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RESEARCH ARTICLE

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Adolescent and young adult acute lymphoblastic leukemia. Final results of the phase II pediatric-like GIMEMA LAL-1308 trial

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Abstract

Adolescents and young adults (AYA) with acute lymphoblastic leukemia (ALL) represent a unique patient population with specific characteristics and needs. Growing evidences suggest that pediatric-inspired approaches improve the outcome in AYA. These results prompted the design of a pediatric AIEOP-BFM ALL 2000-based regimen - the GIMEMA LAL-1308 protocol - for newly diagnosed AYA (range 18-35 years) with Philadelphia negative (Ph-) ALL. The protocol included minimal residual disease (MRD) analysis at two different time-points (TP), that is, at the end of induction IA and consolidation IB, and a modulation in post-consolidation intensity according to MRD. Seventy-six patients were eligible between September 2010 and October 2014. The regimen was well tolerated, with 2.7% induction deaths and no deaths in the post-consolidation phase. The complete response (CR) rate was 92%; the 48-month overall survival (OS) and disease-free survival (DFS) were 60.3% and 60.4%. Both OS and DFS were significantly better in T-ALL than B-ALL. A molecular MRD $<10^{-3}$ at TP1 was associated with a significantly better OS and DFS (77% vs 39% and 71.9% vs 34.4%, respectively);similar results were documented at TP2 (OS and DFS 74.5% vs 30.6% and 71.5% vs 25.7%, respectively). The LAL-1308 results were compared to those from similar historic AYA populations undergoing the two previous GIMEMA LAL-2000 and LAL-0904 protocols. Both OS and DFS improved significantly compared to the two previous protocols. These results indicate that this pediatric-inspired and MRD-oriented protocol is feasible and effective for Ph- AYA ALL patients, and underline the prognostic value of MRD determinations at specific TPs.

Trial registration: clinicaltrials.gov identifier: NCT01156883

1 | INTRODUCTION

In recent years, more intensive approaches have been adopted for the treatment of Ph-negative (Ph-) acute lymphoblastic leukemia (ALL) in adolescents/young adults (AYA), whose outcome remains inferior to that of their younger counterparts.[1-6] Many factors have been shown to contribute to these disparities, the two most important being the difference in therapeutic strategies between pediatric and adult hematologists, and the heterogeneity in ALL biology between children and adults. Although differences in this population clearly exist and the age cut-off of 40 years is somewhat arbitrarily defined, emerging clinical, psychologic and biologic features of the disease suggest that this age group may be a distinct population.[4] In the last decade, the treatment strategies and supportive measures for the treatment of AYA with ALL have been an increasing challenge. Pediatric-inspired regimens have been adopted by multiple national cooperative groups for AYA with various age ranges, up to 55-60 years in some trials. Most studies have reported encouraging results in terms of overall survival (OS) and event-free survival (EFS), and have demonstrated that AYA, and in general patients up to the age of 40, can tolerate therapy similarly to children.[1-6]

Based on these considerations, we designed a pediatric-based regimen at Italian GIMEMA (Gruppo Italiano Malattie EMatologiche dell'Adulto) centers for the treatment of newly diagnosed AYA patients with Ph- ALL (GIMEMA-LAL-1308 protocol). The AIEOP-BFM-(Associazione Italiana di Ematologia e Oncologia Pediatrica; Berlin-Frankfurt-Münster) ALL-2000 regimen, which resulted in a favorable outcome for children with ALL (age 1-18 years),[7,8] was chosen as the backbone for the GIMEMA LAL-1308 trial. The AIEOP-BFM-2000 protocol was a minimal residual disease (MRD)-oriented trial that included MRD analyses at different time-points (TP) at the end of induction and consolidation and modulated post-consolidation intensity according to MRD results.

We hereby report the final analysis of the GIMEMA LAL-1308 protocol and compare the results with those of the two previous adult GIMEMA protocols (LAL-2000 and LAL-0904)[9-11] in a similar historical AYA population.

2 | METHODS

The GIMEMA LAL-1308 protocol was a front-line regimen for patients with Ph- ALL (T-lineage and B-lineage ALL) aged between 18 and 35 years, opened between September 2010 and October 2014. Forty-one Italian GIMEMA centers participated to the study. The eligibility criteria are provided in the supplementary materials.

The protocol was approved by national and local ethical committees prior to patients' enrollment. Informed written consents were obtained according to the Declaration of Helsinki (ICH-GCP) and to each institutional guidelines. The trial was registered with EudraCT number 2009-016075-30.

2.1 | Diagnostic studies

At diagnosis, bone marrow (BM) samples were centrally collected to the Hematology Center in Rome. For all samples, multiparameter flow cytometry (MFC) and molecular screening by multiplex-polymerase chain reactions (PCR) for *ETV6/RUNX1*, *KMT2A/AFF1*, *E2A/PBX1* and *BCR/ABL1* fusion transcripts were performed. For T-ALL, *NUP98/RAP*, *SIL/TAL1* transcripts and *NUP214* rearrangements were also evaluated. Furthermore, 35 of the 55 B-lineage ALL patients, depending on biologic material availability, were retrospectively screened for the "*BCR/ABL1*-like predictor" statistical model, as detailed in supplementary materials.[12]

2.2 | MRD analysis

Minimal residual disease was carried out by real-time quantitative-PCR (RQ-PCR) for immunoglobulin (IG) or T-cell receptor (TR) genes rearrangements and/or MFC (supplementary materials).

