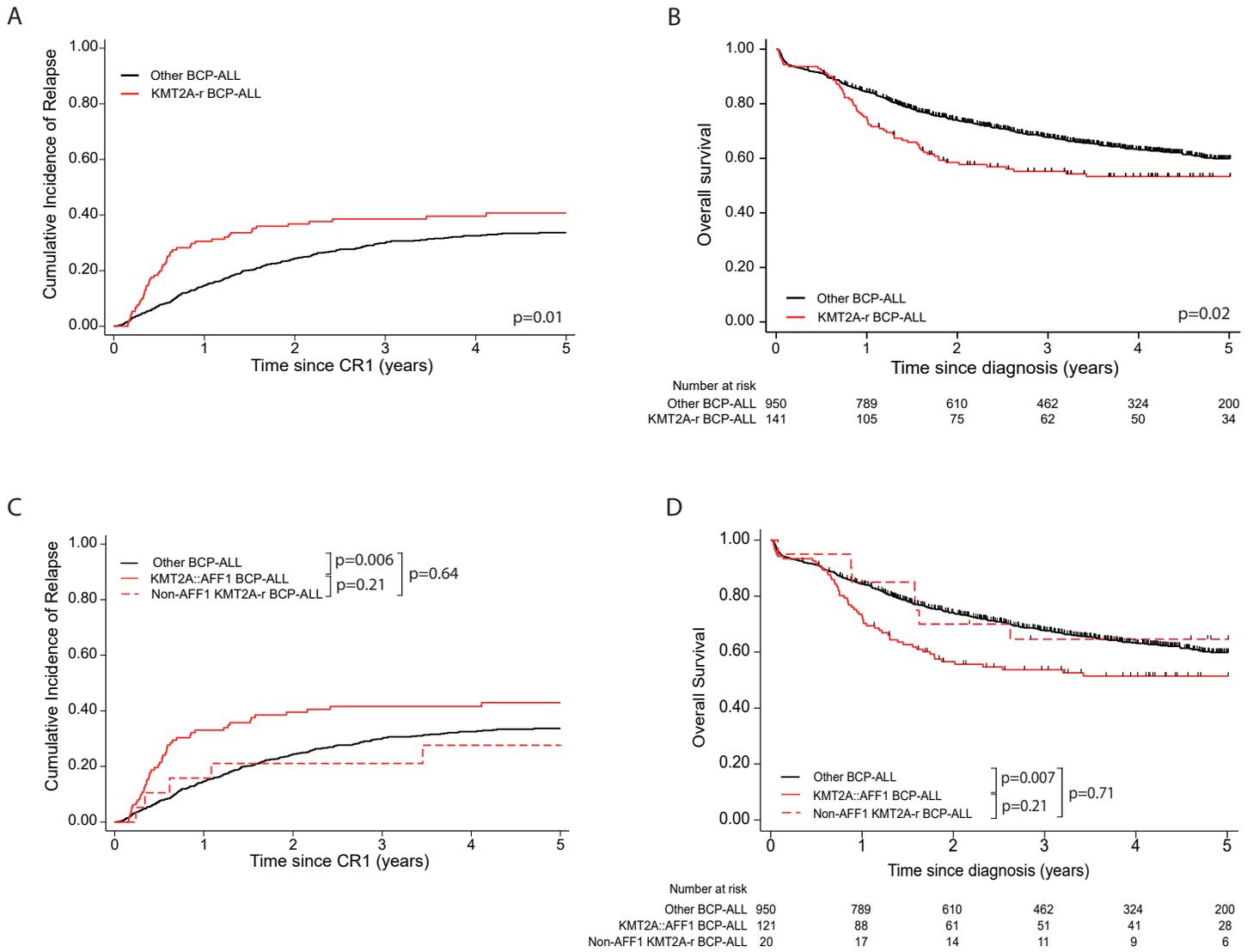


Figure 2

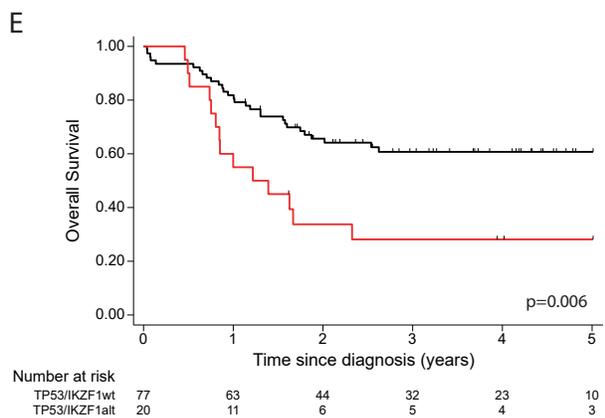
