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Outcome of 421 adult patients with Philadelphia-negative acute lymphoblastic leukemia treated under an intensive program inspired by the GIMEMA LAL1913 clinical trial: a Campus ALL study

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Outcome of 421 adult patients with Philadelphia-negative acute lymphoblastic leukemia treated under an intensive program inspired by the GIMEMA LAL1913 clinical trial: a Campus ALL study

RUNNING HEADS: Real-life study on 421 adult Ph-neg ALL treated with LAL1913 program

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ABSTRACT

The introduction of pediatric-inspired regimens in adult Philadelphia-negative acute lymphoblastic leukemia (Ph- ALL) has significantly improved patients' prognosis. Within the Campus ALL network we analyzed the outcome of adult Ph- ALL patients treated according to the GIMEMA LAL1913 protocol outside the clinical trial, to compare the real-life data with the study results. We included 421 consecutive patients, with a median age of 42 years. The complete remission (CR) rate after the first course of chemotherapy was 94% and a measurable residual disease (MRD) negativity after the third course was achieved in 72% of patients. The 3-year overall survival (OS) and disease-free survival (DFS) were 67% and 57%, respectively. In a multivariate analysis, MRD positivity negatively influenced DFS. In a time-dependent analysis including only very high risk (VHR) and MRD positive cases, transplanted (HSCT) patients had a significantly better DFS than non-HSCT ones (P=0.0017). During induction, grade ≥2 pegaspargase-related hepato-toxicity was observed in 25% of patients (vs 12% in the GIMEMA LAL1913 trial, P=0.0003).

In this large real-life cohort of Ph- ALL, we confirmed the very high CR rate and a superimposable OS and DFS compared to the GIMEMA LAL1913 clinical trial: CR rate after C1 94% vs 85%, P=0.0004; 3-year OS 67% vs 67%, P=0.94; 3-year DFS 57% vs 63%, P=0.17. HSCT confirms its important role in VHR and MRD-positive patients. The rate of pegaspargase-related toxicity was significantly higher in the real-life setting, emphasizing the importance of dose adjustment in the presence of risk factors to avoid excessive toxicity.

INTRODUCTION

No definitive consensus exists on the optimal treatment regimen for adult Philadelphia chromosome-negative acute lymphoblastic leukemia (Ph- ALL) to optimally balance efficacy and toxicity, as shown by the different treatment backbones employed by cooperative study groups.[1-7] Nonetheless, in the last years numerous phase 2 and phase 3 clinical trials from different countries have been associated with rather favorable results compared to previous experiences.[1-7] These improvements have been achieved using intensive pediatric-inspired protocols, new formulations of asparaginase and revised stratification models which included measurable residual disease (MRD) monitoring, in addition to baseline risk factors.[8-10] However, data on the real-life applicability of therapeutic regimens tested in clinical trials, which inherently enroll selected patient populations, are very limited.

Recently, the Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA) published the results of the LAL1913 clinical trial, which included 203 homogeneously treated adult Ph- ALL patients with a pediatric-inspired protocol.[1] After the completion of this study, most Italian hematology centers used the same therapeutic program in their clinical practice while the new protocol for Ph- ALL was under discussion. In this paper, we report the efficacy and safety data of a chemotherapy program performed according to the GIMEMA LAL1913 protocol in adult patients with Ph- ALL treated outside the clinical trial, in a real-life setting.

METHODS

Patients and objectives of the study

We included 421 consecutive adult patients with newly diagnosed Ph- ALL or lymphoblastic lymphoma (LL, with <20% bone marrow blasts) treated according to the GIMEMA LAL1913 protocol,[1] outside the clinical trial, between September 2016 and December 2022. The data were collected from 39 hematology centers that are part of the Campus ALL network in Italy.

The main objectives of the study were to compare the complete remission (CR) rate, the overall survival (OS) and the disease-free survival (DFS) between the real-life cohort (421 cases) and the GIMEMA LAL1913 clinical trial population (203 cases). Secondary endpoints included the evaluation of the treatment toxicity and the allogeneic stem cell transplantation (HSCT) rate according to the risk-group at diagnosis.

Diagnostic procedures such as immunophenotyping, cytogenetics and molecular studies were carried out according to the GIMEMA LAL1913 protocol indications.[1,2] The Philadelphia-like signature was not routinely tested in this real-life population.

In line with the GIMEMA LAL1913 trial, three risk classes were defined at diagnosis (as reported in the supplementary section).

This observational study was approved by the Ethics Committee of Friuli Venezia Giulia, Italy (ethical approval number CEUR-2022-Os-03) and conducted in accordance with the 2008 revision of the Helsinki Declaration.

Treatment protocol

All patients were treated according to the GIMEMA LAL1913 protocol as described by Bassan R et al. and detailed in **Supplemental Table 1**.[1]

Antibiotic, antimycotic and antiviral prophylaxis, and pegaspargase toxicity management were administered according to the policy of each center.

Treatment-related toxicity was evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

MRD analysis

MRD analysis was carried out on bone marrow samples through real-time quantitative (RTq) -PCR for immunoglobulin (IG) or T-cell receptor (TR) gene rearrangements following the EuroMRD guidelines[11] in 3 reference laboratories (as in the GIMEMA LAL1913 trial), or locally through multiparameter flow cytometry (MFC) targeting leukemia associated immunophenotype in patients lacking suitable molecular probes. Similarly to the GIMEMA LAL1913 trial, data on MRD were collected at four specific time points: end of induction week 4 (TP1), weeks 10 (end of course 3, TP2), 16 (end of course 5, TP3) and 22 (end of course 7, TP4). Patients with low positive ($<10^{-4}$) or negative TP2-3 and negative TP4 (or negative TP2-3 when TP4 was missing) were defined as MRD-negative (MRD-neg), while those with TP2-3 $\geq 10^{-4}$ and/or positive TP4 were defined as MRD-positive (MRD-pos), according to the LAL1913 clinical trial.

Statistical analysis

The comparison between baseline characteristics among subgroups was obtained using the Fisher's exact or Chi-squared test for categorical variables, the Student's t-test for normally distributed variables and the Mann-Whitney test for non-normally distributed variables. Logistic regression was used to study variables influencing the obtainment of MRD-negativity at TP2. Median follow-up time was calculated among survivors and was last updated in June 2023.

The response evaluation criteria are reported in the supplementary section.

OS was calculated from the date of diagnosis to the date of the last follow-up or to the date of death by any cause. DFS was calculated from the date of the first CR achievement to the date of the last follow-up, relapse or death by any cause. DFS stratification for MRD followed the definition for MRD-neg and MRD-pos described in the "MRD analysis" section using the available time-points for each patient. OS and DFS were estimated according to the Kaplan-Meier method and the differences between groups were compared with the log-rank test. Univariate and multivariate analyses were carried out by Cox regression for OS and DFS.

Simon-Makuch plot was used to assess the time-dependent effects of HSCT and Mantel-Byar test was used for comparison of survival curves.

The same descriptive statistics were used to compare the characteristics of the real-life and the LAL1913 clinical trial populations. To compare OS and DFS, a subclass matching propensity score was performed (5 quantile classes) considering the following variables: age, sex, risk, lineage, and transplant. All 602 observations were matched, and the real-life data were weighted according to subclassification. Propensity score estimates were calculated using a logistic regression model. A summary of the characteristics of the patients in the propensity score matching is reported in **Supplemental Table 2**.

Statistical significance in all cases was considered for a P value less than 0.05.

RESULTS

<u>Patients' characteristics</u>

The main characteristics of the 421 patients are summarized in **Table 1**. Median age was 42 years (range 18-80) and was significantly lower in T-ALL/LL patients (38.5 vs 45, P=0.0009); 23% (n=97) were older than 55 years; 52.5% (n=221) had B-ALL and 12% (n=50) had LL (of which n=45 T-lineage, P<0.0001).

The median WBC count was significantly higher in T-ALL (P<0.0001), as also the involvement of lymph nodes and mediastinum (42% and 47% of patients, respectively). Central nervous system (CNS) involvement was documented in 9% (n=37) of patients at disease onset (more frequently in T-ALL/LL, 12.5% vs 5%, P=0.0149).