The IG/TR screening was performed according to the EuroMRD guidelines[13]; cases with fusion transcripts (N = 6) were not evaluated for IG/TR. So, MFC was based on the analysis of two antibody combinations at 6/8 colors (supplementary materials). Minimal residual disease was measured at two different TPs: day +33 (TP1, end of induction-IA) and day +78 (TP2, end of consolidation-IB). Patients were considered as MRD responders if MRD values were $<10^{-3}$ for the two markers.

2.3 | Risk group definition and final stratification

Patients were stratified into two risk categories according to baseline molecular features (presence or absence of t(4;11)(q21;q23)/*KMT2A/AFF1*), response to steroid pre-phase, response to induction-IA and molecular response at TP2. Patients were defined as HR if they: a) harbored a t(4;11)/)(q21;q23)/*KMT2A/AFF1*; b) were prednisone poor response (PPR) after pre-phase ($\geq 1 \times 10^9$/L peripheral blasts); c) were not in complete remission (CR) at day +33 and d) were MRD-positive ($\geq 10^{-3}$) at TP2. Although the white blood cell (WBC) count at diagnosis is one of the major factors for risk stratification in pediatric and adult patients with ALL, this parameter was not taken into account for risk stratification,[14] in line with the pediatric AIEOP-BFM ALL-2000 trial. The remainder were considered standard risk (SR).

2.4 | Treatment

Treatment consisted of a 7-day prednisone pre-phase and one intrathecal (i.t.) methotrexate (MTX) dose, followed by induction IA (vincristine, daunorubicin, prednisone and asparaginase [ASP], either pegylated [PEG] or native *E. coli*) and consolidation IB (cyclophosphamide [CTX], standard-dose cytarabine [ARA-C], 6-mercaptopurine [6-MP]). Patients with T-cell ALL and pre-phase prednisone-good-

responders (PGR), received dexamethasone (10 mg/m^2/day) from day +8, in the first and third induction week with subsequent dose tapering in 8 days. Asparaginase administration was based on center drug availability; it consisted of the native *E. coli* form at the dosage of 5000 IU/m^2 every other day for eight doses starting from induction day +12 or PEG ASP (1000 IU/m^2), as unique dose at day +12. The majority of patients received the native form, because the pegylated formulation was still not licensed in Italy at the time of protocol activation. *Erwinia* asparaginase replaced the native *E. coli* form in cases of allergic reaction.

Note, SR patients continued with the post-consolidation and the reinduction phase. In detail, these patients received four bimonthly high-dose MTX (HD-MTX) cycles, followed by protocol IIA (vincristine, doxorubicin, dexamethasone, ASP) and IIB (CTX, standard-dose ARA-C, 6-thioguanine). The HR patients received three blocks of non-cross-resistant drugs, including HD-MTX and/or HD-ARA-C, followed by reinduction, as for SR.

For maintenance, daily 6-MP plus weekly MTX were given for a total of 24 months from diagnosis. For central nervous system (CNS)-directed therapy, i.t. MTX was given during each treatment phase. Cranial radiotherapy was administered to HR or to SR patients with T-cell ALL and an initial WBC count >100 $\times$ 10^9/L (1.800 cGy) or with initial CNS involvement (2.400 cGY). Only patients who did not undergo irradiation received i.t. MTX also during maintenance (every 8 weeks).

Table S1 summarizes all drugs and relative doses administered in the different protocol phases.

A brief summary of the GIMEMA LAL-2000 and LAL-0904 trials is reported in supplementary materials.

2.5 | Indications for allogeneic hematopoietic stem cell transplant

Based on the previous experience of the AIEOP-BFM ALL-2000 protocol, the indications for allogeneic hematopoietic stem cell transplant (HSCT) from a matched family donor or an unrelated donor were the following: a) no CR at day +33; b) PPR associated with T or pro-B ALL phenotype and/or a diagnostic WBC count ≥100 $\times$ 10^9/L; c) t(4;11)/*KMT2A/AFF1*; d) MRD ≥10^{-2} at day +33 and e) MRD ≥10^{-3} at day +78. So, HSCT was planned after at least one HR block; preparative regimens, usually including total-body-irradiation, were carried out according to the center strategy.

2.6 | Patients' evaluation and definitions

Definition of CR and BM relapse are detailed in the supplementary materials. Toxicity was evaluated according to CTCAE v5.0.

2.7 | Protocol objectives

The primary objective was to evaluate safety and feasibility of a pediatric-inspired protocol in AYA Ph- ALL patients. Secondary endpoints included: CR rate after consolidation-IB, disease-free survival (DFS) and OS, and induction/consolidation/post-consolidation and reinduction toxicities (grade 3-4). A further aim was to compare the results of this trial with those obtained with the two previous adult ALL GIMEMA protocols (LAL-2000 and LAL-0904) in a similar AYA patient population.

2.8 | Statistical/analytical issues

Both OS and DFS were calculated using the Kaplan-Meier product limit. Further details are given in supplementary materials.

The analysis of deviance for comparison between FCM and RQ-PCR at TP2 was computed using the Cox regression model for DFS.

The adherence to the schedule was evaluated in terms of patients who completed the planned treatment, including HSCT in HR eligible patients.

