

## **Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia.**

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### Key Points

- In adult ALL, oncogenetic markers and minimal residual disease levels are independent outcome predictors.
- Both factors should be used for individual treatment stratification.

### Abstract

With intensified pediatric-like therapy and genetic disease dissection, the field of adult acute lymphoblastic leukemia (ALL) has evolved recently. In this new context, we aimed to reassess the value of conventional risk factors with regard to new genetic alterations and early response to therapy, as assessed by Ig/TCR minimal residual disease (MRD) levels. The study was performed in 423 younger adults with Philadelphia chromosome-negative ALL in first remission (265 B-cell precursor [BCP] and 158 T-cell ALL), with cumulative incidence of relapse (CIR) as primary endpoint. In addition to conventional risk factors, the most frequent currently available genetic alterations were included in the analysis. A higher specific hazard of relapse was independently associated with post-induction MRD level  $\geq 10^{-4}$  and unfavorable genetic characteristics (*i.e.* *MLL* gene rearrangement or focal *IKZF1* gene deletion in BCP-ALL; and no *NOTCH1/FBXW7* mutation and/or *N/K-RAS* mutation and/or *PTEN* gene alteration in T-ALL). These two factors allowed definition of a new risk classification, which is strongly associated with higher CIR and shorter relapse-free and overall survival. These results indicate that genetic abnormalities are important predictors of outcome in adult ALL, not fully recapitulated by early response to therapy. Patients included in this study were treated in the multicenter GRAALL-2003 and 2005 trials. Both trials were registered at ClinicalTrials.gov (GRAALL-2003, NCT00222027; GRAALL-2005, NCT00327678).

## Introduction

During the last decade, the management of adults with acute lymphoblastic leukemia (ALL) has markedly evolved. Up to the age of 40 to 60 years, most groups are now using pediatric-inspired approaches or even unmodified pediatric protocols.<sup>1-8</sup> Relative to preceding studies, chemotherapy intensity has significantly increased and minimal residual disease (MRD) levels tend to be used to stratify post-remission therapy.<sup>9,10</sup> This evolution has yielded significant improvement in patient outcome, as reported in the first study of our Group for Research on Adult ALL (GRAALL).<sup>11</sup>

This evolving context has made it necessary to reassess conventional risk factors in order to define patients who are at higher risk of relapse in current protocols. Early MRD evaluation needs to be included in this process,<sup>12</sup> as should probably be the most relevant novel genetic markers originally described in childhood ALL.<sup>13</sup> Markers that are of potential prognostic value differ with ALL lineages. In B-cell precursor (BCP) ALL, deletion of the *IKZF1* gene has been reported to be associated with a specific gene expression signature and a worse outcome, with a particularly high incidence in Philadelphia chromosome (Ph)-positive ALL.<sup>14-19</sup> In T-ALL, we and others have reported that mutations of the *NOTCH1* pathway are associated with a better prognosis,<sup>20,21</sup> later refined by taking into account additional bad-prognosis *N/K-RAS* gene mutation or *PTEN* gene alteration.<sup>22</sup>

With the aim of deriving a modern risk model, we therefore reassessed the prognostic significance of conventional and new risk factors, including MRD and oncogenetics, in 423 adult patients with Ph-negative ALL treated in the pediatric-inspired GRAALL trials.

## Methods

### *Treatments*

Between 2003 and 2011, 955 patients with Ph-negative ALL aged 15 to 59 years old were treated in the multicenter GRAALL-2003 and 2005 trials (618 BCP-ALL, 337 T-ALL). GRAALL centers and investigators are listed in the **Supplementary Appendix**. Results of the GRAALL-2003 trial (ClinicalTrials.gov, NCT00222027), which enrolled 225 patients, have been already reported.<sup>11</sup> The GRAALL-2005 trial (ClinicalTrials.gov, NCT00327678) enrolled 730 evaluable patients between 2006 and 2011. Patient outcome was updated in January 2013. Overall, the median follow-up was 4.0 years. Treatment protocols are detailed in a **Supplementary File**. Informed consent was obtained from all patients. Both trials were conducted in accordance with

the Declaration of Helsinki and approved by local and multicenter research ethical committees. Of the 955 patients, 860 (548 BCP-ALL and 312 T-ALL) reached complete remission (CR) after the first induction cycle. Of these, 257 relapsed and 287 died, including 198 deaths after relapse. Three hundred and eleven patients received allogeneic stem cell transplantation (SCT) in first CR (204 BCP-ALL and 107 T-ALL). Of these, 58 relapsed and 98 died, including 47 deaths after relapse. The patient flow chart and main characteristics are given in the **Supplementary Appendix (Figure S1, Table S1)**.

In these GRAALL trials, the following factors were used to classify patients as standard-risk (if no factor) or high-risk patients (if at least one factor): a) central nervous system (CNS) involvement at diagnosis, b) low hypodiploidy/near triploidy on karyotype and/or DNA index analysis, c) complex karyotype (defined as 5 or more unrelated chromosomal abnormalities),<sup>23</sup> d) early resistance to the 1-week steroid prephase, e) poor bone marrow (BM) blast clearance after one additional week of chemotherapy, and f) late CR achieved after the planned salvage course. Additionally, the following factors were also used to define high-risk BCP-ALL patients: a) white blood cell count (WBC)  $\geq 30 \times 10^9/L$ , b) *MLL* gene rearrangement (t[4;11] chromosomal translocation and/or *MLL-AF4* gene fusion, or other *MLL* rearrangement), and c) t(1;19) chromosomal translocation and/or *E2A-PBX1* gene fusion, and d) immature CD10-negative immunophenotype. In the GRAALL-2003 trial only, an MRD level  $\geq 10^{-2}$  was also considered as a high-risk factor, but only one patient fell in this category solely for this reason. These factors are detailed in the **Supplementary Appendix**. Per protocol, allogeneic SCT in first CR was offered to high-risk patients aged 55 years or less, if they had an HLA-identical sibling or 10/10 matched unrelated donor. The use of a 9/10 matched unrelated donor was allowed for patients with *MLL*-rearranged ALL, low hypodiploidy / near triploidy ALL, or late CR.

#### *Minimal residual disease evaluation and study population*

Among the 860 patients who reached CR after the first induction cycle, 423 (49%) benefitted from MRD1 evaluation, based on Ig/TCR gene rearrangements and centrally assessed on BM samples just after the first induction course. The MRD1 time-point was 6 weeks after induction initiation. These 423 patients (260 BCP-ALL and 163 T-ALL patients) represent the study population of this work. Other patients had missing diagnosis and/or MRD1 samples (N= 307 patients), too low blast percentage in the diagnosis sample (N= 25 patients), lack of informative Ig/TCR marker (N= 22 patients), or insufficient sensitivity by EuroMRD criteria (N= 83 patients). They were older, had lower WBC and a slightly longer follow-up than the 423 study patients, but, importantly, no difference was observed between both subsets with respect to CIR (**Table S1**). Three hundred and fifty-five study patients (84%) also had an MRD2 evaluation, assessed

after the first consolidation phase (12 weeks after induction initiation). Methods are detailed in the **Supplementary Appendix**. Briefly: 1) DNA was extracted from diagnostic and follow-up bone marrow samples and its quality was assessed and confirmed by albumin gene assay using standardized real-time quantitative polymerase chain reaction (RQ-PCR);<sup>24</sup> 2) potential Ig/TCR targets were identified using the standardized multiplex PCR established within the BIOMED-2/EuroClonality network;<sup>25</sup> 3) for each patient, preferably two independent Ig/TCR targets with a sensitivity of at least  $10^{-4}$  and a quantitative range of  $10^{-4}$  for at least one of the two targets were selected for MRD level monitoring. All MRD data were assessed according to the guidelines developed within the EuroMRD group.<sup>24</sup>

### *New genetic markers evaluation*

Among study patients, 216/260 BCP-ALL patients (83%) were studied centrally for *IKZF1* gene deletions, using breakpoint-specific multiplex PCR, as described,<sup>26</sup> and multiplex-ligation probe assay (MLPA). Methods are detailed in the **Supplementary Appendix**. A univariable analysis of the impact of *IKZF1* gene deletions was also performed in a larger subset of 324 BCP-ALL patients in first CR with available *IKZF1* gene status, even if not monitored for MRD levels. This analysis, which is provided in the **Supplementary Appendix**, explains why only focal *IKZF1* gene deletions were evaluated as a potential risk factor in the present study. Among T-ALL patients, 125/163 (77%) could be classified centrally according to the four-gene oncogenetic classifier we recently reported. We have indeed shown that a favorable genetic profile is defined by the presence of *NOTCH1/FBXW7* mutation without *N/K-RAS* mutation or *PTEN* alteration, while high-risk profiles are defined by the absence of *NOTCH1/FBXW7* mutation and/or presence of *N/K-RAS* mutation and/or *PTEN* alteration.<sup>22</sup> Methods are detailed in the **Supplementary Appendix**. No difference in patient characteristics and outcome was detected between patients evaluated or not evaluated for these new genetics markers, in BCP- and T-ALL separately.