As for cytogenetics/genetics (evaluable in 81% of patients, n=342), 15 patients had a *KMT2A*;11q23 rearrangement, 45 had other adverse karyotypes, while a t(1;19)/*TCF3::PBX1* translocation was detected in 5 patients and a hyperdiploidy in 15. As specified in the "Methods" section, the Philadelphia-like signature was not routinely tested.

Overall, 49% of patients were standard risk (SR), 10% high risk (HR) and 41% very high risk (VHR). T-ALL/LL patients displayed VHR features more frequently (52% vs 30% of B-ALL/LL, P<0.0001).

The median follow-up of the entire population was 24.6 months. At the last follow-up, 306 patients (73%) were alive (251/306 - 82% in CR1) and 115 (27%) had died (64/115 - 56% due to underlying disease, 24/115 - 21% due to transplant-related mortality, 9/115 - 8% deaths during induction, 5/115 - 4% deaths in CR during subsequent courses of chemotherapy, 13/115 - 11% due to other causes).

Treatment and response

All 421 patients received the first course of therapy (C1) and 358 (85%) of them were able to continue the treatment up to the third course (C3). Prior to C3 we recorded 15 deaths, 9 during induction (2%), 3 during consolidation (7 of which due to infection) and 3 unrelated to disease or therapy, while 40 patients switched to an alternative treatment, 26 (65%) due to refractoriness or early progression (14 after C1 and 12 after C2) and 14 (35%) due to adverse events (10 after C1 and 4 after C2). Eight patients had a short follow up (too early) and did not undergo C3 at data cut off. Overall, only 6% of the entire patient population (26/421) was refractory after C2.

The morphologic CR rate after C1 was 94% (356/379) and after C2 95% (329/347) of evaluable patients (not evaluable patients were those with LL without marrow involvement and those in which the bone marrow study was not performed). The early death rate was 3% (n=12) of the whole population.

After C3, 146 patients (35%) underwent a HSCT in first line; in 16% of patients (n=24), the procedure was preceded by immunotherapy for MRD persistence (Blinatumomab in 22 cases, Inotuzumab in 2 cases). The two main indications for HSCT were: VHR disease (70.5%, n=103) and MRD positivity (21%, n=31). HSCT was more frequently carried out in T-ALL patients (47% vs 31%, P=0.002). Overall, 129 SR-MRD-neg patients were able to proceed to maintenance. Globally, 39 patients were treated with immunotherapy (35 Blinatumomab, 3 Inotuzumab, 1 Daratumumab) for MRD persistence after first-line chemotherapy.

MRD study was available in 381 patients (90.5%); 71% (n=269) were monitored by RTq-PCR for Ig/TR gene rearrangements, and the remaining 29% (n=112) by MFC. The rates of MRD negativity at TP1 and TP2 were, respectively, 46% and 67% of the evaluable patients (72% when excluding LL patients without MRD study on bone marrow).

A summary of the MRD response at the different time points is provided in **Table 2**, and did not differ between B-ALL and T-ALL patients. A multivariate logistic regression analysis including age, risk category, lineage, ECOG score, and CNS involvement was carried out to study variables influencing the obtainment of MRD negativity at TP2, and we found that the presence of a HR or a VHR risk class was the only factor associated with failure to achieve MRD negativity (OR 0.38, CI 0.22 – 0.64, P=0.0003).

Side effects and toxicities

Chemotherapy dose reductions beyond those established by the LAL1913 protocol in patients >55 years were required during C1 in 118 patients (28%), due in 50% of patients (59) to either toxicity or infection.

Table 3 summarizes the pegaspargase-related toxicity. During C1, 382 patients (91%) received pegaspargase, and 49% of them (189) developed a grade ≥2 related toxicity (mainly hepatic toxicity) while thrombosis, pancreatic toxicity and hypersensitivity reaction were rare (as reported in **Table 3**). The global rate of grade ≥2 pegaspargase-related toxicity at C2 was 32% (101/314). Pegaspargase was not administered at C2 in 12% of patients (47/382) due to previous related toxicity at C1. In addition, a drug dose reduction was required during C2 in 27% of patients receiving pegaspargase (86/314). During C5 and C6, the global rate of grade ≥2 pegaspargase-related toxicity was 38% and 30%, respectively. A pegaspargase dose reduction at C5 and C6 was required in 28% (50/177) and 35% (45/129) of cases, respectively. The drug was omitted at C5, due to the previous related toxicity, in 9% of patients and in 19% of patients at C6 (**Table 3**).

Pegaspargase-free courses (C3, C4, C7 and C8) were administered at the programmed full doses of chemotherapy in 93%, 93%, 89% and 87% of patients, respectively.

Infectious complications were more frequently recorded during C1. Bacteremia/sepsis, being the most common infection, was observed in 14% of patients (59), followed by pneumonia in 11% (45); 20 cases of pneumonia (5% of the whole population) were mycotic. Moreover, during C1, 22% (91) of patients developed febrile neutropenia. In the following courses the number of patients developing bacteremia/sepsis was lower (between 1% and 9%); the courses with the highest number of events observed were C3, C6 and C7 (respectively, 7%, 7% and 9%). Also, the number of patients developing pneumonia was lower (between 0% and 4%) with similar percentages in the different courses. The number of patients developing febrile neutropenia beyond C1 ranged between 3% and 21% of patients, and again a higher number of events was observed at C3, C6 and C7 (respectively, 17%, 18% and 21%).

Survival analysis and prognostic factors

The 3-year OS probability was 67% (median not reached), without significant differences between patients aged ≤40 and those aged 41-55 (76% vs 63%, P=0.28). However, both these groups had a significantly higher 3-year OS than patients aged >55 (55%, Logrank test P=0.0007 vs patients aged ≤40 and P=0.041 vs patients aged 41-55), as shown in **Figures 1A** and **1C**.

The 3-year DFS probability was 57% (median not reached), without significant differences between patients aged ≤40 and patients aged 41-55 (61% vs 60%, P=0.77). Again, both these groups had a significantly higher 3-year DFS than patients aged >55 (46%, Logrank test P=0.011 vs patients aged ≤40 and P=0.050 vs patients aged 41-55), as shown in **Figures 1B** and **1D**.

Figure 2A shows the DFS curves for MRD-neg and MRD-pos patients. The 3-year DFS was 67% in MRD-neg patients vs 32% in MRD-pos ones (Logrank test P <0.0001), respectively.

In univariate analysis, a younger age predicted a better OS, while CNS involvement and MRD-pos predicted a worse OS. A younger age (≤55 years) also predicted a better DFS in univariate analysis, while MRD-pos, CNS involvement, high leucocyte count (>30x10^9/l), adverse cytogenetics, the presence of a *KMT2A* rearrangement and the VHR risk class per se predicted a worse DFS. In multivariate analysis for OS and DFS, significance was retained only for MRD-pos **(Figure 3).**

To better analyze the effect of HSCT, a time-dependent analysis was performed for DFS. HSCT did not show a benefit when considering the whole population, but when we considered just VHR or MRD-pos patients, i.e. those who were transplant candidate according to the GIMEMA LAL1913 protocol, the impact of HSCT was significant (Mantel-Byar P=0.0017). In **Figure 2B** we report the Simon-Makuch plot for DFS of VHR and MRD-pos patients according to HSCT.

<u>Comparison with the results of the GIMEMA LAL 1913 trial</u>

As shown in **Table 4**, we compared the most important findings of this real-life observational study (including 421 cases) and the results of the GIMEMA LAL1913 clinical trial (including 203 cases).[1] The real-life population was slightly older, although the difference was not significant (median age 42 vs 40 years, P=0.5, with patients older than 55: 23% vs 19%, P=0.33) and included a higher number of T-ALL/LL (47.5% vs 31.5%, P=0.0002). Moreover, in the real-life population cohort we observed a non-significantly higher proportion of HR+VHR patients (51% vs 43%, p=0.09).

The CR rate at TP1 was higher in the real-life population (94% vs 85%, P=0.0004), but the rate of MRD negativity at both TP1 and TP2 was lower (respectively 46% vs 56%, P=0.04, and 72% vs 80%, P=0.04).