3 | RESULTS

3.1 | Patients' study group

Seventy-six patients were eligible for the study. Table S2 shows the baseline characteristics of the patients treated with the GIMEMA LAL-1308, compared with those of patients enrolled in the previous GIMEMA LAL-2000 (n = 162) and 0904 (n = 135) protocols, within the same age range. The three cohorts of patients showed similar diagnostic clinical and biologic characteristics: no significant differences were found in gender, median age, initial WBC, platelet count and hemoglobin level; the distribution of patients according to the immunophenotype was similar in the three protocols (T-cell ALL: 27.6%, 32.1% and 31.1%, respectively, in GIMEMA LAL-1308, 2000 and 0904 trial; P = .526); two patients, both enrolled in the GIMEMA LAL-2000, had a biphenotypic leukemia. The incidence of t(4;11)/*KMT2A/AFF1* was 5.5%, 3.8% and 4.5% in LAL-1308, LAL-2000 and LAL-0904 protocols (P = .83). Moreover, in the current trial, two T-ALL patients harbored *SIL/TAL1* and nine with B-lineage ALL (26%) were classified as *BCR/ABL1*-like ALL.

3.2 | Patients' flow, response to induction and feasibility

Prednisone response at day 8 was available in 72 (95%) of the 76 eligible patients; 16 (22%) were PPR and 56 (78%) PGR. At the end of induction IA, one patient went off treatment for a medical decision and 68 (90.7%) achieved a CR; there were two induction deaths (2.7%; one encephalopathy and one for unknown reasons) and five patients (6.7%) were resistant to phase IA. Seventy-three patients proceeded to consolidation IB. Sixty-nine (92%) were in morphologic CR after phase IB, while four (5%) were still resistant. Two cases with available material could be studied retrospectively with the

BCR/ABL1-like predictor and one proved positive. Three patients in CR subsequently withdrew from the study (two due to toxicity, one for protocol violation). At the end of consolidation IB, because of the MRD results (see below), 30 patients were considered HR and 40 SR.

With regard to the 30 HR patients, four did not receive consolidation treatment (one who underwent a HSCT and three for a medical decision). Twenty-six patients received a consolidation block; thereafter, three patients underwent a HSCT and three withdrew from the protocol (one for relapse, one for toxicity and one for refusal). Twenty and 17 patients, respectively, received the second and third block: three patients were allografted after the second and five after the third block; two patients were lost to follow-up. Ten patients received a reinduction, and three of them were subsequently allografted. Finally, seven HR patients carried on with maintenance phase, since they did not fulfill HSCT eligibility criteria.

As for the 40 SR patients, five did not receive HD-MTX (two underwent a HSCT for a medical decision, one was lost to follow-up, one refusal and one toxicity). Of the 35 patients who received HD-MTX, 29 continued with reinduction and maintenance phases, whereas six went off trial due to a medical decision in two, toxicity in one, refusal in one and relapse in two. Overall, a HSCT was carried out in 15 HR and two SR patients (Figure 1).

3.3 | Comparison with previous GIMEMA trials

One of the aims of the trial was to compare the results obtained with this pediatric-based strategy with those achieved in previous GIMEMA trials in a similar AYA population. The CR rate was 84.4% and 83.2%, and the incidence of refractoriness 11.7% and 12%, respectively, in the GIMEMA LAL-2000 and LAL-0904 as opposed to 90.7% and 4%, respectively, in the current study (overall *P* value = .543; LAL-1308 vs LAL-2000 *P* = .262; LAL-1308 vs LAL-0904 *P* = .253) (Table S3).

3.4 | MRD status

The MRD analysis at day +33 (TP1) was available in 64 and 49 patients by MFC and RQ-PCR, respectively; at day +78 (TP2), 66 patients were evaluable by MFC and 50 by RQ-PCR. The dynamics of MRD clearance at days +33 and + 78 are shown in Figure 2. At TP1, 58% of evaluable patients (37/64) were MRD responders ($<10^{-3}$) by MFC; similarly, 57% (28/49) were also MRD responders by RQ-PCR. At TP2, a MRD response ($<10^{-3}$), assessed by MFC and RQ-PCR, was obtained in 82% and 76% of patients, respectively. Thus, according to MRD levels, in the final stratification, 30 (43%) patients were considered as HR and 40 (57%) as SR.

3.5 | Remission duration and survival

Twenty-five patients (10 HR and 15 SR) relapsed after a median time of 16 months (range 0.8-49.1); 21 relapses occurred in the BM and four in the CNS.