### *Statistical methods*

The primary endpoint was cumulative incidence of relapse (CIR) after censoring patients who received allogeneic SCT in first CR at time of SCT. CIR was estimated taking into account death in first CR as a competing risk. Cause-specific hazard ratios (HRs) with 95% confidence interval (95% CI) were given as measures of association between each variable and CIR. In BCP-ALL patients, variables that entered the prognostic analysis were as follows: WBC  $\geq 30 \times 10^9/L$ , CNS involvement, CD10-negative immature phenotype, t(4;11) translocation or *MLL/AF4* or

other *MLL* gene rearrangement, t(1;19) translocation or *E2A-PBX1* rearrangement, low hypodiploidy/near triploidy, complex karyotype, focal *IKZF1* gene deletion, and MRD1 level  $\geq 10^{-4}$ . In T-ALL patients, variables that entered the prognostic analysis were as follows: WBC  $\geq 100 \times 10^9/L$ , pro-T/mature-T phenotype, CNS involvement, complex karyotype, *TLX1* gene overexpression defined as *TLX1* over *ABL* expression ratio  $> 1$  as described,<sup>27</sup> high-risk genetic profile as defined above, and MRD1 level  $\geq 10^{-4}$ . The analysis was then repeated without censoring patients who received allogeneic SCT in first CR at SCT date. Variables associated with a P value lower than 0.15 in univariable analysis, either when using SCT censoring or not, entered the multivariable analysis performed by the Cox models.<sup>28</sup> Proportional-hazards assumptions were checked by testing that the log hazard-ratio functions were constant over time. Absence of unacceptable collinearity was checked by calculating the variance inflation factors (VIFs) for each co-variable,<sup>29</sup> considering a value of 4 as the maximum acceptable level of VIF. Secondary endpoints included relapse-free survival (RFS) and overall survival (OS) from CR. RFS and OS from CR were estimated by the Kaplan-Meier method<sup>30</sup> and compared by the log-rank test.<sup>31</sup> Statistical analyses were performed using the Stata/IC 12.1 software (StataCorp, College Station, TX, USA). All tests were two-sided, and a P value  $< 0.05$  was considered statistically significant.

## Results

### *Patients*

The main characteristics of the 423 study patients are given in **Table 1**. Two hundred and sixty patients had BCP-ALL and 163 had T-ALL. Their median age was 31.2 years. Overall, 273 of them (65%) could be classified at high-risk according to conventional protocol criteria. A total of 158 patients (107 BCP-ALL and 51 T-ALL) actually received allogeneic SCT in first CR. Among them, 35 relapsed and 54 died, including 25 deaths in first CR. Among the remaining 265 patients, 92 relapsed and 78 died, including 8 deaths in first CR.

### *Early response evaluation*

At the post-induction MRD1 time-point, the numbers of patients with no detectable MRD1, detectable MRD1 level lower than  $10^{-4}$ , and MRD1 level  $\geq 10^{-4}$  were 196 (46.3%), 69 (16.3%), and 158 (37.4%), respectively. The proportion of patients with an MRD1 level  $\geq 10^{-4}$  was 42.7% in BCP-ALL (111/260) and 28.8% in T-ALL (47/163). **Figure 1A** illustrates CIR according to MRD1 level. At 5 years, CIR was estimated at 22.9% (95% CI, 17-31) versus 30.8% (95% CI, 18-49) in

patients with negative MRD1 or MRD1 less than  $10^{-4}$  ( $P = 0.24$ ), while it was 60.4% (95% CI, 48-73) in those with MRD1  $\geq 10^{-4}$ . The  $10^{-4}$  MRD1 cutoff was thus retained for prognostic analysis. With regard to the primary CIR endpoint, cause-specific HR was 3.20 (95% CI, 2.11-4.84) for patients with MRD1 level  $\geq 10^{-4}$  ( $P < 0.001$ ). It was 3.46 (95% CI, 2.00-6.00;  $P < 0.001$ ) in BCP-ALL patients and 2.93 (95% CI, 1.50-5.71;  $P = 0.002$ ) in T-ALL patients. Similar results were obtained when transplanted patients were not censored at SCT time (not shown).

Post-consolidation MRD2 level evaluation was available for 355 patients. As expected, MRD2 and MRD1 levels strongly correlated in this cohort. Only 4 out of 249 patients with an MRD1 level  $< 10^{-4}$  had an MRD2 level  $\geq 10^{-4}$ , while 81% of the patients with an MRD2 level  $< 10^{-4}$  (245/302) had an MRD1 level  $< 10^{-4}$  ( $P < 0.001$ ). Overall, 265 patients achieved a MRD response lower than  $10^{-4}$  at MRD1, while 57 achieved it at MRD2 only, and 49 did not achieve it at either time-point. At 5 years, CIR was estimated at 24.7% (95% CI, 19-32) in patients who reached a MRD level lower than  $10^{-4}$  at MRD1, while it was 56.0% (95% CI, 39-75) and 57.8% (95% CI, 37-81) in those who reached this level at MRD2 only or never reached it, respectively ( $P = 0.14$ ) (**Figure 1B**). Similar results were obtained when transplanted patients were not censored at SCT time (not shown).

With regard to the primary CIR endpoint, resistance to the steroid prephase and poor BM blast clearance were both associated with a higher specific hazard of relapse (cause-specific HR, 1.69 [1.05-2.71] and 2.20 [1.45-3.35];  $P = 0.031$  and  $< 0.001$ , respectively). For both criteria, HRs were relatively similar in BCP- (1.87 and 2.15, respectively) and T-ALL patients (1.72 and 2.28, respectively). Similar results were obtained when transplanted patients were not censored at SCT time (not shown). The MRD1 response was nonetheless a better predictor of relapse than earlier morphological response assessment. This is illustrated in **Figures 1C and 1D**. For both assessments, MRD1 response allowed to significantly discriminate high-risk *versus* good-risk patients in the two subsets of patients defined by their early morphological response. Conversely, early morphological assessment did not significantly define high-risk *versus* good-risk patients among good or poor MRD1 responders. After adjustment on resistance to the steroid prephase, only the MRD1 response remained significantly predictive of a higher CIR, either in the whole population ( $P < 0.001$ ) or in BCP- and T-ALL patients separately ( $P < 0.001$  and  $P = 0.017$ , respectively). After adjustment on poor BM blast clearance, again only MRD1 response remained significantly predictive of a higher CIR, either in the whole population ( $P < 0.001$ ) or in BCP- and T-ALL patients separately ( $P < 0.001$  and  $P = 0.044$ , respectively). This was also true when not using SCT censoring. For all these reasons, MRD1 response at the  $10^{-4}$  level was the only response-related factor considered for further prognostic analysis.