Importantly, OS and DFS were similar in the two studies, with a 3-year OS of 67% vs 67%, P=0.94, and a 3-year DFS of 57% vs 63%, P=0.17, respectively (**Figure 4**). When weighed according to the propensity score performed, the 3-year OS and DFS were 67% and 55%, respectively (with P=0.94 and P=0.17, compared to the GIMEMA LAL1913 trial data). The rate of HSCT in first line was higher in the real-life setting (35% vs 28%), thought without reaching a significant difference (P=0.09).

Finally, we compared pegaspargase-related adverse events during C1, and we observed a higher rate of grade ≥2 hepatic toxicity in patients treated in the real-life setting compared to those included in the LAL1913 trial (25% vs 12%, P=0.0003), while the rates of grade 3 pancreatic toxicity and the thrombotic events were similar in the two cohorts (3% vs 1%, P=0.26, and 2% vs 2%, P=1.00).

DISCUSSION

Pediatric-inspired protocols have improved the outcome of Ph- ALL in adults,[8] as demonstrated by several trials yielding comparable results, with CR rates around 90% and OS and DFS rates above 60% at 3-5 years, despite the differences in trial design and asparaginase formulations and dosage.[1-7] In trials including older adults (>55 years), this patient population witnesses worse results, with inferior CR rates and survival.[1-4] This is likely due to the difficulties in delivering optimal chemotherapy doses, increased complication rates, and a different disease biology compared to younger patients.[3,4,12]

Despite the significant number of clinical trials using pediatric-inspired protocols in adult Ph- ALL, very limited data are available on the feasibility, toxicities and outcome of these protocols in the context of the daily clinical practice outside of clinical trials.[13-16] Since the completion of the GIMEMA LAL1913 trial, the results of which have been recently published,[1] most Italian hematology centers have adopted this pediatric-inspired therapeutic program as the standard of care for the clinical management of newly diagnosed adult Ph- ALL. The current study conducted within the Campus ALL network and involving 39 hematology centers in Italy was thus aimed at analyzing the feasibility and performance of the LAL1913 program in the real-life in terms of tolerability and outcome, and to compare these results with those obtained in the original clinical trial.[1] To our knowledge, this multicenter real-life study that included 421 adult Ph-ALL patients homogeneously treated according to a pediatric-inspired protocol (GIMEMA LAL1913)[1] is the largest so far available.

Some differences emerged between the characteristics of the real-life population compared to the clinical trial one. The real-life cohort included more T-ALL (47.5% vs 31.5%) and a higher proportion of HR+VHR patients, albeit the difference was not significant (51% vs 43%). The median age and the proportion of patients aged >55 were comparable in the two cohorts (23% in real-life vs 19% in clinical trial, P=0.33).

In consideration of the abovementioned differences, a high CR rate after C1 (94%) was observed in the real-life setting, resulting even higher than the rate reported in the GIMEMA LAL1913 clinical trial (85%, p=0.0004). However, the rate of MRD-neg patients at both TP1 and TP2 was lower in the real-life cohort compared to the clinical trial results (respectively 46% vs 56%, P=0.04, and 72% vs 80%, P=0.04), and this can be explained by the higher number of HR and VHR patients included in our study, considering that this was the only variable that significantly influenced the achievement of a MRD negativity a TP2. It is noteworthy that the OS and DFS observed in our real-life population were comparable to the results reported in the GIMEMA LAL1913 clinical trial (3-year OS 67% vs 67%, P=0.94; 3-year DFS 57% vs 63%, P=0.17). The CR and MRD-neg rates, OS and DFS were also in line with other published prospective clinical trials.[3,4,6]

Similarly to other studies, age had an impact on OS in this real-life analysis,[1-4,14-16] but unlike the GIMEMA LAL1913 clinical trial, patients aged 41-55 years fared the same as patients aged ≤40 years, and only patients aged >55 years showed a significantly reduced survival.[1]

Besides, this study confirmed that biological features of the disease at diagnosis (cytogenetics, leukocytosis, *KMT2A* rearrangements) played an important role in DFS in univariate analysis, contributing to the definition of HR and VHR classes. This effect was not evident in the GIMEMA LAL1913 clinical trial, in which only patients with *KMT2A* rearranged ALL showed a significantly worse outcome.[1]

We also confirmed the crucial role of MRD monitoring in the clinical practice and its important prognostic impact on OS and DFS as observed in many clinical trials.[1,2,14,17-19]

Our real-life data also highlighted the prognostic impact of first-line HSCT in patients with unfavorable risk factors (VHR and/or MRD-pos), with results comparable to those reported in the GIMEMA LAL1913 clinical study. In our population, a proportion of patients was bridged to HSCT procedure with immunotherapy for MRD positivity, an option that was not available in the GIMEMA LAL1913 trial, but given that this approach is becoming standard in the clinical practice and that several trials (such as the ongoing GIMEMA LAL2317) are exploring a sequential chemo-immunotherapy approach, the role of HSCT may change in the near future.[17,20,21]

In terms of tolerability, the highest rate of pegaspargase-related toxicity in our study was observed at C1, with 49% of patients experiencing at least one grade ≥2 adverse event. In particular, during C1 25% of patients in this study developed grade ≥2 hepatic toxicity compared to 12% of patients in the

GIMEMA LAL1913 clinical trial (P=0.0003). Overall, the pegaspargase-related toxicity observed compares favorably to other international reports in the literature.[22,23] This finding may reflect less attention to risk factors for pegaspargase-related toxicity (such as obesity, hepatopathies) and/or a less stringent patient selection in the real-life setting than in the GIMEMA LAL1913 clinical trial.

Infectious complications are a significant concern during the management of Ph- ALL patients.[24] In our analysis, infectious complications mainly occurred at C1, with 14% of patients developing bacteremia/sepsis and 11% of patients developing pneumonia (mycotic in nearly half of the cases). While pneumonia represents an uncommon event in subsequent courses, the number of patients who developed bacteremia/sepsis reached 9% of cases, in line with previous studies involving Ph-ALL patients treated with intensive protocols.[6,7,13] Despite the low rate of early mortality observed in our study (3%, 12/421), infections were the main cause of death, affecting more than half of cases. This suggests the need to improve infection surveillance, prophylactic measures, and antimicrobial therapy.

In summary, our study demonstrates the feasibility and favorable outcome of a pediatric-inspired therapeutic regimen in a large real-world setting with CR rates and OS and DFS similar to those reported in the originator clinical trial (GIMEMA LAL1913). Moreover, our analysis confirms the important role of HSCT in patients with high risk factors or MRD positivity. Therefore, outside of clinical trials efforts should be made to obtain a detailed disease characterization, keeping up with recently identified molecular subgroups, and to strictly monitor MRD at the appropriate time points to better identify patients with risk factors for early HSCT referral.[9-10]

A limitation of our study is the lack of availability of the Ph- like signature for the majority of the patients, that should be more widely tested in the common clinical practice.[25-28] Besides, the widespread use of immunotherapy in patients with pre-transplant MRD persistence could improve the outcome of transplant, and we are waiting for the results of the studies testing these approaches in patients with baseline high risk features[29] or in all cases, including MRD-negative patients, where blinatumomab also appears to be effective.[30]

The tolerability of pegaspargase in the real-life setting remains an important concern, given the cornerstone role of this drug in the therapy regimen, and indeed our study is limited by the lack of a precise correlation analysis between pegaspargase dosage and response. Future studies are needed to individualize the dosage of the drug to be given to each patient with a tolerated dose while remaining within the efficacy range.[8,20,22,31,32] Probably, the widespread availability of asparaginase activity monitoring could be useful to optimize dose calculation.[33]

A detailed analysis of infectious complications was beyond the purpose of this study (and will be detailed in a subsequent report), but this remains an important issue to address, as infections are an important cause of morbidity and mortality, and efforts should be made to uniform anti-infectious prophylaxis, especially on the anti-mycotic front.[24,34]

Finally, elderly patients still show inferior outcomes compared to younger patients, even observing a median OS of 45 months, and future studies should aim at identifying the optimal age cut-off to define the “elderly” and to design better and tailored induction therapies incorporating frontline immunotherapy to reduce toxicity and improve the outcome.[35-37]

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TABLE 1. Characteristics of the 421 study patients.