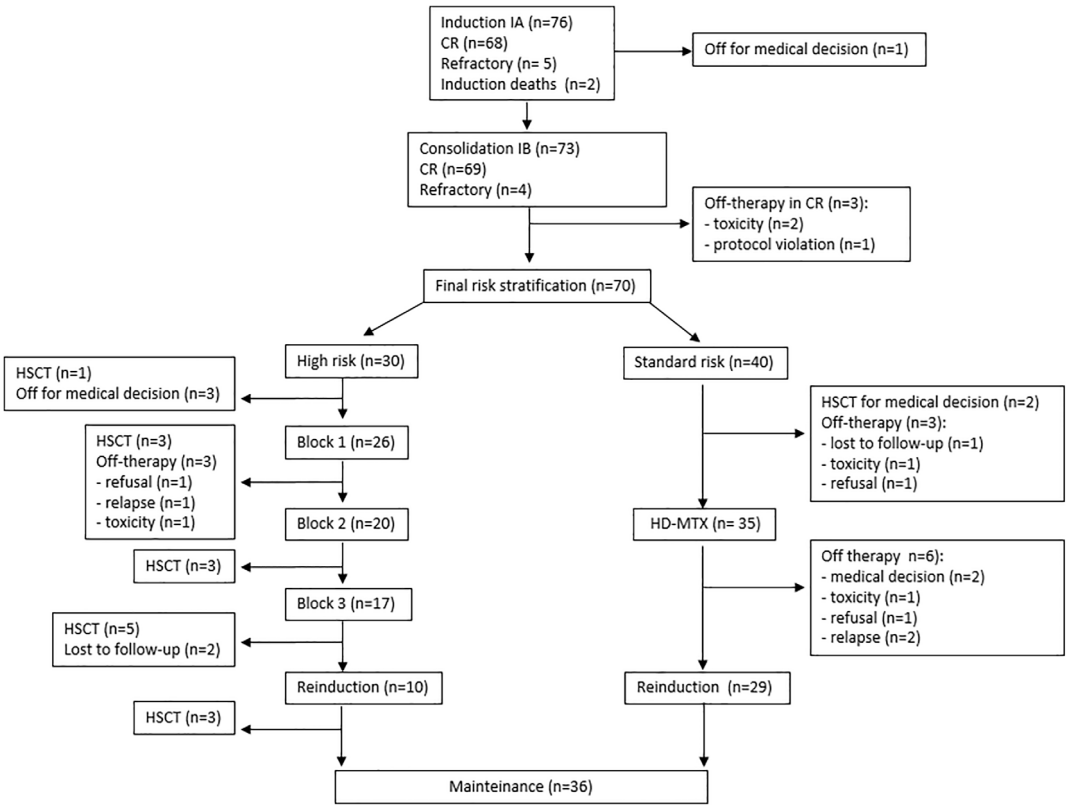

FIGURE 1 Flow chart of patients enrolled in the GIMEMA LAL-1308 protocol

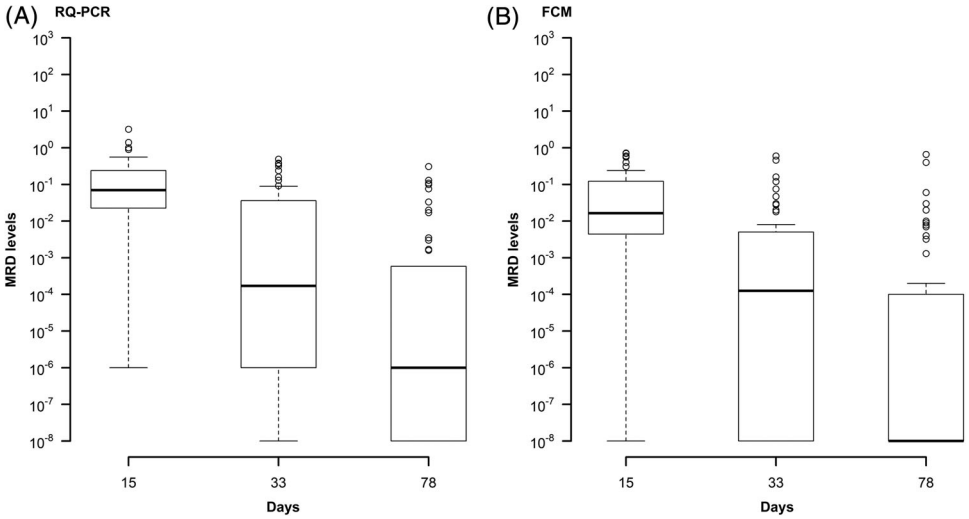

FIGURE 2 GIMEMA-LAL-1308 protocol: dynamics of MRD clearance according to RQ-PCR and FCM at days +15, +33 and + 78

Note, HR relapses comprised eight B-lineage and two T-lineage ALL; among B-lineage ALL, one patient was *KMT2A/AFF1*, four were PPR and three had MRD levels of 10^{-3}, 10^{-2} and 10^{-1}, respectively. Within T-lineage ALL, one was a late responder and the other showed high MRD levels at day +78. Four relapses occurred after HSCT (two B-lineage; two T-lineage).

Regarding SR patients, all relapses occurred in B-lineage ALL; four had MRD levels between 10^{-3} and 10^{-4}.

Finally, of the 20 relapsed patients evaluable for the *BCR/ABL1*-like predictor, five (25%) were classified as *BCR/ABL1*-like. Within this subset, one patient was molecularly MRD positive at TP2, one had MRD levels between 10^{-3} and 10^{-4} and three patients were MRD responders.

The 48-months OS and DFS were 60.3% (95%CI: 49.1-74) and 60.4% (95%CI: 49.4-73.8), respectively, with a median follow-up of 48 months (range 0.8-82.1). Both the OS and DFS were significantly better compared to those of the previous GIMEMA LAL-2000 and LAL-0904 protocols in the similar AYA population (OS 55.6% - 95% CI: 47.1-65.6 and 45.8% - 95%CI: 38-55.3; P = .037; DFS 49.2% - 95%CI: 40.4-60 and 34.1% - 95%CI: 26.3-44.2; P = .002; respectively (Figure 3A-B).

The 48 months-OS between SR and HR patients was statistically different (73.4%, 95%CI: 58.5-92 and 52.6% - 95%CI: 36.8-75.3; P = .032), while no statistical difference was recorded in the DFS between the two groups (66.6% - 95%CI: 52.8-84, and 54.2% - 95% CI: 37.5-78.3; P = .514) (Figure S1), thus indicating that the use of an intensified regimen can partly overcome the unfavorable outcome of HR cases.

When we analyzed the outcome according to the immunophenotype (T-lineage vs B-lineage ALL), T-ALL patients showed a better OS and a statistically improved DFS compared to B-lineage ALL (OS 75.2% - 95% CI: 58.5-96.7 vs 54.2% -95% CI: 41-71.7; P = .27; DFS 88.2% - 95%CI: 74.2-100 vs 50% -95%CI: 37.4-66.9; P = .005). Finally, *BCR/ABL1*-like cases, tended to have an inferior OS compared to non-*BCR/ABL1*-like cases (38.1% - 95% CI: 15.7-92.4 vs 50.7% - 95% CI: 34.2-75.3, data not shown).