#### *Focal IKZF1 gene deletion in BCP-ALL patients*

Among the 216 patients studied for *IKZF1* gene status, a focal *IKZF1* gene deletion was detected in 54 patients (25%), a complete *IKZF1* gene in 15 patients, while the 147 remaining patients had no *IKZF1* gene deletion. Deletion was mono-allelic in the large majority of cases. Among the 54 patients with focal deletion, 28 (52%) had a deletion of exons 4-7 and 22 (41%) had a deletion of exons 2-7 or 4-8, while the 4 remaining patients had various other deletions that were undetectable by the multiplex PCR system and only identified by MLPA analysis. As explained in the **Supplementary Appendix**, only focal *IKZF1* deletions were evaluated as a potential risk factor. Actually, the outcome of patients presenting a complete deletion did not differ from that of patients without any deletion (**Figure S2A**). On the other hand, the type of focal deletion did not seem to significantly influence CIR (**Figure S2B**). At 5 years, CIR was estimated at 53.9% (95% CI, 38-72) in patients with focal *IKZF1* gene deletion *versus* 28.6% (95% CI, 20-40) in other patients (**Figure 2**). The corresponding cause-specific HR was 2.65 (95% CI, 1.48-4.73;  $P=0.001$ ). Similar results were obtained when transplanted patients were not censored at SCT time.

#### *NOTCH1/FBXW7/RAS/PTEN gene status in T-ALL patients*

Among the 163 T-ALL study patients, 136 were studied for *NOTCH1/FBXW7* mutation and a mutation was found in 90 (66%). One hundred twenty-eight of these 136 patients were also tested for *N-RAS* and *K-RAS* mutation, which was found in 13 patients (including 4 patients without *NOTCH1/FBXW7* mutation). One hundred and seventeen of these 128 patients also were tested for *PTEN* genomic alteration, which was found in 12 patients (including 7 patients without *NOTCH1/FBXW7* mutation). In these 117 patients, 22 (19%) had either *N/K-RAS* mutation or *PTEN* alteration, no patient having both. Overall, 125 patients (77%) could be eventually classified according to our 4-gene (*NOTCH1*, *FBXW7*, *N/K-RAS*, *PTEN*) classification. Among them, 60 patients (48%) had a high-risk genetic profile, defined by the absence of *NOTCH1/FBXW7* mutation and/or *N/K-RAS* mutation and/or *PTEN* alteration.<sup>22</sup> At 5 years, CIR was 55.7% (39-73) in patients with high-risk genetic profile *versus* 15.4% (7-32) in other patients. The corresponding cause-specific HR was 5.33 (95% CI, 2.14-13.26;  $P<0.001$ ). Similar results were obtained when transplanted patients were not censored at SCT time.

### Prognostic analysis

The prognostic value of conventional risk factors was then examined in this population of patients treated with a pediatric-inspired protocol, first in univariable analysis and then in multivariable analysis, against MRD1 response and the genetic markers mentioned above. Results are summarized in **Table 2**. Based on univariable analysis results, covariables that entered the multivariable analysis were as follows; 1) in BCP-ALL patients: WBC  $\geq 30.10^9/L$ , *MLL* gene rearrangement, *IKZF1* gene deletion, and MRD1 level  $\geq 10^{-4}$ ; 2) in T-ALL patients; WBC  $\geq 100.10^9/L$ , pro-T/mature-T phenotype, CNS involvement, high-risk genetic profile, and MRD1 level  $\geq 10^{-4}$ . As shown in **Table 2**, the following factors were jointly selected as being associated with a worse outcome with regard to the primary CIR endpoint: 1) *MLL* gene rearrangement, *IKZF1* gene deletion, and MRD1 level  $\geq 10^{-4}$  in BCP-ALL patients; 2) high-risk genetic profile and MRD1 level  $\geq 10^{-4}$  in T-ALL patients. The incidence of BCP-ALL patients with an MRD1 level  $\geq 10^{-4}$  was higher in the presence of focal *IKZF1* gene deletion (65% versus 36%,  $P=0.001$ ), while it did not significantly increase in patients with *MLL*-rearranged ALL (48% versus 42%;  $P=0.56$ ). In T-ALL patients, there was a trend towards a higher incidence of patients with an MRD1 level  $\geq 10^{-4}$  in patients with a high-risk genetic profile (37% versus 21.5%,  $P=0.08$ ).

### New risk classification

Based on these results, high-risk patients could be defined as patients with MRD1 level  $\geq 10^{-4}$ ; and/or unfavorable genetics, defined as: 1) t(4;11) translocation or other *MLL* gene rearrangement and/or *IKZF1* gene deletion in BCP-ALL; and 2) no *NOTCH1/FBXW7* mutation and/or *N/K-RAS* mutation and/or *PTEN* alteration in T-ALL patients. With this new definition, the overall percentage of high-risk patients is 59%, in both ALL lineages. With regard to the primary CIR endpoint, the cause-specific HR was 4.38 (95% CI, 2.47-7.76) for high-risk versus standard-risk patients ( $P<0.001$ ). It was 3.89 (95% CI, 1.91-7.90;  $P<0.001$ ) in BCP-ALL patients and 5.31 (95% CI, 2.00-14.07;  $P=0.001$ ) in T-ALL patients. Without censoring at SCT time, the cause-specific HR was 3.78 (95% CI, 2.27-6.30) for high-risk versus standard-risk patients ( $P<0.001$ ). It was 3.17 (95% CI, 1.72-5.83;  $P<0.001$ ) in BCP-ALL patients and 5.33 (95% CI, 2.07-13.7;  $P=0.001$ ) in T-ALL patients.

**Figure 3** illustrates CIR without SCT censoring according to the four patient subsets that can be defined by genetic characteristics and MRD response (*i.e.* genetics-/MRD-, genetics-/MRD+, genetics+/MRD- and genetics+/MRD+), in BCP- and T-ALL patients separately. As shown, MRD

level seemed to be the predominant predictor in BCP-ALL patients, further refined by genetic features (**Figure 3A**). In T-ALL patients, conversely, the oncogenetic classification seemed to be the predominant predictor, further refined by MRD response (**Figure 3B**). In both lineages, patients with high-risk genetic characteristics and poor MRD1 response experienced a worse outcome. Finally, **Figure 4** shows the impact of this new risk classification, based on oncogenetics and/or MRD1 level only, on RFS and OS from CR, in BCP- and T-ALL patients separately and without SCT censoring.

## Discussion

In the present study, we reassessed the value of conventional and new risk factors, including MRD response and newly described genetic markers, in adult patients with Ph-negative ALL in first CR. We confirm that, as in children, early MRD response is a powerful risk factor that should be used in adult patients treated in modern protocols including prospective treatment stratification on the individual risk. This has been already observed in six studies,<sup>32-37</sup> published between 2000 and 2013 and including in total more than 1,000 patients (102, 196, 116, 212, 161 and 580 patients, respectively). In these studies, MRD levels were evaluated at various early time-points using either flow cytometry<sup>32,34</sup> or Ig/TCR gene amplification in 4 studies.<sup>33,35-37</sup> In all studies but one, MRD was identified as a strong predictor of outcome after adjustment on conventional risk factors in multivariable analysis. However, none of these studies included the more recently identified genetic ALL markers, such as *IKZF1* gene deletions in BCP-ALL or NOTCH1 pathway gene mutations in T-ALL, as undertaken here.

Most groups still use conventional risk factors, like WBC, immunophenotype and standard cytogenetics, in the definition of high-risk *versus* standard-risk patients.<sup>37,38</sup> Although the treatment protocol may have an impact on prognostic factors, the present study provides strong evidence that, at least when using a pediatric-inspired protocol, most conventional risk factors could be safely abandoned in future trials that will rely on prospective MRD monitoring and up-to-date oncogenetic characterization. In fact, the single conventional factor that remained of significant value in our study was the presence of *MLL* rearrangement, including the recurrent t(4;11) chromosomal translocation.