	All patients (n=421)	B-ALL/LL (n=221)	T-ALL/LL (n=200)
Age (years), median (range)	42.0 (18-80)	45.0 (18-80)	38.5 (18-72)
≤ 40, n (%)	199 (47)	87 (39)	112 (56)
>40-55, n (%)	125 (30)	66 (30)	59 (29.5)
>55, n (%)	97 (23)	68 (31)	29 (14.5)
Gender (male), n (%)	248 (59)	111 (50)	137 (68.5)
Diagnosis, n (%)			
ALL	371 (88)	216 (98)	155 (77.5)
LL	50 (12)	5 (2)	45 (22.5)
ECOG PS, n			
0;1;2;3;4;NA	212;147;42;15;3;2	122;77;19;3;0;0	90;70;23;12;3;2
Hemoglobin (g/dl), median (range)	10.7 (4.1-17.2)	9.3 (4.1-15.1)	12.3 (4.2-17.2)
WBC (10^9/l), median (range)	9.9 (0.2-626.9)	6.9 (0.2-626.9)	12.2 (0.5-538.0)
≤ 30 (%)	304 (72)	170 (77)	134 (67)
>30-100 (%)	66 (16)	31 (14)	35 (17.5)
>100 (%)	51 (12)	20 (9)	31 (15.5)
PB blasts (%), median (range)	38.0 (0.0-100.0)	40.5 (0.0-100.0)	30.0 (0.0-100.0)
BM blasts (%), median (range)	80.0 (0.0-100.0)	85.0 (0.0-100.0)	73.0 (0.0-100.0)
Platelets (10^9/l), median (range)	78 (4-753)	63.5 (8-400)	115 (4-753)
Hepatomegaly, n (%)	27 (6)	12 (5)	15 (7.5)
Splenomegaly, n (%)	44 (10.5)	18 (8)	26 (13)
Lymphadenopathy, n (%)	110 (26)	26 (12)	84 (42)
Mediastinal mass, n (%)	96 (23)	2 (1)	94 (47)
CNS involvement, n (%)	37 (9)	12 (5)	25 (12.5)
Other involved sites, n (%)	5		
testis/ovary:skin	1:4	-	-
Immunophenotype, n (%)			
B: pro, common, pre, MPAL, UND	221 (52.5)	39,130,23,5,24	
T: ETP, pro, pre, cortical, mature, MPAL, UND	200 (47.5)		40,15,23,57,13,4,48
Citogenetics/genetics, n (%)			
Evaluable, n (%)	342 (81.2)	191 (86.4)	151 (75.5)
Normal	174 (51)	78	96
Adverse	60 (17)	45	15
- t(4;11)/*KMT2A::AFF4*, t(11;19)	15	15	-
- Other[a]	45	30	15
Non-adverse	108 (32)	67	40
- t(1;19)/*E2A::PBX1*	5	5	-
- Hyperdiploid	15	15	0
- Other non-adverse	88	48	40
Risk stratification, n (%)			
Evaluable 420/421 (99.8%)			
Standard risk (SR)	207 (49)	120 (54)	87 (44)
High risk (HR)	42 (10)	34 (15)	8 (4)
Very high risk (VHR)	171 (41)	67 (30)	104 (52)

Abbreviations: ALL, acute lymphoblastic leukemia; LL, lymphoblastic lymphoma; ECOG PS, Eastern Cooperative Oncology Group performance status; NA, not available; WBC, white blood cells; PB, peripheral blood; BM, bone marrow; CNS, central nervous system; UND, undefined; ETP: early T precursor

[a] other than t(4;11)/KMT2A rearrangement: 11q23, +8, -7, del6q, t(8;14) abnormalities, low hypodiploidy (30-39 chromosomes), near triploidy (60-78 chromosomes) or complex karyotype with ≥5 unrelated anomalies

TABLE 2. Summary of response according to different time points.

	All patients (n=421)	**B-ALL (n=216)**	**T-ALL (n=155)**	**LL (n=50)**
Response at TP1 (in available cases)	**379**	**209**	**147**	**23**
MRD-pos or unk CR (%)	181 (48)	108 (52)	66 (45)	7 (30)
MRD-neg CR (%)	175 (46)	91 (43.5)	71 (48)	13 (57)
No CR (%)	23 (6)	10 (5)	10 (7)	3 (13)
Response at TP2 (in available cases), n (%)	**344***	**169**	**128**	**47***
MRD-pos CR (%)	70 (20)	44 (26)	25 (19.5)	1 (2)
MRD-neg CR (%)	231 (67)	115 (68)	96 (75)	20 (43)
MRD-unk CR (%)	23 (7)*	4 (2)	2 (2)	17 (36)*
No CR (%)	20 (6)*	6 (4)	5 (4)	9 (19)*
Response at TP3 (in available cases), n (%)	**168**	**89**	**64**	**15**
MRD-pos CR (%)	15 (9)	7 (8)	8 (12.5)	0
MRD-neg CR (%)	144 (86)	77 (86.5)	52 (81)	15 (100)
MRD-unk CR (%)	4 (2)	4 (4.5)	0	0
No CR (%)	5 (3)	1 (1)	4 (6)	0
Response at TP4 (in available cases), n (%)	**141****	**69**	**43**	**29****
MRD-pos CR (%)	7 (5)	2 (3)	4 (9)	1 (3)
MRD-neg CR (%)	112 (79)	61 (88)	39 (91)	12 (41)
MRD-unk CR (%)	19 (13.5)**	5 (7)	0	14 (48)**
No CR (%)	3 (2)**	1 (1.5)	0	2 (7)**

Abbreviations: ALL, acute lymphoblastic leukemia; LL, lymphoblastic lymphoma; MRD-pos, measurable residual disease positive; MRD-neg, measurable residual disease negative; MRD-unk, measurable residual disease unknown; CR, complete remission

*including 23 patients evaluated only with PET scan (17 CR, 6 not CR) with bone marrow MRD not evaluable

** including 16 patients evaluated only with PET scan (14 CR, 2 not CR) with bone marrow MRD not evaluable

TABLE 3. Summary of pegaspargase-related toxicity

Study parameter	Course 1	Course 2	Course 5	Course 6
Patients	421	382	203	167
Had pegaspargase, n (%)	382/421 (91)	314/382 (82)	177/203 (87)	129/167 (77)
Not administered for previous pegaspargase toxicity, n (%)	NA	47/382 (12)	19/203 (9)	31/167 (19)
Pegaspargase dosing, n (%)				
• Reduced	61/382 (16)	86/314 (27)	50/177 (28)	45/129 (35)
• Full Dose	321/382 (84)	228/314 (73)	127/177 (72)	84/129 (65)
Had pegaspargase-related toxicity grade ≥2, n (%)	189/382 (49)	101/314 (32)	67/177 (38)	39/129 (30)
• Hepatobiliary G≥2/≥3, n (%)	96 (25) / 59 (15)	47 (15) / 15 (5)	36 (20) / 17 (10)	19 (15) / 10 (8)
• Pancreatic G≥2/≥3, n (%)	21 (6) / 11 (3)	1 (0.5) / 1 (0.5)	4 (2) / 3 (2)	0 / 0
• Thrombosis G≥2/≥3, n (%)	7 (2) / 6 (2)	5 (2) / 4 (1)	0 / 0	0 / 0
• Coagulopathy G≥2/≥3, n (%)	81 (21) / 24 (6)	57 (18) / 13 (4)	37 (21) / 10 (6)	22 (17) / 6 (5)
• Metabolic G≥2/≥3, n (%)	13 (3) / 9 (2)	7 (2) / 4 (1)	6 (3) / 4 (2)	2 (2) / 1 (1)

Abbreviations: NA, not applicable

TABLE 4. Comparison between the study results and the GIMEMA LAL1913 clinical trial.

