Imatinib treatment of paediatric Philadelphia chromosome-positive acute lymphoblastic leukaemia (EsPhALL2010)

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Abstract: Background. EsPhALL2004 study showed a 10% advantage in disease-free survival for short, discontinuous use of imatinib after induction in Philadelphia-chromosome-positive acute lymphoblastic leukaemia (ALL) Ph+ALL patients receiving BFM therapy and HSCT. Other contemporary studies showed an advantage from continuous protracted exposure to imatinib challenging the indications to transplant. The EsPhALL2010 study was designed to assess if imatinib given from day 15 of induction and continuously throughout chemotherapeutic treatment allowed to maintain or improve the outcome obtained in EsPhALL2004 despite decreasing the number of patients undergoing HSCT.

Methods. Patients aged 1-17 years recruited into national frontline trials for acute lymphoblastic leukaemia and with t(9;22)(q34;q11) were eligible to the trial. Patients with abnormal renal or hepatic function, or an active systemic infection, were ineligible. Patients received imatinib 300/mg/m² continuously from day 15 of induction, during chemotherapy. Minimal residual disease (MRD) was measured by IG/TR. Eligibility to HSCT depended on early morphological response and MRD. Imatinib was recommended throughout the first year after transplant. The co-primary endpoints were event-free survival and overall survival. Analyses included all patients enrolled (intention-to-treat population). The trial is registered with the European Clinical Trials Database (EudraCT 2004-001647-30) and with ClinicalTrials.gov, number NCT00287105 and is completed.

Findings. 155 evaluable patients were registered between Jan 1, 2010 and Dec 31, 2014. 151 (97%) achieved CR1 after induction and 4 after consolidation phase IB. 59 patients (38%) underwent HSCT in CR1. The 5-year event-free survival and overall survival were 57.0 (SE 4.1) and 71.8 (SE 3.8), respectively. 14 (14.6%) deaths occurred in CCR in 96 patients treated with chemotherapy only (mainly during HR blocks and the subsequent DI), and 11 (18.6%) in 59 HSCT patients. The 5-year cumulative incidence of relapse was 26.9% (SE 3.7, 8 relapses in 59 transplanted patients and 32 relapses in 96 patients who received chemotherapy). In patients MRD negative at end of IB, there were no relapses in those transplanted (N=17) and 9 relapses in those (N=30) treated with chemotherapy. In patients with positive MRD at end of IB, 5 relapses each occurred in 15 transplanted and 12 not transplanted patients. WBC count at diagnosis $\geq 100 \times 10^9/L$ was associated with a poor prognosis (HR 3.47, 95% CI 1.74-6.89, $p=0.0004$). Serious adverse events (N=154) were reported in 80 (51.6%) of 155 patients. The primary toxicity was infection (61 [39.4%] patients of 155, mostly bacterial); gastrointestinal disorders occurred in 10 (6.5%) patients and osteonecrosis in 8 (5.2%). Serious adverse events occurred mainly during HR Blocks and DIs, including 14 fatal events (in phase IB [N=1], HR Blocks [N=6], first DI [N=6] and second DI [N=1]).

Interpretation. Imatinib given since day 15 of induction markedly improved CR rates to 97% from 78% in EsPhALL2004. Though HSCT rates were significantly lower (38% versus 81%), outcomes were similar in the two studies: 5-year EFS and survival were 57.0% and 71.8% versus 60.3% and 71.6%, respectively. Our data suggest that imatinib given early and continuously with intensive chemotherapy may be associated with very severe toxicity. WBC $\geq 100 \times 10^9/L$ was the most relevant independent prognostic factor.

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Treatment of Pediatric Philadelphia Positive Acute Lymphoblastic Leukemia with imatinib: an Intergroup, Open-label, Single-Arm Clinical Trial (EsPhALL2010)

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Research in context

Evidence before this study.

We searched PubMed for journal articles to July 20, 2018 without date limitations and with the terms “Ph+ALL” and “children” and “TKI or imatinib”. We found 32 publications in childhood Ph+ALL, but many of these were case reports or based on retrospective small-scale series. We therefore considered three relevant contemporary studies which evaluated with different strategies imatinib combined with front-line chemotherapy and HSCT. In the COG AALL0031 study, continuous imatinib exposure (340 mg/m²/day) improved outcome in cohort 5 patients with a 3-year event-free survival (EFS) of 80% markedly better than historical controls treated without TKI (35%), with no significant toxicities. Three-year EFS was similar for patients in cohort 5 treated with chemotherapy plus imatinib (88%) or sibling donor HSCT (57%). These results were confirmed by a successive report with longer-term follow-up. The Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) reported on the Ph+ALL04 study in which patients received 2 weeks of imatinib (340 mg/m²/day) monotherapy phase between the 3-phase high risk chemotherapy and HSCT. All patients were eligible to HSCT in CR1 and the 4-year event-free survival was 78%. The randomized EsPhALL2004 study showed a 4-year disease-free survival of 73% for patients exposed to short, discontinuous use of imatinib (300 mg/m²/day) after induction and receiving BFM high risk chemotherapy and HSCT, with 10% advantage with respect to outcome of patients who did not receive imatinib. Imatinib was well tolerated. These results suggest that a long and continuous use of imatinib concurrently with chemotherapy may challenge the indications to transplant.

Added value of this study.