3.6 | Outcome by MRD status

Patients with a MRD value $<10^{-3}$ at TP1, evaluated by MFC and RQ-PCR, had a significantly better 48-month OS compared to those with a MRD $\geq 10^{-3}$. More in detail, when MRD was evaluated by MFC, OS was 74.7% (95% CI: 59.3-94) vs 41.5% (95% CI: 25.4-67.7) (P = .002); when evaluated by RQ-PCR, the OS was 77.1% (95% CI: 60.6-98.1) vs 39% (95% CI: 21.3-71.2) (P = .004). Likewise, the MRD status, at the same TP and with the same cut-off, influenced DFS: by MFC 73.5% (95% CI: 60.1-90) for patients with MRD $<10^{-3}$ vs 42.6% (95% CI: 25.8-70.4), for those with MRD $\geq 10^{-3}$ (P = .025), and by RQ-PCR: 71.9%, (95% CI: 56.2-92) vs 34.4% (95% CI: 17.6-67.3 (P = .013) for MRD levels $<10^{-3}$ and $\geq 10^{-3}$, respectively.

Along the same line, MFC-MRD results at TP2 had a prognostic impact on OS - 67.1%, (95% CI: 54.3-83) vs 27.2%, (95%CI: 9.1-81.3, P = .002) for patients $<10^{-3}$ and $\geq 10^{-3}$, respectively - and on DFS that resulted 63.9% (95% CI: 51.9-78.8) for patients with MRD $<10^{-3}$ compared to 29.6% (95% CI: 9.3-94) for those with levels $>10^{-3}$ (P = .04) (Figure 4E-F).

The MRD molecular status at TP2, strongly influenced OS and DFS; with a cut-off of 10^{-3}, positive patients showed a worse OS and DFS compared to those who achieved lower MRD levels: OS 30.6%, (95% CI: 11.9-78.2) vs 74.5%, (95% CI: 61.3-90.4) (P < .001) and DFS 25.7%, (95% CI: 7.8-84.5 vs 71.5% (95% CI: 58-88.1) (P < .001) (Figure 4A-B).

Since at present the established molecular MRD cut-off values are below 10^{-4}, survival analyses were repeated using this cut-off level: indeed, evaluations at TP1 and TP2 by RQ-PCR influenced both OS and DFS. At TP1 25/49 (51%) patients resulted $\geq 10^{-4}$ and 24/49 (49%) $<10^{-4}$; the OS was 77.1% (95% CI: 58.8-100) vs 46.1% (95% CI: 29.1-72.9), for cases below and above the 10^{-4}, respectively, and DFS was 76.1% (95% CI: 59.8-96.8) vs 37.7% (95% CI: 21.6-65.8). At TP2, 20/50 (40%) patients resulted $\geq 10^{-4}$ and 30/50 (60%) $< 10^{-4}$; the OS and DFS were 78.1% (95% CI: 64-95.3) vs 29.3% (95% CI: 13.5-63.6) and 70.2% (95% CI: 54.5-81.4) vs 23.9% (95% CI: 9.6-59.8)

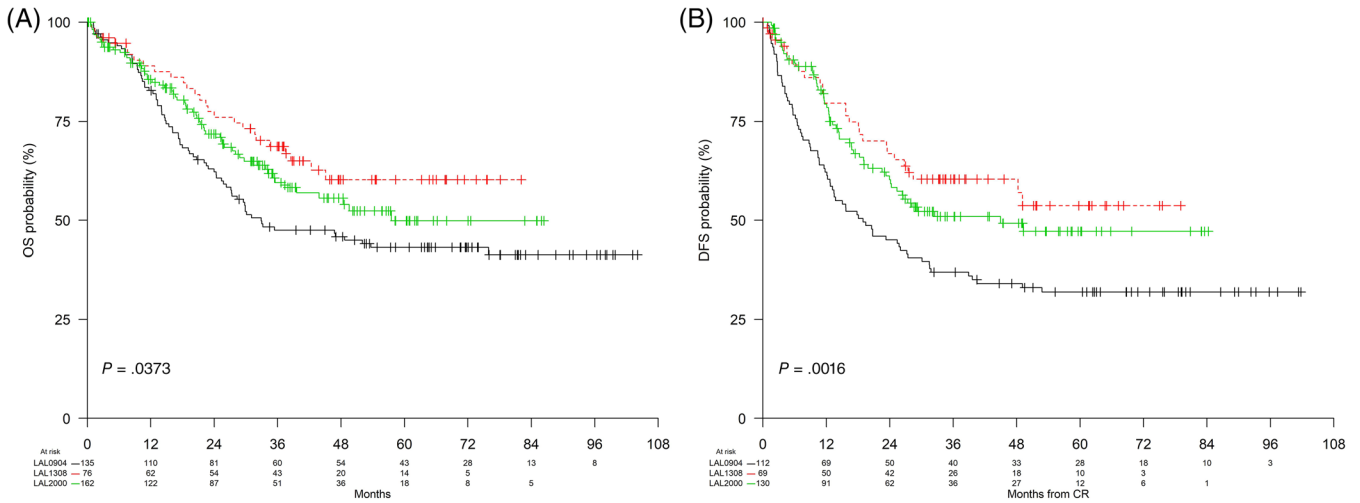

FIGURE 3 Overall survival and disease-free survival in the GIMEMA LAL-1308, LAL-0904 and LAL-2000 protocols [Color figure can be viewed at wileyonlinelibrary.com]