The present study also confirms the prognosis impact of new ALL genetic subsets in a large multivariate setting including MRD. The number of subsets that have been characterized and evaluated here is still limited, with respect to the long list of genetic events that have been described in this disease.<sup>13</sup> It nonetheless includes the most frequent events described to date as

influencing patient outcome. Interestingly, even if these genetic anomalies may influence MRD response, they were independently associated with a higher relapse risk in both lineages, meaning that the MRD response does not totally recapitulate the intrinsic risk. One may regret the absence of the so-called *BCR-ABL*-like ALL subset in the list of markers evaluated here in BCP-ALL patients. This latter subset has been defined recently in pediatric cohorts on the basis of a gene expression signature highly similar to that of Ph-positive ALL associated to a poor outcome.<sup>14,39</sup> Nearly half of so-defined *BCR-ABL*-like cases had *IKZF1* deletions and a lower proportion had a high *CRLF2* gene expression level. Recently, the Dutch pediatric group has reported that both the *BCR-ABL*-like signature and *IKZF1* gene deletion had independent prognostic value, while high *CRLF2* gene expression did not.<sup>40</sup> The British-American adult Intergroup has also reported an inferior outcome associated with *IKZF1* deletions in univariable analysis.<sup>41</sup> For the time being, screening for *IKZF1* deletions, which can be performed at the individual patient level by routine laboratory assays, appears nonetheless to be the best marker to be used, as prospective multicenter treatment stratification based on gene expression signature does not represent a simple approach.

The statistical independence of high-risk genetics and poor MRD response in predicting relapse underlines the remarkable heterogeneity of ALL, not to mention the fact that even in a given genetic subset the leukemic clone could be heterogeneous.<sup>42-44</sup> One may imagine why MRD may not entirely recapitulate the risk of relapse in this context. The number of BM cells that can be sampled and analyzed limits the detection of residual leukemic cells. Depending on the genetic event and the clonal hierarchy, good apparent treatment response with favorable MRD kinetics may still be associated with the persistence of very low levels of cells endowed with leukemic stem cell capacities that could initiate ALL recurrence in a given patient. Upon examination of the respective roles of genetics and MRD in both lineage subgroups, MRD seems to be the prominent factor in patients with BCP-ALL, while the *NOTCH1/FBXW7/RAS/PTEN* genetic profile seems to be more important in patients with T-ALL (**Figure 3**).

It thus appears that an accurate genetic characterization of the disease and a prospective evaluation of the bone marrow MRD response may both be required to optimally define individual patient risk. Combination of both factors essentially allows identification of an important fraction of patients with a very good outcome when treated with a pediatric-inspired protocol (5-year OS from CR, approximately 80% and 90% in good-risk BCP- and T-ALL patients, respectively; **Figure 4**). How they can be used for treatment stratification will depend on the therapeutic option proposed. This should be first elucidated for allogeneic SCT in first CR. By landmark analysis, the German group recently showed that poor MRD responders benefited

from SCT in first CR, suggesting that good MRD responders did not.<sup>37</sup> Whether this is true within each different ALL genetic subset remains an open issue.

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### **Authorship Contributions and Disclosure of Conflicts of Interest.**

#### *Author contributions.*

Conception and design : KB, VA, FH, TL, XT, YC, NB, ED, PC, AB, OR, JPV, MCB, ML, EMI, NI and HD ; Administrative support : VL ; Provision of study materials or patients : FH, TL, XT, YC, NB, PC, AB, OR, JPV, NI and HD ; Collection and assembly of data : KB, VA, MLB, JMC, NG, BS, ED, HC, TF, VL, MCB, ML, EMI, NI and HD ; Data analysis and interpretation : all authors ; Manuscript writing : KB, SC, VA, NI and HD ; Final approval of the manuscript : all authors.

#### *Disclosure of conflicts of interest*

The authors have nothing to disclose.

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**Table 1. Study patient characteristics.**

	All patients (N=423)	BCP-ALL patients (N=260)	T-ALL patients (N=163)
<b>Patient-related characteristics</b>			
Trial, 2003/2005	103/320	66/194	37/126
Median age, years (range)	31.2 (15.2-59.9)	34.5 (15.2-59.9)	29.6 (16.3-57.0)
Gender, M/F	264/159	147/113	117/46
<b>Disease-related characteristics</b>			
Median WBC, 10 <sup>9</sup> /L (range)	15.4 (0.4-456)	9.3 (0.4-396)	32.1 (1.4-456)
CNS involvement *, Y/N/unknown	33/386/4	15/242/3	18/144/1
Complex karyotype *, Y/N/failure/unknown	32/322/65/4	18/200/40/2	14/122/25/2
Low hypodiploidy/near triploidy *, Y/N/unknown	11/363/49	11/219/30	0/144/19
WBC ≥30 x 10 <sup>9</sup> /L (BCP-ALL) *, Y/N	-	67/193	-
CD10-negative immature ALL *, Y/N/unknown	-	67/183/10	-
<i>MLL</i> gene rearrangement (t[4;11] or other) *, Y/N/unknown	-	29/224/7	-
t(1;19) *, Y/N/unknown	-	14/230/16	-
<i>IKZF1</i> gene deletion, Y/N/unknown	-	54/162/44	-
WBC ≥100 x 10 <sup>9</sup> /L (T-ALL), Y/N	-	-	37/126
Pro-T/mature-T ALL °, Y/N/unknown	-	-	22/129/12
<i>TLX1</i> overexpression, Y/N/unknown	-	-	29/98/36
<i>NOTCH1/FBXW7</i> gene mutation, Y/N/unknown	-	-	90/46/27
High-risk <i>NOTCH1/FBXW7/RAS/PTEN</i> genetics, Y/N/unknown	-	-	60/65/38
<b>Response-related characteristics</b>			
Resistance to steroid prephase *, Y/N/unknown	102/320/1	42/218/0	60/102/1
Poor early BM blast clearance *, Y/N/unknown	169/245/9	107/146/7	62/99/2
MRD1 level ≥10 <sup>-4</sup> , Y/N (%)	158/265 (37.4%)	111/149 (42.7%)	47/116 (28.8%)

BCP: B-cell precursor; CNS: central nervous system; WBC: white blood cell count; BM: bone marrow; Y/N: yes/no; °: according to the European Group for the Immunological classification of Leukemias (EGIL); \*: risk factor used in GRAALL trials.

**Table 2. Cause-specific hazards of relapse.**

	Patients, N/tested (SCT patients)	SCT censoring		No SCT censoring	
		Cause-specific HR (95% CI)	P value	Cause-specific HR (95% CI)	P value
<b>Univariable analysis</b>					
<b>BCP-ALL</b>					
WBC $\geq 30 \times 10^9/L$ *	67/260 (39/107)	1.85 (1.01-3.38)	0.046	1.90 (1.19-3.02)	0.007
CNS involvement *	15/257 (9/107)	1.41 (0.44-4.56)	0.56	0.87 (0.32-2.40)	0.79
CD10-negative immature ALL *	67/250 (38/102)	0.90 (0.45-1.79)	0.76	0.75 (0.43-1.29)	0.29
<i>MLL</i> gene rearrangement (t[4;11] or other) *	29/253 (18/105)	2.11 (0.94-4.73)	0.07	1.50 (0.79-2.85)	0.22
t(1;19) *	14/244 (9/103)	1.15 (0.28-4.76)	0.85	1.04 (0.38-2.84)	0.95
Low hypodiploidy/near triploidy *	11/230 (5/100)	1.43 (0.44-4.60)	0.55	1.23 (0.45-3.37)	0.69
Complex karyotype *	18/258 (5/107)	1.04 (0.75-1.44)	0.83	1.01 (0.76-1.36)	0.92
<i>IKZF1</i> gene deletion	54/216 (20/88)	2.65 (1.48-4.73)	0.001	2.06 (1.25-3.39)	0.004
MRD1 level $\geq 10^{-4}$	111/260 (59/107)	3.45 (2.00-6.00)	<0.001	3.07 (1.92-4.90)	<0.001
<b>T-ALL</b>					
WBC $\geq 100 \times 10^9/L$	37/163 (13/51)	1.65 (0.82-3.33)	0.16	1.77 (0.98-3.21)	0.06
CNS involvement *	18/162 (14/51)	2.83 (0.82-9.72)	0.10	1.89 (0.89-4.04)	0.10
Pro-T/mature-T ALL	22/151 (9/47)	1.34 (0.52-3.47)	0.55	1.93 (0.95-3.90)	0.07
Complex karyotype *	14/161 (8/51)	0.86 (0.54-1.35)	0.51	0.82 (0.54-1.24)	0.34
<i>TLX1</i> overexpression	29/127 (5/43)	0.92 (0.41-2.10)	0.85	0.77 (0.35-1.67)	0.51
High-risk <i>NOTCH1/FBXW7/RAS/PTEN</i> genetics	60/125 (20/39)	5.33 (2.14-13.26)	<0.001	4.70 (2.14-10.32)	<0.001
MRD1 level $\geq 10^{-4}$	47/163 (25/51)	2.93 (1.50-5.71)	0.002	2.50 (1.44-4.37)	0.001
<b>Multivariable analysis</b>					
<b>BCP-ALL</b>					
MRD1 level $\geq 10^{-4}$	-	3.21 (1.67-6.18)	<0.001	2.49 (1.43-4.32)	0.001
<i>IKZF1</i> gene deletion	-	2.43 (1.29-4.60)	0.006	1.75 (1.0-3.05)	0.05
<i>MLL</i> gene rearrangement (t[4;11] or other) *	-	3.15 (1.13-8.80)	0.028	1.73 (0.79-3.77)	0.17
WBC $\geq 30 \times 10^9/L$ *	-	1.01 (0.46-2.24)	0.98	1.37 (0.76-2.47)	0.30
<b>T-ALL</b>					
High-risk <i>NOTCH1/FBXW7/RAS/PTEN</i> genetics	-	5.59 (1.82-17.19)	0.003	4.39 (1.75-11.03)	0.002
MRD1 level $\geq 10^{-4}$	-	2.50 (1.06-5.87)	0.036	3.13 (1.51-6.50)	0.002
WBC $\geq 100 \times 10^9/L$	-	1.34 (0.54-3.35)	0.53	1.51 (0.70-3.26)	0.29
CNS involvement *	-	2.49 (0.47-13.3)	0.29	1.38 (0.51-3.74)	0.53
Pro-T/mature-T ALL	-	1.01 (0.33-3.09)	0.98	1.22 (0.53-2.80)	0.63