	Campus ALL study (421 patients)	**GIMEMA LAL1913 trial (203 patients)**	**P**
Age (years), median (range)	42.0 (18-80)	39.8 (18-65)	0.5000
≤40, n (%)	199 (47)	103 (51)	0.4773
>40-55, n (%)	125 (30)	61 (30)	0.9867
>55, n (%)	97 (23)	39 (19)	0.3300
Diagnosis, n (%)			
ALL	371 (88)	183 (90)	0.5702
LL	50 (12)	20 (10)	0.5200
B-ALL/T-ALL	**221/200**	**139/64**	**0.0002**
WBC (10^9/l), median (range)	9.9 (0.2-626.9)	7.1 (1.5-347.3)	
≤30 (%)	304 (72)	159 (78)	0.1251
>30-100 (%)	66 (16)	31 (15)	0.9910
>100 (%)	**51 (12)**	**13 (6)**	**0.0395**
CNS involvement, n (%)	37 (9)	19 (9)	0.9559
Risk stratification, n (%)			
Standard risk (SR)	207 (49)	115 (58)	0.0994
High risk (HR)	42 (10)	20 (10)	0.9175
Very high risk (VHR)	171 (41)	68 (33)	0.0951
CR at TP1 (%)	**94**	85	**0.0004**
MRD negativity at TP2 (cases available) (%)	**72**	**80**	**0.0401**
Refractory patients before course 3 (%)	6	3	0.2030
First line HSCT rate (%)	35	28	0.0921
Median follow-up (months)	25	39	
3-year OS (%)	67	67	0.9400
3-year DFS (%)	57	63	0.1700
Pegaspargase toxicity (%)			
Grade ≥2 hepatic toxicity at C1	**25**	**12**	**0.0003**
Grade ≥3 pancreatic toxicity at C1	3	1	0.2592
Grade ≥3 thrombosis at C1	2	2	0.9988

Abbreviations: ALL, acute lymphoblastic leukemia; LL, lymphoblastic lymphoma; WBC, white blood cells; CNS, central nervous system; CR, complete remission; MRD, measurable residual disease; HSCT, allogeneic stem cell transplantation; OS, overall survival; DFS, disease free survival

Figure legends

FIGURE 1. Overall survival (OS) and disease-free survival (DFS). A and B) OS and DFS of the entire population. C and D) OS and DFS stratified for age (≤40, 41-55, >55).

Abbreviations: NR, not reached

FIGURE 2. Disease-free survival (DFS) according to MRD and transplant (HSCT) realization. A) DFS stratified for MRD status. B) Simon-Makuch plot of DFS of VHR and MRD-positive patients according to HSCT

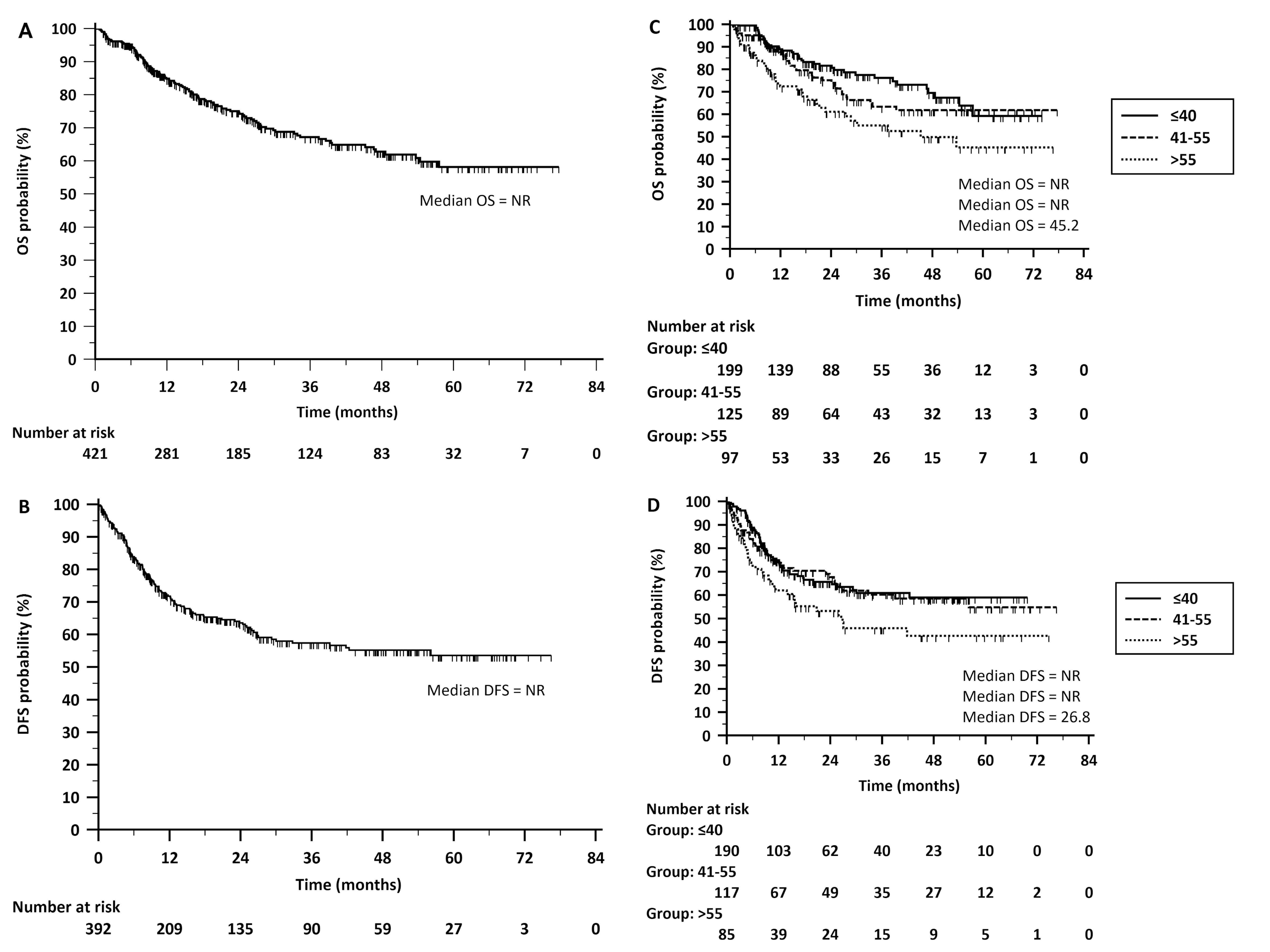
Abbreviations: MRD, measurable residual disease; HSCT, allogeneic stem cell transplantation; VHR, very high risk

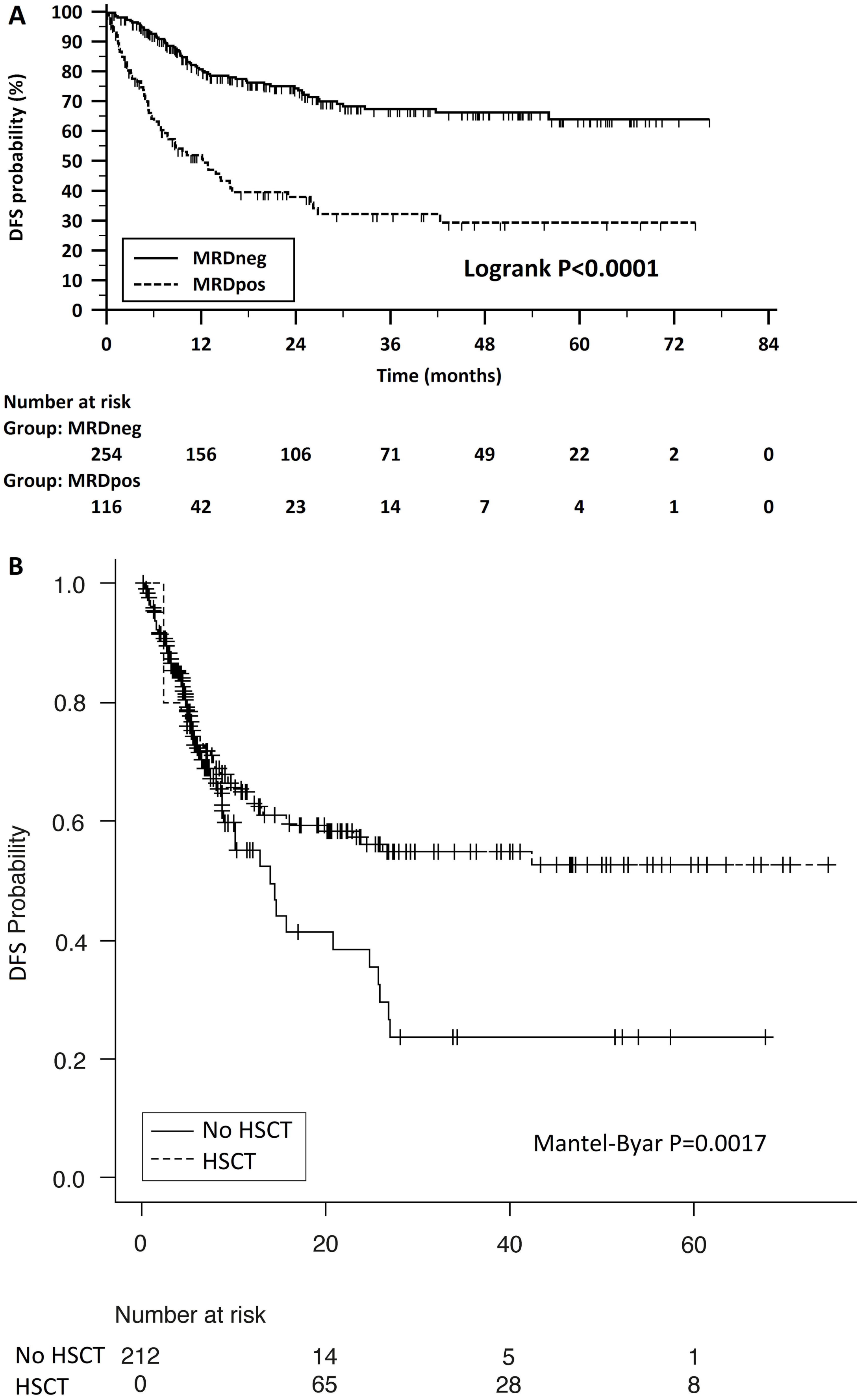
FIGURE 3. Univariate and multivariate analysis. A) Univariate for OS. B) Univariate analysis for DFS. C) Multivariate analysis for OS. D) Multivariate analysis for DFS