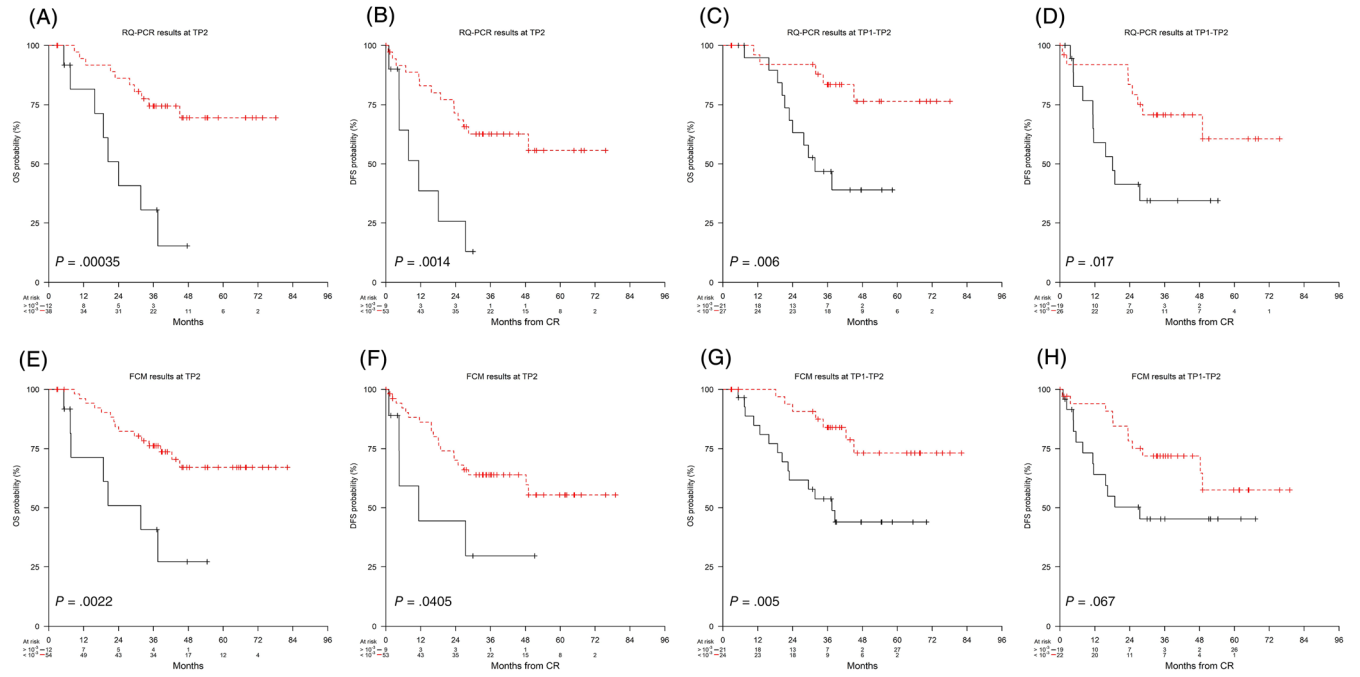

FIGURE 4 GIMEMA-LAL-1308 protocol: (A-B) 48-months overall survival and disease-free survival according to RQ-PCR and (E,F) FCM for all patients; (C,D) 48-months overall survival and disease-free survival for subgroup negative at TP1 and TP2 by RQ-PCR and (G,H) FCM [Color figure can be viewed at wileyonlinelibrary.com]

respectively, for patients with MRD level < 10^{-4} or ≥ 10^{-4} (P = .02 and P < .01, respectively).

Data at TP1 and, more importantly, at TP2 clearly confirm that MRD is the most important prognostic factor. Moreover, the comparison between MFC and RQ-PCR, carried out by analysis of deviance, confirms a better discriminative power of RQ-PCR over MFC (P value <.001).

To further investigate the role of MRD, we conducted a subanalysis on OS and DFS subdividing patients as follows: those who were MRD at both TP1 and TP2 "persistently negative", using two cut-offs, that is, <10^{-3} and < 10^{-4} by FCM and RT-PCR and those who were either positive at both time points or at one of them. Considering the cut-off of 10^{-3}, 35 patients proved negative by FCM. The 48-month OS was 73.1% vs 44% for persistently negative vs 44% for the other patients (Figure 4G, P = .005). Similarly, 27 patients had a RQ-PCR value <10^{-3}; the 48-month OS were 76.5% vs 39% (Figure 4C, P = .006). Along the same line, using the cut-off 10^{-4}, 30 patients were negative by FCM and 27 by RQ-PCR. The 48-month OS were better for cases with value below 10^{-4} compared with cases above in both methodology (51.6% vs 69.5% for FCM and 83.2% vs 44.3% for RQ-PCR) (data not shown). The DFS results were superimposable to those documented in OS (Figure 4D-H).

3.7 | Univariate and multivariate analysis

Finally, we performed an univariate analysis on DFS and OS. Univariate Cox regression analysis considering the major risk variables (age, WBCs, hemoglobin, platelets, phenotype, prednisone response and risk class and MRD value $<10^{-3}$ at TP1 and TP2 by MFC and RQ-PCR), identified as risk factors for DFS MRD at TP1 and TP2 by both MFC and RQ-PCR and cell lineage (B-lineage and T-lineage), whereas univariate analysis for OS revealed as risk factors the MRD values at TP1 and TP2 by both MFC and RQ-PCR and risk stratification (Tables S4A and S5A).