The endpoint was cumulative incidence of relapse, after censoring patients who received allogeneic SCT in first CR or not, in BCP-ALL and T-ALL subsets, separately. Univariable and multivariable cause-specific hazard ratios (HR) and P values are given; \*: risk factors used in GRAALL trials.

## Figure legends.

### Figure 1. CIR according to early response.

CIR, after censoring patients who received allogeneic SCT in first CR at SCT time, is shown: **(1A)** according to post-induction MRD1 level (evaluated at week 6 after initiation of the first induction cycle); **(1B)** according to post-induction MRD1 and post-consolidation MRD2 (evaluated at week 12 after initiation of the first induction cycle) levels, using a  $10^{-4}$  MRD cutoff at both time-points; **(1C)** according to resistance or sensitivity to the steroid prephase and MRD1 level, showing that MRD1 response may discriminate high- versus good-risk patients in prephase resistant ( $P = 0.016$ ) as well as in sensitive ( $P < 0.001$ ) patients; conversely, resistance or sensitivity to the steroid prophase did not significantly define high- versus good-risk patients among those with low or high MRD1 level ( $P = 0.30$  and  $0.55$ , respectively); **(1D)** according to early BM blast clearance and MRD1 level, showing that MRD1 response may discriminate high- versus good-risk patients both in patients with poor ( $P = 0.031$ ) or good ( $P < 0.001$ ) early BM blast clearance; conversely, early BM blast clearance did not significantly define high- versus good-risk patients among patients with low or high MRD1 level ( $P = 0.06$  and  $0.67$ , respectively).

### Figure 2. CIR according to *IKZF1* gene deletion in BCP-ALL study patients.

CIR, after censoring patients who received allogeneic SCT in first CR at SCT time, is shown in BCP-ALL patients according to the presence of focal *IKZF1* gene deletion.

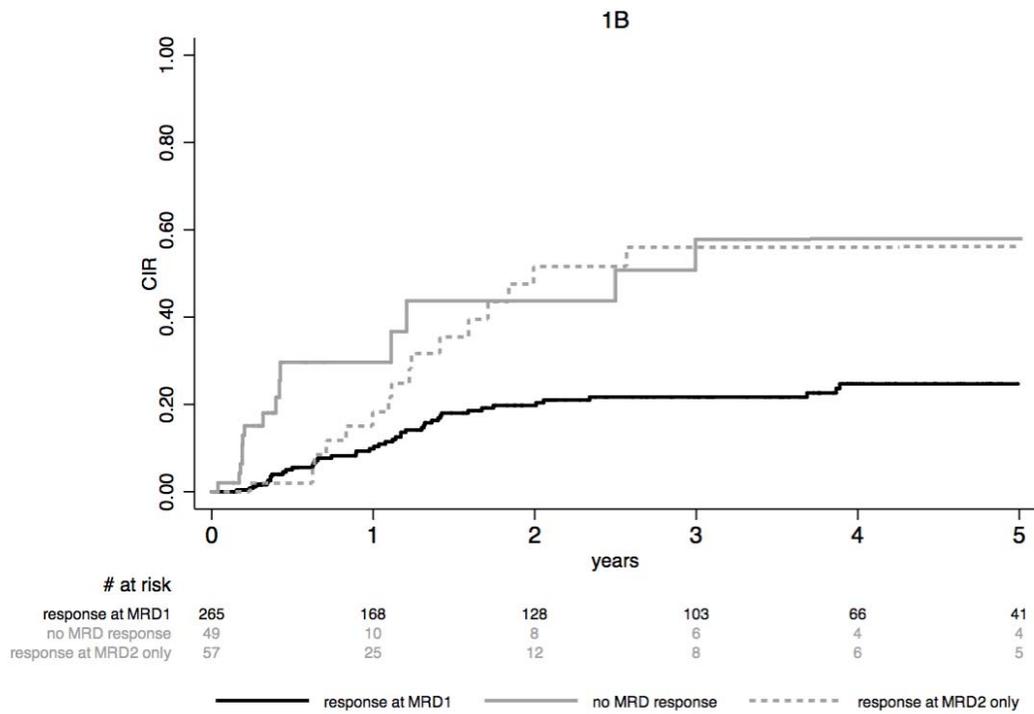
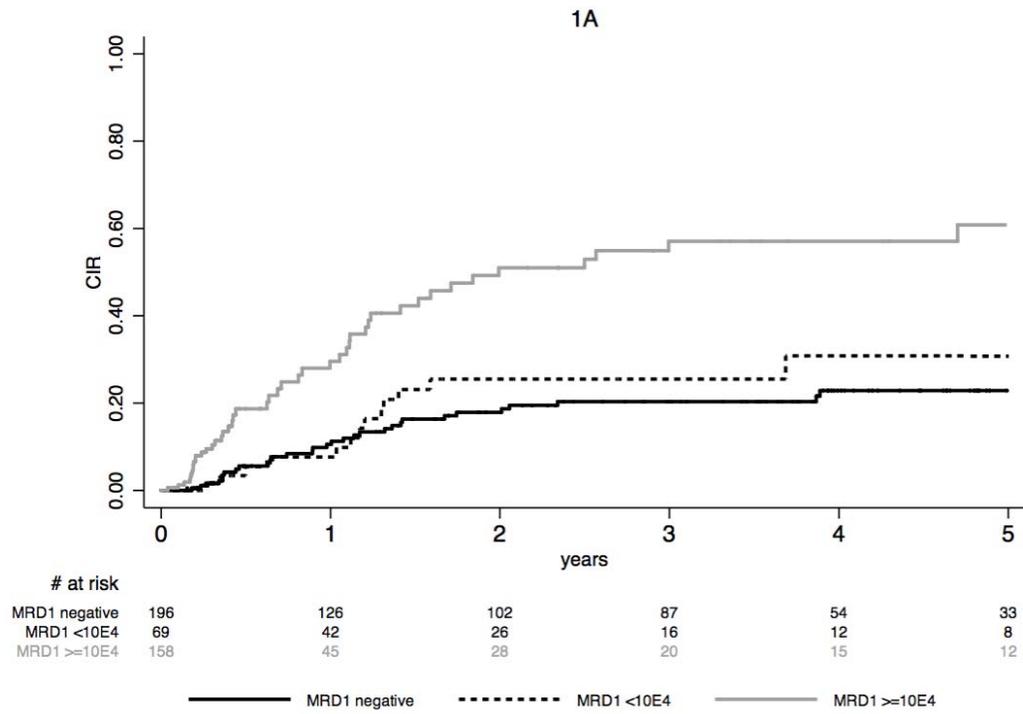
### Figure 3. CIR according to high-risk genetics and MRD response.