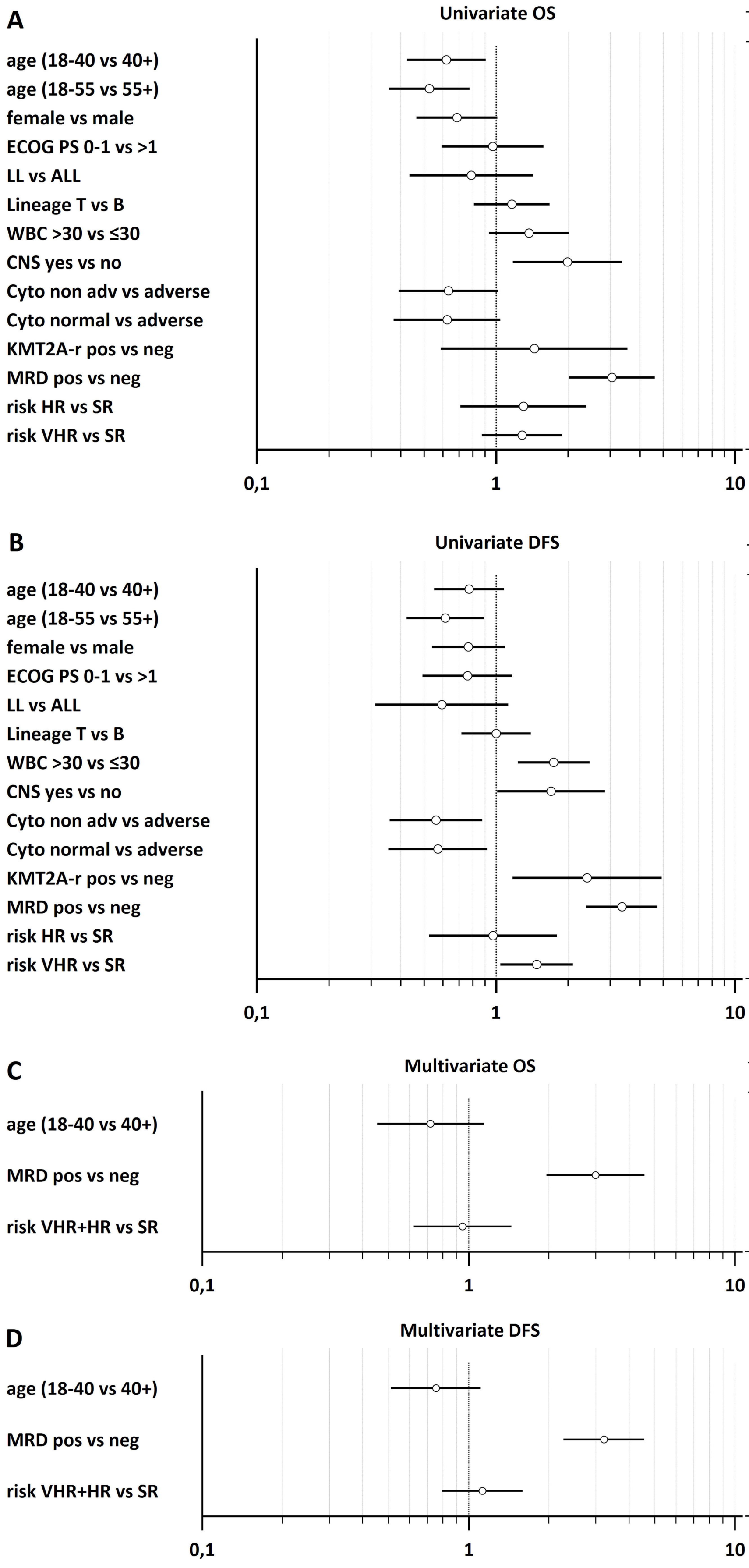
Abbreviations: OS, overall survival; DFS, disease-free survival; ECOG PS, Eastern Cooperative Oncology Group performance status; LL, lymphoblastic lymphoma; ALL, acute lymphoblastic leukemia; WBC, white blood cell; CNS, central nervous system; Cyto, cytogenetics; MRD, measurable residual disease; SR, standard risk; HR, high risk; VHR, very high risk

FIGURE 4. Comparison of overall survival (OS) and disease-free survival (DFS) between the study population (421 cases) and the GIMEMA LAL1913 clinical trial population (203 cases).

Abbreviations: RL-LAL1913, real life population; LAL1913, GIMEMA LAL1913 clinical trial


A
OS probability (%)
Time (months)
Median OS = NR
Number at risk
421 281 185 124 83 32 7 0
B
DFS probability (%)
Time (months)
Median DFS = NR
Number at risk
392 209 135 90 59 27 3 0
C
OS probability (%)
Time (months)
≤40
41-55
>55
Median OS = NR
Median OS = NR
Median OS = 45.2
Number at risk
Group: ≤40
199 139 88 55 36 12 3 0
Group: 41-55
125 89 64 43 32 13 3 0
Group: >55
97 53 33 26 15 7 1 0
D
DFS probability (%)
Time (months)
≤40
41-55
>55
Median DFS = NR
Median DFS = NR
Median DFS = 26.8
Number at risk
Group: ≤40
190 103 62 40 23 10 0 0
Group: 41-55
117 67 49 35 27 12 2 0
Group: >55
85 39 24 15 9 5 1 0


A
DFS probability (%)
100
90
80
70
60
50
40
30
20
10
0
MRDneg
MRDpos
Logrank P<0.0001
0
12
24
36
48
60
72
84
Time (months)
Number at risk
Group: MRDneg
254 156 106 71 49 22 2 0
Group: MRDpos
116 42 23 14 7 4 1 0
B
DFS Probability
1.0
0.8
0.6
0.4
0.2
0.0
No HSCT
HSCT
Mantel-Byar P=0.0017
0
20
40
60
Number at risk
No HSCT 212 14 5 1
HSCT 0 65 28 8



A — Univariate OS

	HR	CI	P
age (18-40 vs 40+)	**0,6205**	**0,4251-0,9056**	**0,0138**
age (18-55 vs 55+)	**0,5260**	**0,3563-0,7766**	**0,0013**
female vs male	0,6863	0,4649-1,0131	0,0594
ECOG PS 0-1 vs >1	0,9677	0,5926-1,5804	0,8963
LL vs ALL	0,7878	0,4345-1,4283	0,4343
Lineage T vs B	1,1647	0,8091-1,6766	0,4145
WBC >30 vs ≤30	1,3746	0,9336-2,0238	0,1087
CNS yes vs no	**1,9909**	**1,1758-3,3711**	**0,0108**
Cyto non adv vs adverse	0,6329	0,3917-1,0228	0,0631
Cyto normal vs adverse	0,6243	0,3733-1,0441	0,0740
KMT2A-r pos vs neg	1,4445	0,5878-3,5500	0,4251
MRD pos vs neg	**3,0551**	**2,0210-4,6182**	**<0,0001**
risk HR vs SR	1,3019	0,7100-2,3871	0,3962
risk VHR vs SR	1,2843	0,8728-1,8898	0,2065