In multivariate analysis, the only parameter that retained statistical significance in both OS and DFS was MRD by RQ-PCR at TP2 (Tables S4B and S5B).

3.8 | Safety and feasibility

Overall, 70% of patients maintained the adherence to the protocol scheme with high tolerability and without excessive toxicity.

A full assessment of toxicity was carried out for all treatment phases (Table S6). Over 90% of patients developed a grade 4 neutropenia during induction therapy, partly sustained by the leukemia itself; sepsis and other infections (grade ≥ 3) occurred in 10 patients (13%) during the neutropenic phase. Grade 3-4 ASP-related adverse events during induction were: elevated alanine aminotransferase levels in five patients, acute pancreatitis in two, fatal encephalopathy in one and deep vein thrombosis in three. Transient neurotoxicity was observed in two patients. Throughout phase IB, 10 infections (grade 3), one pleural effusion and one hepatotoxicity were reported; in two cases, infections led to a drop out from the study. The SR post-consolidation phase was associated with gastrointestinal disorders (grade 3) in three patients, methotrexate-related vasculitis in one and an increase in serum creatinine levels (grade 3) in three patients, requiring a prolongation in the folic acid rescue. Adverse events such as neutropenia, thrombocytopenia, febrile neutropenia (38%), hepatic toxicity (8%), occurred frequently during post-consolidation blocks, although no patient died due to adverse events. One patient presented hepatotoxicity (grade 3) in the first block; four patients developed MTX-related kidney injury, during the second course, which completely resolved. Severe mucositis requiring parenteral nutrition, were reported in two patients during HD-MTX blocks. Infections were observed in 31% of patients; in five cases severe febrile neutropenia required prolonged antibiotic treatment.

An avascular bone necrosis was confirmed in one patient and one presented with hip pain without X-ray abnormalities. Bone symptoms occurred after maintenance discontinuation.

4 | DISCUSSION

The application of pediatric-based treatment regimens has enabled improvement of the outcome of AYA patients with ALL.[1-6] The present GIMEMA-LAL-1308 study is the first Italian multicenter experience with a pediatric-based protocol designed and applied specifically to Ph- ALL patients aged between 18 and 35 years. As mentioned above, the model of inspiration was the AIEOP-BFM LAL-2000 pediatric protocol, both for the chemotherapy schema and MRD risk stratification. The GIMEMA LAL-1308 differs from the previous GIMEMA LAL 2000 and 0904 protocols: indeed, the latter did not contemplate the pediatric strategy such as the higher cumulative doses of immunosuppressive drugs (asparaginase, vincristine and steroids), the lower exposure to myelotoxic dugs (anthracycline and etoposide) and the greater use of intrathecal prophylaxis. In addition, other differences with adult regimens were the limitation of HSCT in the first-line strategy and the absence of the reinduction phase that was pioneered by the BFM group and confirmed, in a large phase 3 randomized trial, to be a critical component of ALL therapy.[15] More importantly, the GIMEMA LAL-2000 and 0904 did not consider patients' stratification according to the MRD levels, whereas the AIEOP-BFM LAL-2000 was the first pediatric protocol to demonstrate in a large cohort of patients the prognostic value of MRD at specific TPs for stratification and intensive adapted therapy.[7] In line with this, in the GIMEMA LAL-1308 trial, the evaluation of MRD retained the prognostic value on outcome and was crucial for treatment decisions.

Since one of the major issues related to the application of a pediatric strategy to AYA and adults is the compliance to treatment and drug-related toxicity, the primary endpoint of the GIMEMA LAL-1308 was to evaluate these aspects. Our results show that 70% of patients maintained the adherence to the protocol scheme with high tolerability and without excessive toxicity, in line with other similar studies[16-18] A higher rate of CR, a lower induction death rate and a significantly better OS and DFS compared to the results achieved in the same age group with the previous GIMEMA trials were observed.

Despite some differences in the age ranges and follow-up duration, our results are comparable with those achieved by other multicenter adult ALL studies that adopted pediatric trials in AYA. The Spanish Programa Espanol de Tratamientos en Hematologia was the first group to report the outcome of 81 patients (15-30 years) with Ph- ALL treated with the pediatric ALL-96 study.[19] In this protocol, only SR patients were included; the 6-year EFS and OS were 61% and 69%. The same study group, more recently, published the results of the ALLRE08 PETHEMA trial that confirmed the superiority of the pediatric regimen on the AYA population with Ph-negative SR ALL, with a better OS and EFS in adolescents compared with young adults.[17] The largest pediatric regimen-based experience, to date, is that of the German Multicenter Group for Adult ALL (GMALL) on 1529 patients aged 15-35 years. The 5-year OS rates were 73%, 69% and 60% for patients aged 15 to 17 years, 18 to 25 years and 26 to 35 years, respectively.[20] The pediatric NOPHO ALL 2008 (Nordic Society of Pediatric Hematology Oncology) protocol was successfully administered to all ALL patients from age 1 to 45 years. Despite the increased risk of acute toxicities in the age group between 18 and 45 years, the treatment was feasible

with a 5-year EFS of 74%.[21] Finally, the CALGB recently reported the results of their 10 403 trial, in which the doses and schedule were identical to the COG AALL0232-HR-protocol; 295 AYA patients (16-39 years) were treated and the estimated 3-year EFS and OS were 59% and 73%, respectively.[18] However, not all investigators have reached the same conclusions with regard to the superiority of the pediatric-inspired regimens for AYA ALL. The MD Anderson Group observed no differences in the 5-year OS using a BFM regimen compared retrospectively with the results obtained with the hyper-CVAD and augmented hyper-CVAD (60% OS rates in the two groups).[22] However, CD20-positive patients in the comparator hyper-CVAD group received either rituximab or ofatumumab. This might explain the higher than expected 60% OS in the hyper-CVAD treatment group.