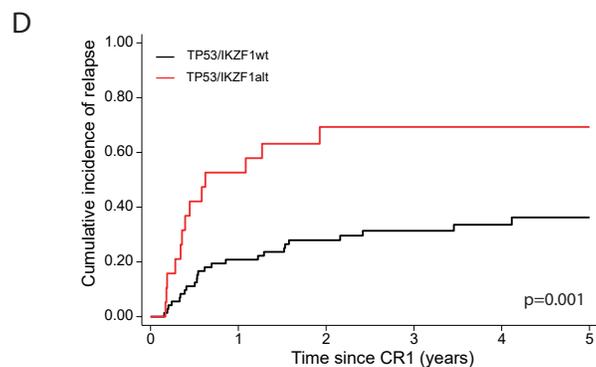
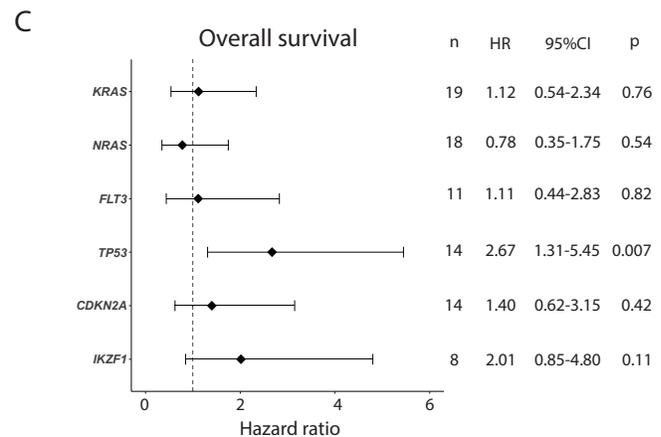
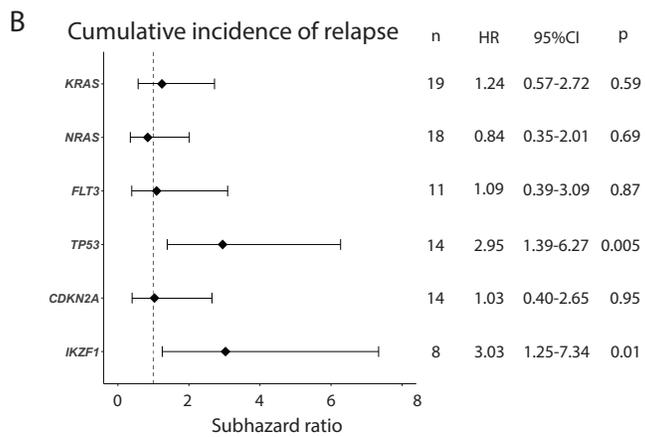