B — Univariate DFS

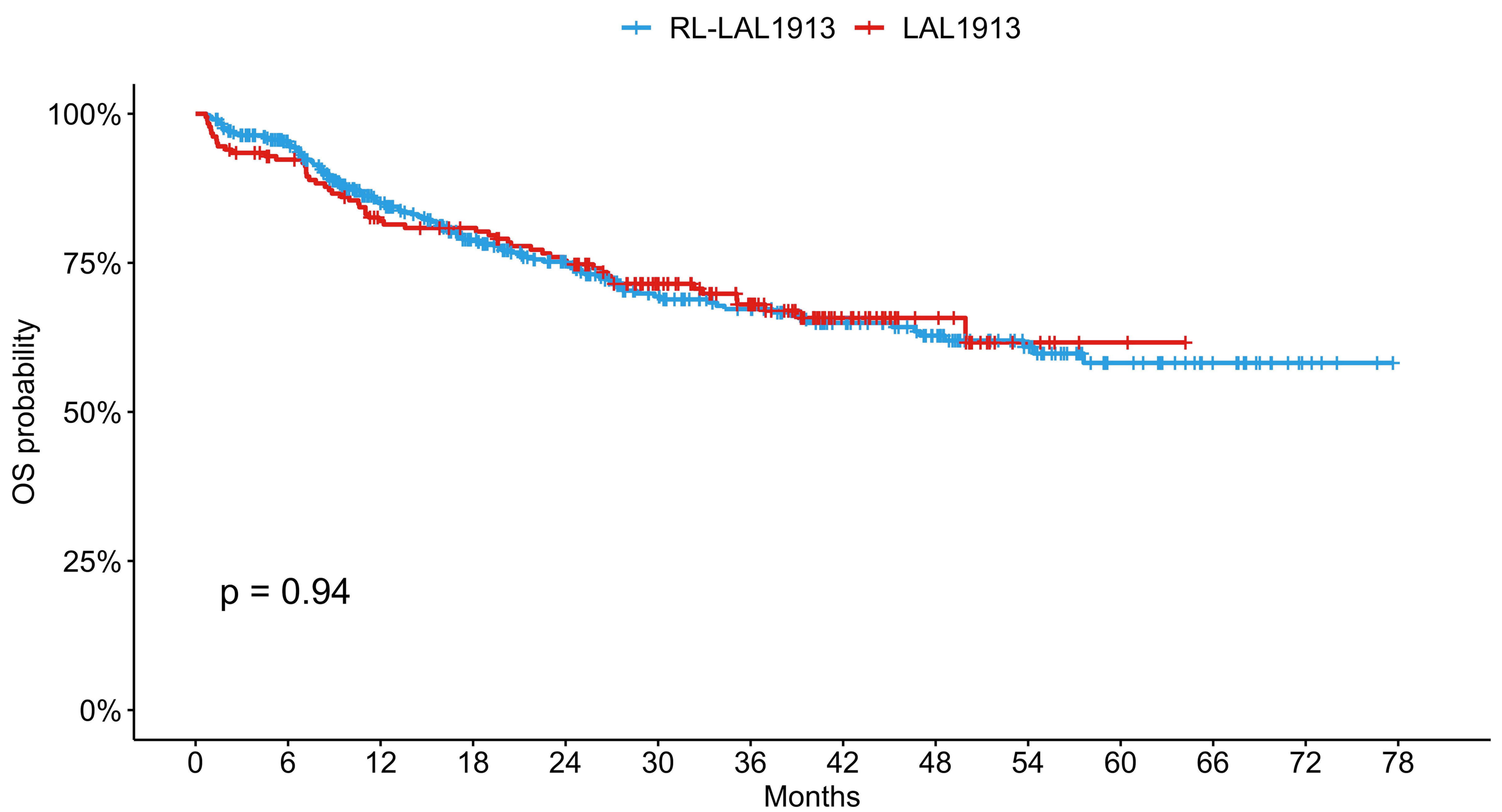
	HR	CI	P
age (18-40 vs 40+)	0,7724	0,5515-1,0819	0,1315
age (18-55 vs 55+)	**0,6133**	**0,4226-0,8899**	**0,0104**
female vs male	0,7662	0,5394-1,0884	0,1390
ECOG PS 0-1 vs >1	0,7593	0,4391-1,1690	0,2134
LL vs ALL	0,5939	0,3129-1,1270	0,1127
Lineage T vs B	1,0009	0,7169-1,3975	0,9958
WBC >30 vs ≤30	**1,7432**	**1,2329-2,4646**	**0,0018**
CNS yes vs no	**1,7000**	**1,0107-2,8596**	**0,0466**
Cyto non adv vs adverse	**0,5610**	**0,3589-0,8769**	**0,0116**
Cyto normal vs adverse	**0,5709**	**0,3549-0,9183**	**0,0215**
KMT2A-r pos vs neg	**2,4052**	**1,1722-4,9353**	**0,0173**
MRD pos vs neg	**3,3633**	**2,3864-4,7000**	**<0,0001**
risk HR vs SR	0,9703	0,5252-1,8001	0,9292
risk VHR vs SR	**1,4804**	**1,0442-2,0988**	**0,0284**

C — Multivariate OS

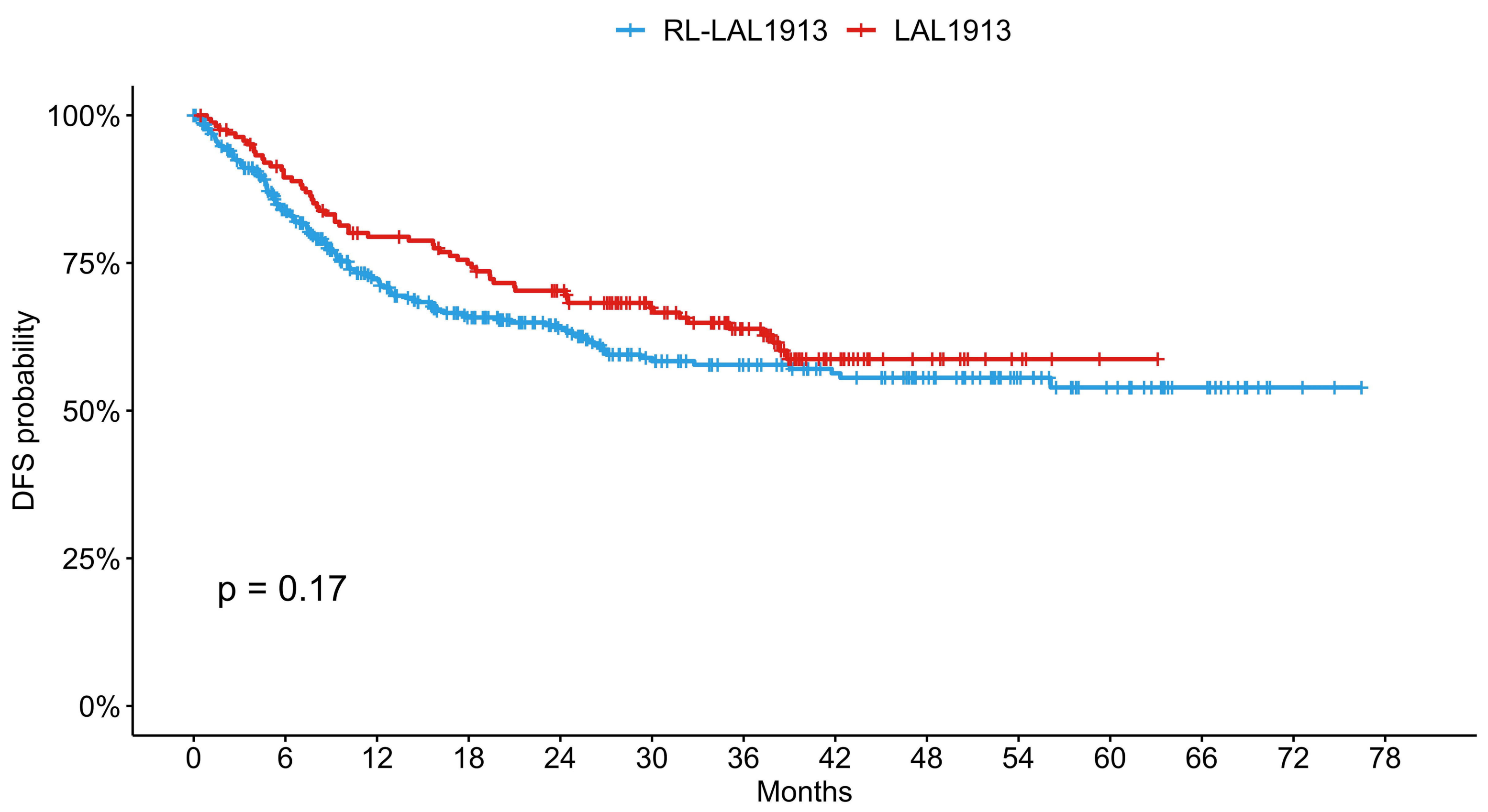
	HR	CI	P
age (18-40 vs 40+)	0,7190	0,4528-1,1417	0,1641
MRD pos vs neg	**2,9925**	**1,9599-4,5693**	**<0,0001**
risk VHR+HR vs SR	0,9486	0,6222-1,4461	0,8071