CIR is shown for patients also investigated for relevant oncogenetic events, according to the presence of high-risk genetic characteristics (*MLL* gene rearrangement and/or focal *IKZF1* gene deletion in BCP-ALL patients; high-risk *NOTCH1/FBXW7/RAS/PTEN* profile in T-ALL patients) and MRD1 level (using a  $10^{-4}$  cut-off): **(3A)** in BCP-ALL patients, MRD1 level discriminates high-risk patients in good- as well as high-risk genetic subgroups (HR, 2.69 [1.28-5.68] and 2.63 [1.23-5.63];  $P = 0.009$  and  $0.013$ , respectively), while no significant difference in CR was observed in patients with low MRD1 level whatever their genetic characteristics ( $P = 0.18$ ); **(3B)** in T-ALL patients, a high-risk genetic profile discriminated high-risk patients whatever the level of MRD1 (HR, 4.27 [1.52-12.01] and 4.04 [1.17-13.97];  $P = 0.006$  and  $0.027$ , respectively), while no significant difference in CIR was observed in patients with a good-risk genetic profile whatever their MRD1 level ( $P = 0.19$ ).

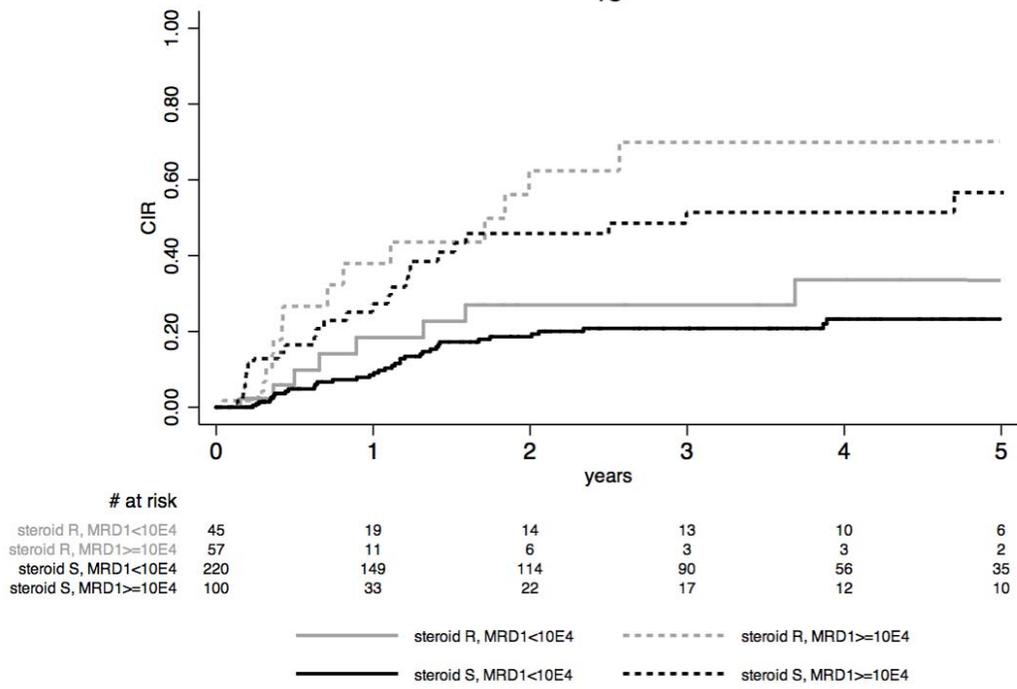
**Figure 4. RFS and OS from CR according to the new risk classification.**

RFS and OS from CR are shown for patients also investigated for relevant oncogenetic events (*MLL* gene rearrangement and *IKZF1* gene deletion in BCP-ALL; *NOTCH1*/*FBXW7*/*RAS*/*PTEN* anomalies in T-ALL), according to the new risk classification. High-risk patients are defined here as those with high-risk oncogenetics and/or MRD1 response  $\geq 10^{-4}$ : **(4A)** RFS in BCP-ALL patients (77 versus 44% at 5 years; HR, 2.84 [95% CI, 1.72-4.70];  $P < 0.001$ ); **(4B)** OS from CR in BCP-ALL patients (79 versus 50% at 5 years; HR, 2.78 [95% CI, 1.61-4.80];  $P < 0.001$ ); **(4C)** RFS in T-ALL patients (86 versus 52% at 5 years; HR, 4.20 [95% CI, 1.85-9.51];  $P = 0.001$ ); **(4D)** OS from CR in T-ALL patients (91 versus 62% at 5 years; HR, 4.14 [95% CI, 1.58-10.83];  $P = 0.004$ ).