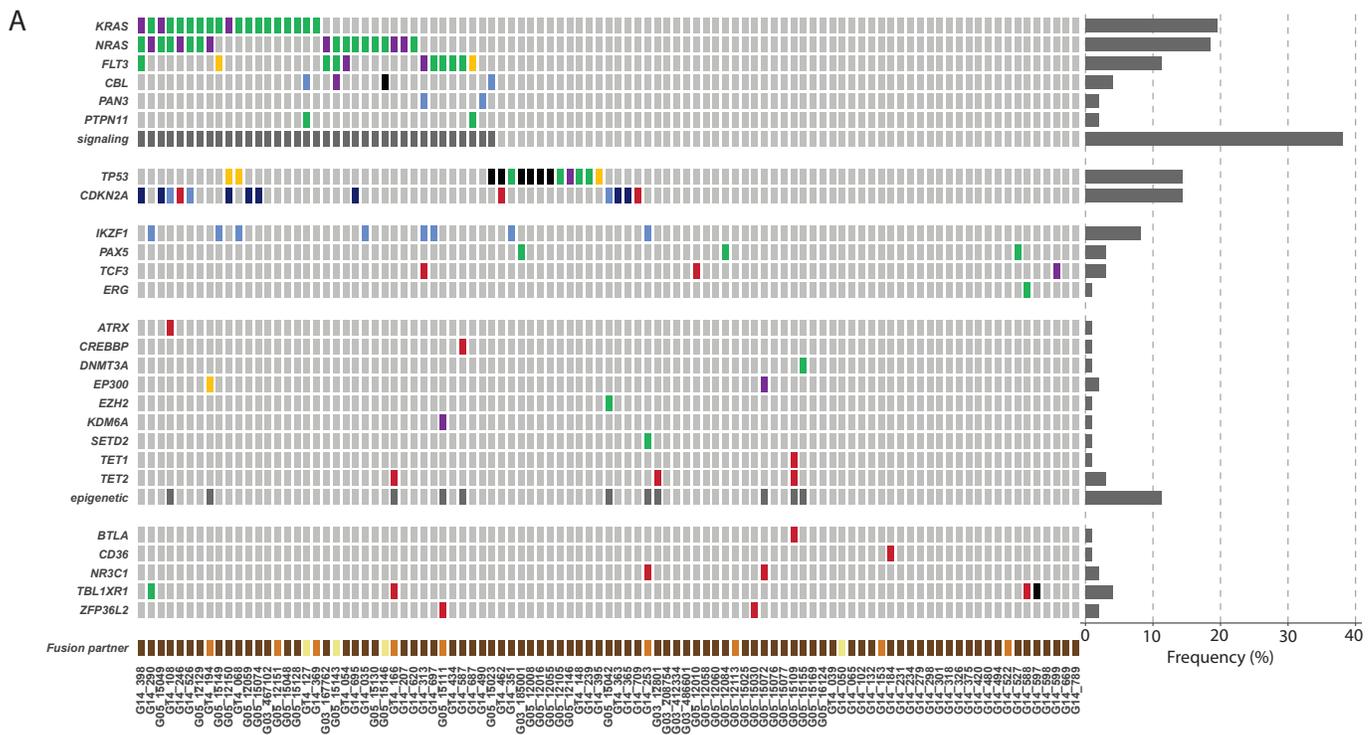
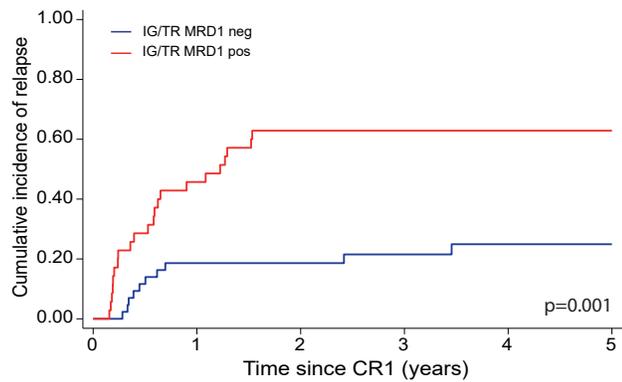
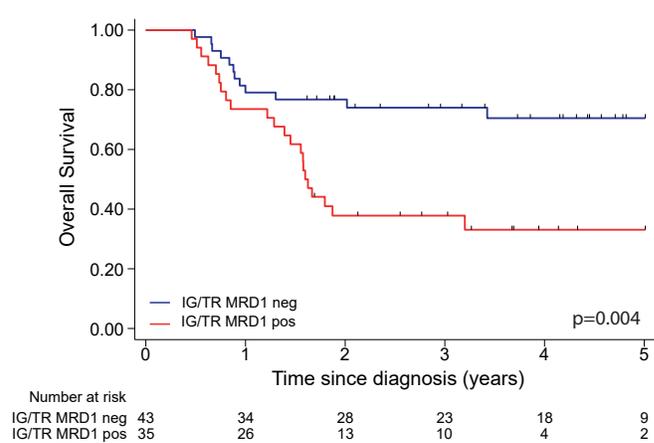