D — Multivariate DFS

	HR	CI	P
age (18-40 vs 40+)	0,7531	0,5108-1,1103	0,1543
MRD pos vs neg	**3,2157**	**2,2671-4,5612**	**<0,0001**
risk VHR+HR vs SR	1,1246	0,7931-1,5948	0,5119



RL-LAL1913
LAL1913
100%
75%
50%
25%
0%
OS probability
p = 0.94
0
6
12
18
24
30
36
42
48
54
60
66
72
78
Months



RL-LAL1913
LAL1913
100%
75%
50%
25%
0%
DFS probability
p = 0.17
0
6
12
18
24
30
36
42
48
54
60
66
72
78
Months

SUPPLEMENTAL METHODS

Risk classification according to the GIMEMA LAL1913 clinical trial:

- Very high risk (VHR): WBC count >100 x10^9/L or adverse cytogenetics/molecular biology such as t(4;11)/MLL rearrangement at 11q23, +8, -7, del6q, t(8;14), low hypodiploidy with 30-39 chromosomes, near triploidy with 60-78 chromosomes, karyotype with >5 unrelated anomalies, or an early/late non-cortical immunophenotype EGIL T-I/II/IV (CD1a negative) for T-precursor ALL
- High risk (HR): complete remission after the second cycle or, for B precursor ALL, a WBC count >30x10^9/L or a pro-B immunophenotype.
- Standard risk (SR): for B-precursor ALL a WBC count <30x10^9/L, for T-precursor ALL a WBC count <100x10^9/L and a cortical immunophenotype EGIL T-III (CD1a+).

Response evaluation criteria: the CR was defined as the disappearance of clinical and laboratory signs of ALL/LL, including extramedullary disease if previously detected; a transfusion-free status with neutrophils >1.0x10^9/l and platelets >100x10^9/l; and a normocellular or regenerating bone marrow with blast cell content <5%. A recurrence was defined as the reappearance of >5% marrow leukemic cells and/or an extramedullary involvement. In cases of LL without marrow involvement and therefore MRD monitoring, the evaluation of response was carried out with PET scans at TP2 and TP4. Early death was defined as death not due to disease occurring before the third course of CHT.

SUPPLEMENTAL TABLE 1. Treatment protocol.

Treatment phase	Drugs	Dosing	Days
Prephase	Prednisone Cyclophosphamide	20 mg/m^2 q12h 300 (200 if age >55) mg/m^2	-5 to -1 -3 to -1
Course 1 (C1)	Idarubicin Vincristine Dexamethasone Pegaspargase IT prophylaxis	12 (9 if age >55) mg/m^2 1.4 mg/m^2 (max. 2 mg) 5 mg/m^2 q12h 2000 (1000 if age >55) UI/ m^2	1,2 1,8,15,22 1-5, 15-19 10 1,15
Course 2,4,6 (C2, C4, C6)	Vincristine Idarubicin Cyclophosphamide Dexamethasone Cytarabine Pegaspargase Mercaptopurine IT prophylaxis	1.4 mg/m^2 (max. 2 mg) 12 (9 if age >55) mg/m^2 1000 mg/m^2 5 mg/m^2 q12h 75 mg/m^2 2000 (1000 if age >55) UI/ m^2 60 mg/m^2	1,8 (no course 2) 1 1 1-5 2-5 8 (no course 4) 1-10 1 (and 15, course 2)
HD courses 3,7 (C3, C7)	Methotrexate Cytarabine	2500 (B), 5000 (T), 1500 (if age >55) mg/m^2 over 24 hours 2000 mg/m^2	1 3,4
HD course 5 (C5)	Methotrexate Pegaspargase Mercaptopurine	2500 (B), 5000 (T), 1500 (if age >55) mg/m^2 over 24 hours 2000 (1000 if age >55) UI/ m^2 25 mg/m^2	1 3 8-18
Course 8 (C8)	Vincristine Idarubicin Dexamethasone Cyclophosphamide Prednisone IT prophylaxis	1.4 mg/m^2 (max. 2 mg) 10 (7.5 if age >55) mg/m^2 5 mg/m^2 q12h 300 (200 if age >55) mg/m^2 20 mg/m^2 q12h	1,8 1,8 1-5 1-3 8-12 1,15
Maintenance courses M1,3,5,7,9,11	Cyclophosphamide Mercaptopurine Methotrexate IT prophylaxis	100 mg/m^2 75 mg/m^2 15 mg/m^2	1-4 8-28 8,15,22 1 (courses 3,5)
Maintenance courses M2,4,6,8,10,12	Vincristine Prednisone Mercaptopurine Methotrexate IT prophylaxis	1 mg/m^2 (max. 2 mg) 20 mg/m^2 q12h 75 mg/m^2 15 mg/m^2	1 1-5 8-28 8,15,22 1 (courses 2,4)
Maintenance courses M13-24	Mercaptopurine Methotrexate	75 mg/m^2 15 mg/m^2	1-28 1,8,15,22

Abbreviations: IT, intrathecal

SUPPLEMENTAL TABLE 2. Characteristics of the patients in the propensity score matching

Characteristic	**Overall**, N = 602	**RL-LAL1913**, N = 419	**LAL1913**, N = 183	**p-value**
Age, median (range)	42 (18 - 80)	42 (18 - 80)	41 (18 - 65)	0.5
Sex, n (%)				0.6
Male	349 (58%)	246 (59%)	103 (56%)	
Female	253 (42%)	173 (41%)	80 (44%)	
WBC, median (range)	9 (0 - 627)	10 (0 - 627)	6 (0 - 347)	**0.001**
Unknown	1	1	0	
Lineage, n (%)				**<0.001**
B	359 (60%)	221 (53%)	138 (75%)	
T	243 (40%)	198 (47%)	45 (25%)	
Risk, n (%)				0.6
SR	302 (50%)	207 (49%)	95 (52%)	
HR	63 (10%)	42 (10%)	21 (11%)	
VHR	237 (39%)	170 (41%)	67 (37%)	
Cytogenetics, n (%)				**0.026**
Normal	219 (48%)	173 (51%)	46 (39%)	
Non adverse	148 (32%)	108 (32%)	40 (34%)	
Adverse	93 (20%)	60 (18%)	33 (28%)	
Unknown	142	78	64	
CR, n (%)				0.2
CR	556 (92%)	391 (93%)	165 (90%)	
no CR	46 (7.6%)	28 (6.7%)	18 (9.8%)	
Transplant, n (%)	200 (33%)	144 (34%)	56 (31%)	0.4
Time to transplant, median (range)	6.12 (2.93 - 28.88)	6.04 (2.93 - 28.88)	6.41 (3.72 - 12.20)	0.2
Unknown	402	275	127	