**Figure 1.**



1C



1D

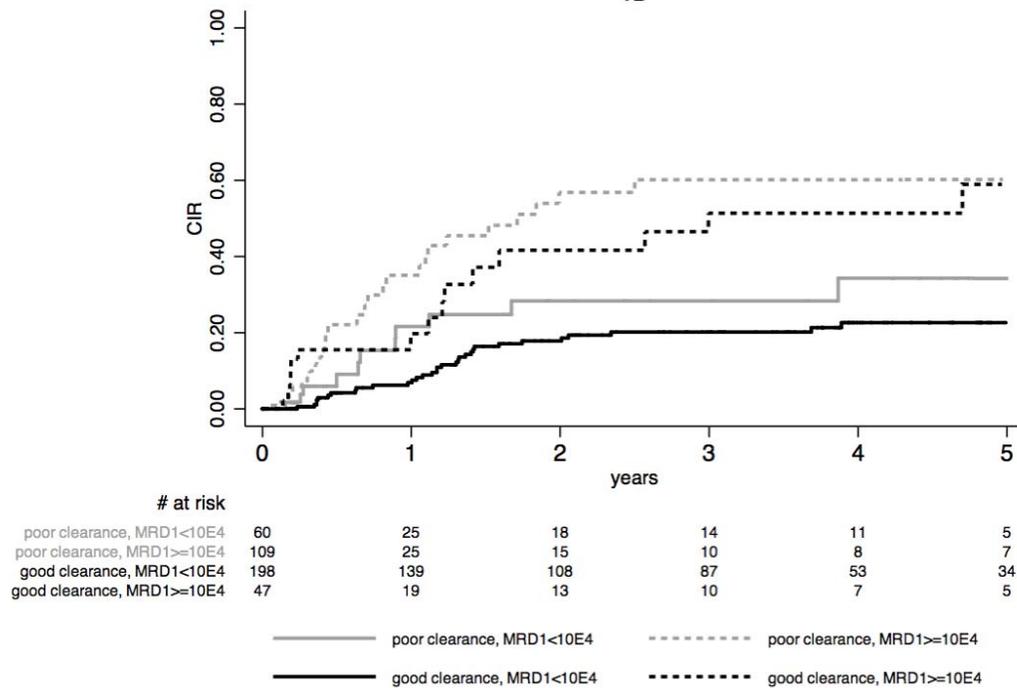
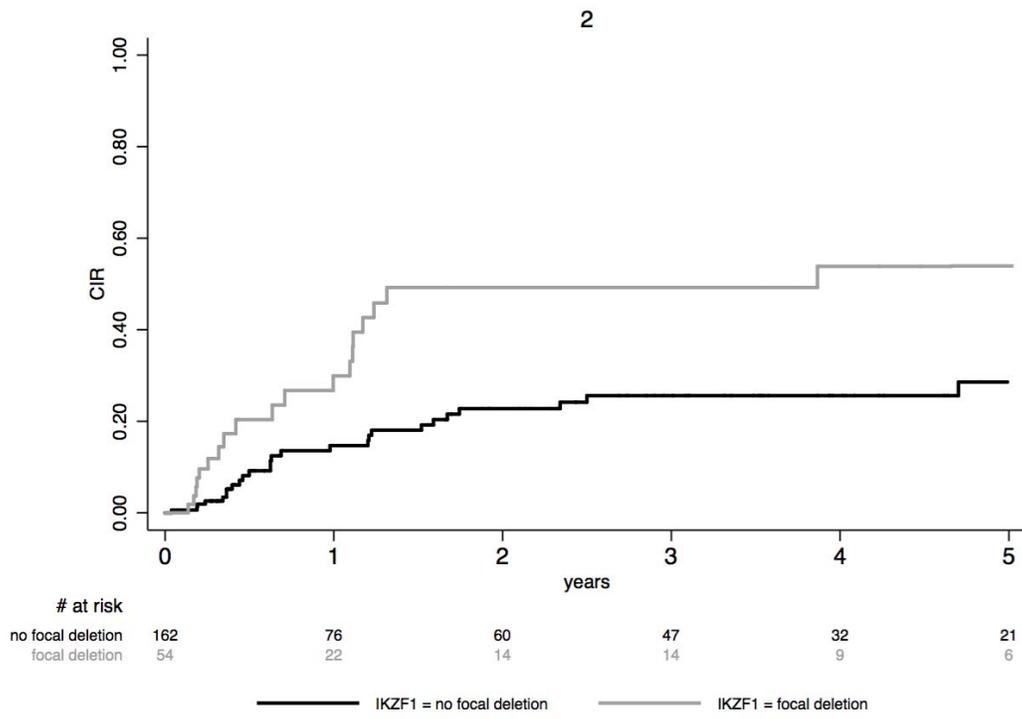
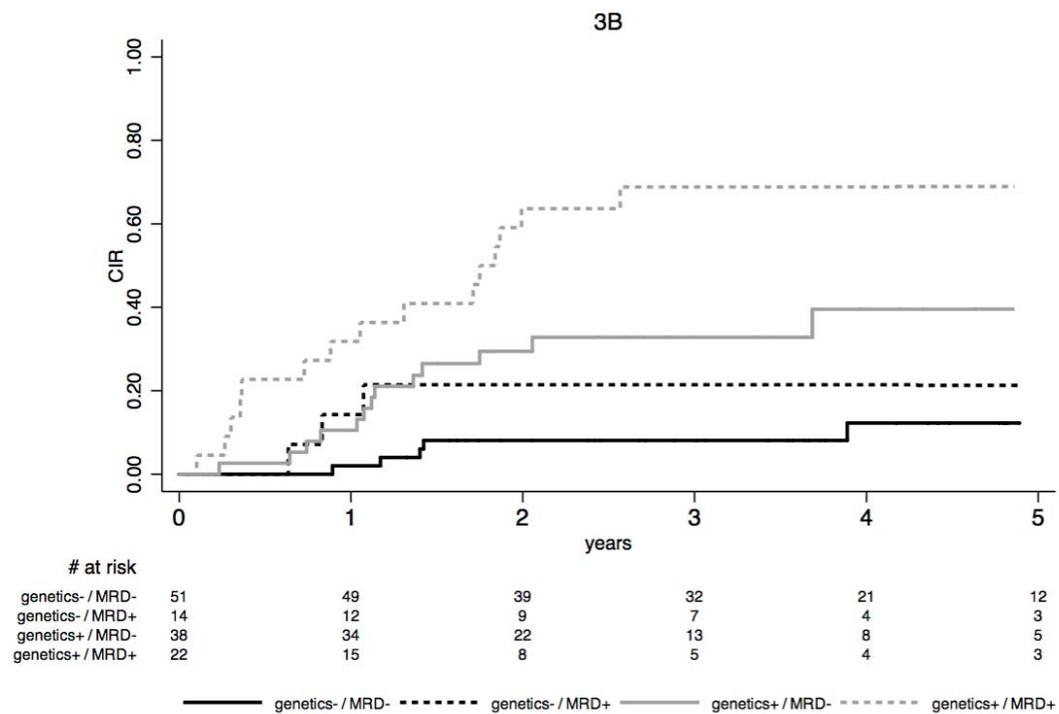
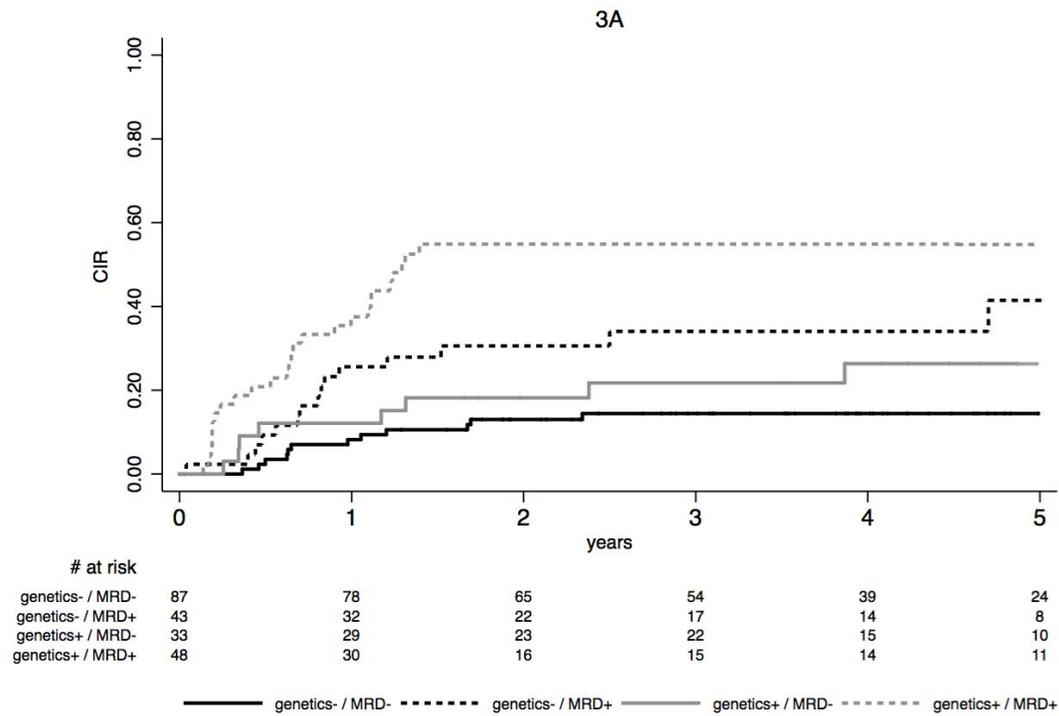