Figure 3

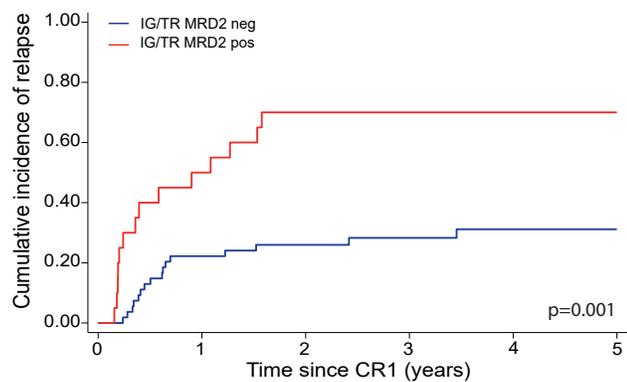
A



B



C



D

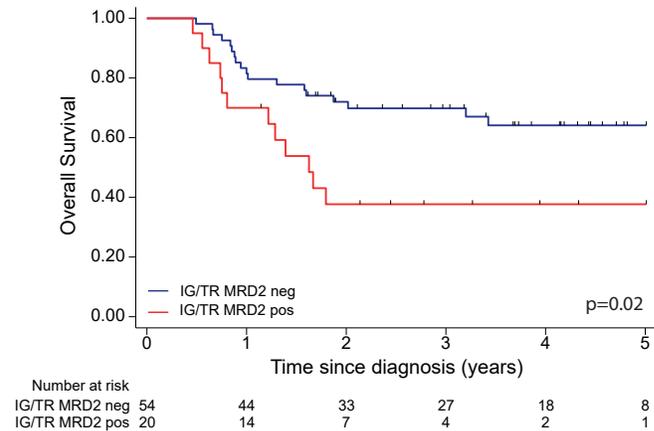


Figure 4

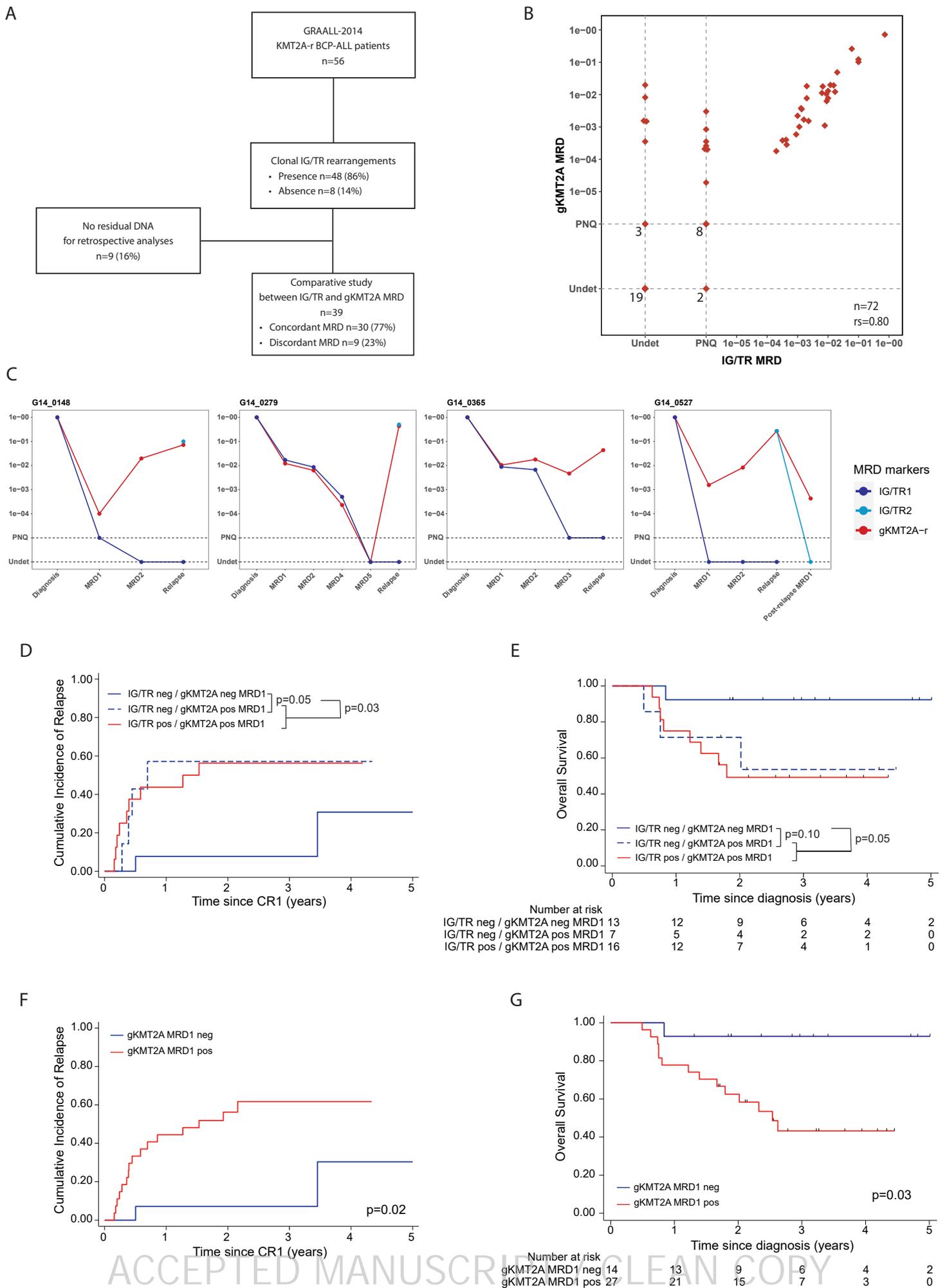


Figure 5