Our pediatric-based protocol was a MRD-oriented trial. Early MRD assessment has now a clear role in determining which patients benefit from specific adjustments in first-line treatment. In our study, molecular MRD at day +78 was strongly predictive of the disease outcome; with a 10^{-3} cut-off, both OS and DFS were statistically different for patients with molecular response compared to those with a persistent MRD positivity: OS 74.5.1%, vs 30.6%, DFS 71.5% vs 25.7 ($P < .001$). Even stronger results were observed with the lower molecular MRD cut-off at 10^{-4}; this value is now accepted as the optimal cut-off, but since this trial was inspired by a pediatric regimen,[7] the same cut-off point at 10^{-3} was maintained at the time of the design of the study. Indeed, OS and DFS were 78.1% (95% IC 64-95.3) vs 29.3% (95% IC 13.5-63.6) and 70.2% (95% IC 54.5-81.4) vs 23.9% (95% IC 9.6-59.8), respectively, for patients with MRD levels at day +78 $< 10^{-4}$ or $\geq 10^{-4}$. These results are in line with two reports from the GRAAL group, which showed that - in the context of a trial based on transplant allocation - a MRD negativity, at both the 10^{-3} and 10^{-4} cut-off points, is predictive of outcome.[23,24]

In the present study, we also performed MFC-MRD, mostly because MFC is less time consuming and can be applied to all cases, while ASO-primer RQ has a failure rate of approximately 10%. Overall, also MFC was strongly predictive of outcome, but with a lower prediction rate, indicating that MFC should be reserved only to cases lacking a molecular marker.

For patients who do not achieve an early MRD clearance, intensified therapy alone does not appear to confer an improvement in prognosis if MRD persists upon consolidation completion. In the study by Borowitz et al.,[25] pediatric and AYA patients undergoing the COG AALL0232 protocol were assessed for MRD by MFC at the end of induction and consolidation and were assigned to receive more intensive therapy, if their MRD was >0.1% at the end of induction. Patients with a MRD positivity persisting at the end of consolidation fared worse (39% EFS), in spite of intensified treatment.

In our study, the intensified consolidation with HD chemotherapy resulted in a better OS and DFS in T-lineage compared to B-lineage ALL (OS T-ALL 75.2% vs B-ALL 54.2%; DFS: T-ALL 88.2% vs B-ALL 50%), confirming that treatment intensification is pivotal for improving outcome in T-ALL, and is capable of overcoming the historically poor outcome of this subset, also in line with previous results.[26] Within B-lineage ALL, an increased incidence of *BCR/ABL1*-like ALL (30%), a subset with a gene expression profile similar to that of Ph + ALL and a diverse range of genetic alterations that can activate kinase signaling, has been reported in the AYAs population. In the CALGB 1043 study, 31% of the evaluable patients proved Ph-like and these patients had a significantly worse outcome with an estimated 3-year EFS of only 42%.[18] Non-randomized pediatric/adolescent programs are evaluating the feasibility of defining *BCR/ABL1*-like ALL in real-time in order to add a TKI or other inhibitors to chemotherapy.[27] In our study, a proportion of B-lineage ALL cases with available diagnostic material could be evaluated retrospectively using the recently described *BCR/ABL1*-like predictor.[12] Nine patients were predicted as *BCR/ABL1*-like ALL: while the numbers are too small to draw any definitive conclusion, it is important to underline that six of them (67%) were either refractory or relapsed.

In conclusion, in our experience, a pediatric-based and MRD-oriented treatment proved feasible and was associated with encouraging long-term results in AYA patients with Ph- ALL. The standardized assessment of MRD at specific TPs had a strong prognostic impact. Note, MRD should be monitored in the management of AYA ALL patients and used in clinical trials, not only to risk stratify patients, but also to determine which patients could be candidate to newer treatment strategies. Importantly, the identification of *BCR/ABL1*-like cases should be carried out as soon as possible, since it might further help in refining patients' stratification and may prompt the use of targeted therapies. Finally, the possibility of increasing therapeutic efficacy in B-lineage ALL by combining newer drugs (immunotherapy) in the front-line chemotherapy needs to be evaluated particularly in AYA patients. Indeed, in a recent study, blinatumomab administered to ALL patients with high MRD in CR1 or CR2 was associated to a 91% MRD negativity rate in 32 AYA patients (18-34 years) after only one cycle.[28] The ongoing GIMEMA LAL 2317 protocol for Ph- B-lineage ALL patients that incorporates blinatumomab in the front-line treatment will conclusively clarify this aspect.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

A.M.T. designed the study, enrolled patients analyzed data and wrote the manuscript, M.C. analyzed data and wrote the manuscript, A.V. enrolled patients and collected clinical samples, A.P. analyzed the data A.G., I.D.S., M.C., D.P.M.S., L.E. performed diagnostic studies and MRD evaluation. M.M. performed translational studies, M.L.M., B.M., M.L., M.D.A., A.C., enrolled patients. V.C. participated in the study design and revised the paper. P.F. and M.V. carried out regulatory activity S.C. enrolled patients and wrote the manuscript, R.F. designed the study, critically revised the paper and approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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