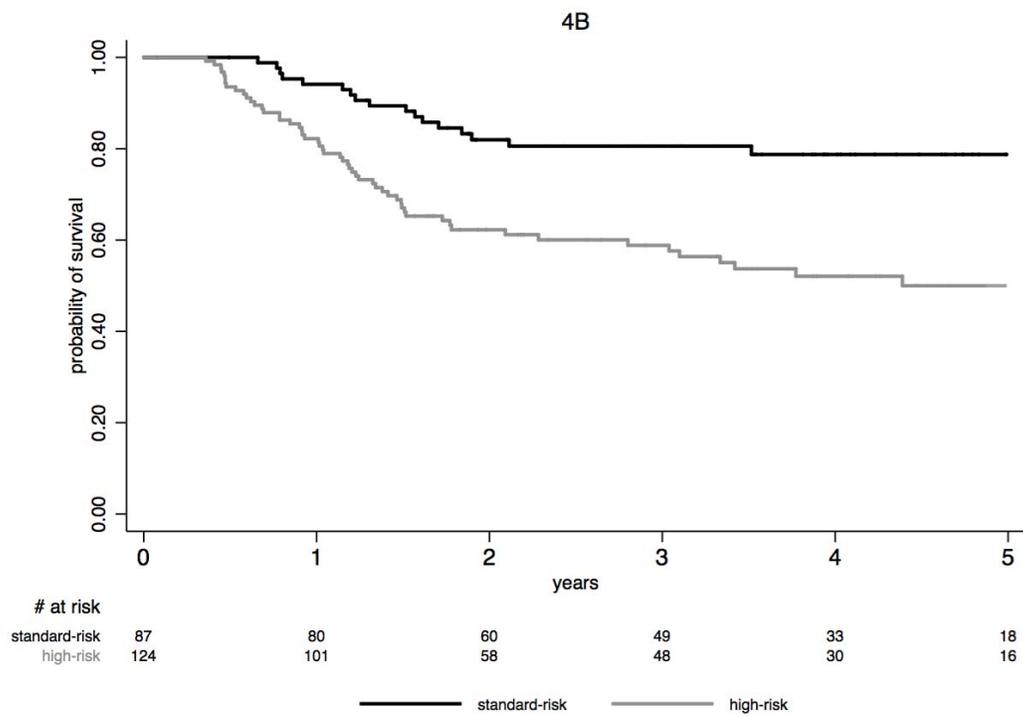
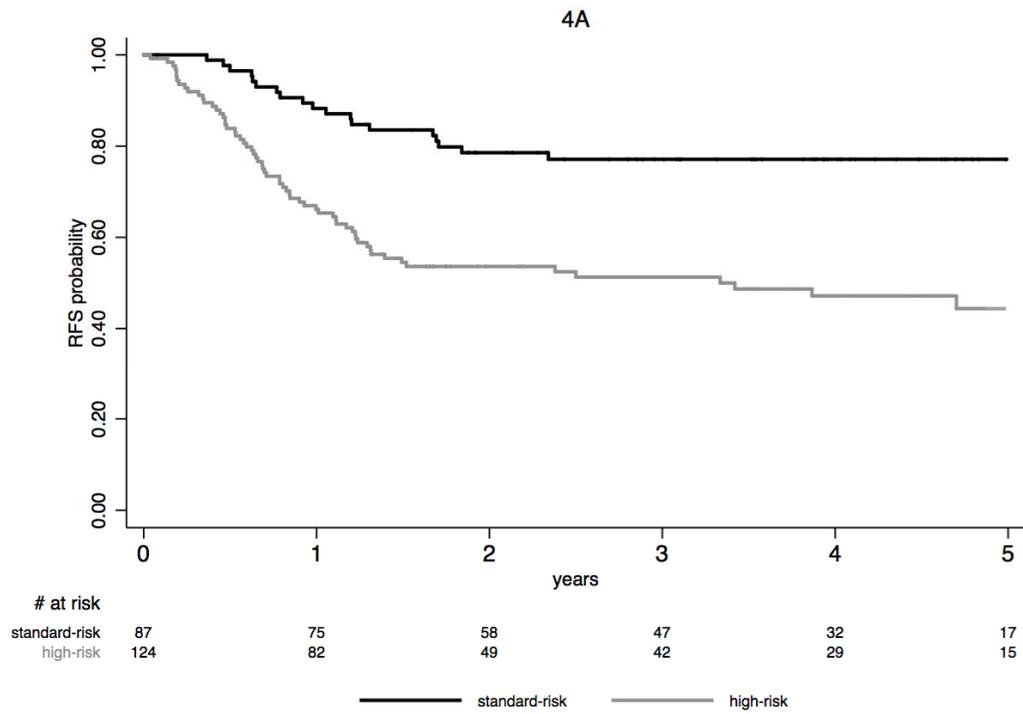
Figure 2.

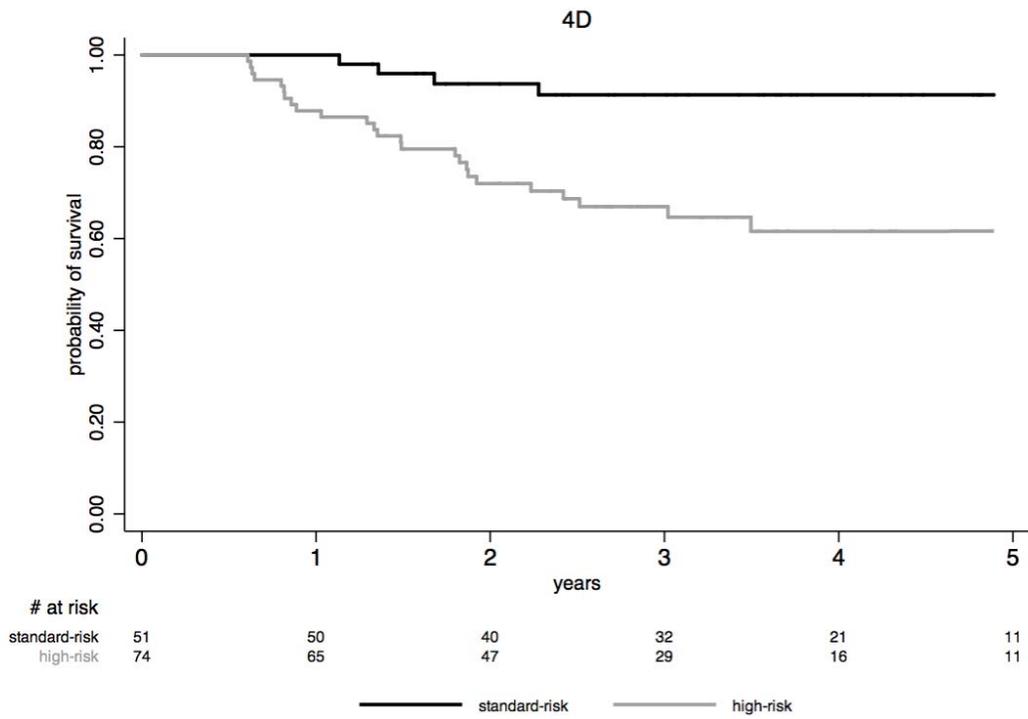
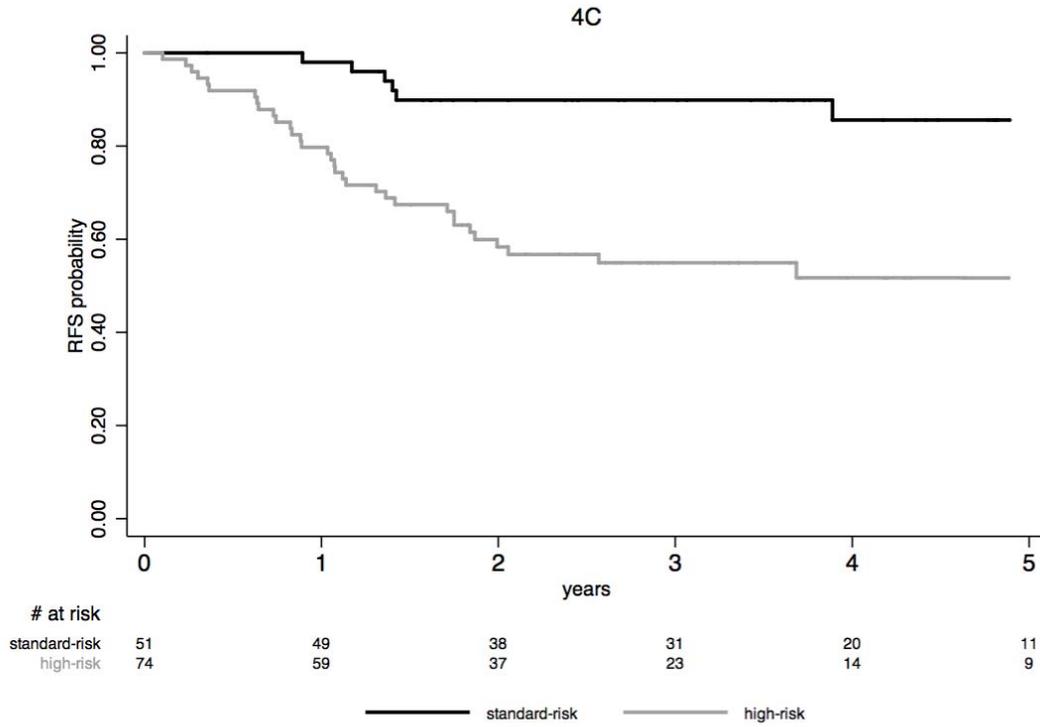


**Figure 3.**



**Figure 4.**







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## **Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia**

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