This prospective trial introduces the early exposure to continuous imatinib administration (since day 15 of induction) in a protocol with BFM chemotherapy backbone and transplantation. This study allows to evaluate the impact on early response and event-free survival and the role of MRD and transplant in this context. Another relevant aspect is to assess the associated toxicity in comparison with the previous experience of a discontinuous imatinib administration. This study conducted by a large consortium allows to recruit a considerable number of patients in a very rare subgroup of pediatric ALL.

Implications of all the available evidence.

The early exposure to imatinib (since day 15 of IA) markedly improved the response to induction therapy. Continuous, protracted exposure to imatinib clearly delayed the time of relapses in Ph+ALL, yet a plateau in EFS for untransplanted patients was not achieved. The risk of relapse was very low in patients with early MRD negativity who underwent HSCT. High WBC count was found to be the most important prognostic factor either in patients treated with chemotherapy only or transplant. Imatinib given early and continuously with intensive chemotherapy was associated with very severe toxicity. Toxicity observed in this study justifies further investigations on the efficacy of less intensive chemotherapy associated with early and protracted exposure to imatinib, on the role of transplant and on immunological innovative approaches for patients at higher risk of relapse.

Abstract

Background. EsPhALL2004 study showed a 10% advantage in disease-free survival for short, discontinuous use of imatinib after induction in Philadelphia-chromosome-positive acute lymphoblastic leukaemia (ALL) patients receiving BFM therapy and hematopoietic stem cell transplantation (HSCT). Other contemporary studies showed an advantage from continuous protracted exposure to imatinib challenging the indications to transplant. The EsPhALL2010 study was designed to assess if imatinib given from day 15 of induction and continuously throughout chemotherapeutic treatment allowed to maintain or improve the outcome obtained in EsPhALL2004 despite decreasing the number of patients undergoing HSCT.

Methods. Patients aged 1-17 years recruited into national frontline trials for acute lymphoblastic leukaemia and with t(9;22)(q34;q11) were eligible to the trial. Patients with abnormal renal or hepatic function, or an active systemic infection, were ineligible. Patients received imatinib 300/mg/m² continuously from day 15 of induction, during chemotherapy. Minimal residual disease (MRD) was measured by IG/TR. Eligibility to HSCT depended on early morphological response and MRD. Imatinib was recommended throughout the first year after transplant. The co-primary endpoints were event-free survival and overall survival. Analyses included all patients enrolled (intention-to-treat population). The trial is registered with the European Clinical Trials Database (EudraCT 2004-001647-30) and with ClinicalTrials.gov, number NCT00287105 and is completed.

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Introduction

Approximately 3-5% of pediatric acute lymphoblastic leukemia (ALL) patients present with the t(9;22)(q34;q11.2) translocation or Philadelphia chromosome (Ph+). Historically, Ph+ ALL was associated with a poor prognosis, with long-term event-free-survival (EFS) rates of ~30%.¹⁻⁸ Hematopoietic stem cell transplantation (HSCT) was associated with a superior EFS compared with chemo alone (44% vs. 34%, $p=0.05$) in the pre-tyrosine kinase inhibitor (TKI) era.^{3,4} There is now a general consensus that Ph+ ALL patients with a favorable early response to the combination of chemotherapy and TKI can be spared HSCT in first complete remission (CR). The COG AALL0031 study tested the combination of imatinib (beginning after Induction) along with an intensive chemotherapy backbone; patients with an HLA-matched sibling were allocated to HSCT in first CR. The 3-year EFS rate for all 44 Ph+ ALL patients who received imatinib continuously (340 mg/m²/day), irrespective of HSCT, was 80% (SE 11%), markedly better than historical controls treated without TKI (35% [4%], $p<0.0001$).⁹ The 5-year disease-free-survival (DFS) of 28 untransplanted patients was 70% (SE 12%), similar to that of patients treated with HSCT, either from a sibling donor ($n=21$, 5-year DFS 65% [SE 11%]) or from an unrelated donor ($n=13$, 5-year DFS 59% [SE 15%], $p=0.77$).¹⁰

The following COG study AALL0622 evaluated dasatinib on a chemotherapy backbone similar to that of AALL0031 with HSCT recommended for high risk patients and for any standard risk patient with a matched family donor. No relevant difference in results was observed, but a negative prognostic role was shown for IKZF1 deletions.¹¹

Contemporarily, the European intergroup study of Ph+ ALL conducted from 2004 to 2009 (EsPhALL2004) investigated the post-induction treatment with imatinib. Patients were risk-stratified on the basis of early response to therapy and Good Risk (GR) patients were randomized to the use of imatinib while poor Risk (PR) subjects were all given imatinib. The BFM ALL high risk (HR) chemotherapy backbone was used as post induction therapy and imatinib discontinued at maintenance or at HSCT. HSCT was recommended for GR patients with a matched related or unrelated donor, and for PR patients from any donor. Overall, 80% of patients received an allogeneic HSCT in CR1. In the analysis by intention-to-treat, imatinib was associated with a trend toward better 4-year DFS in GR patients (73% vs. 62%, $p=0.24$); the as-treated analysis showed a 4-year DFS of 75% for GR patients receiving imatinib and 56% for those who did not ($p=0.06$).¹²

In 2010, the EsPhALL trial was amended to give imatinib continuously from day 15 of induction and to decrease the number of patients undergoing HSCT, which was limited to poor MRD responders from 2012. The results of the EsPhALL2010 protocol are here reported and compared with those of EsPhALL2004.

Methods

Study design and participants

EsPhALL2010 is a prospective, single arm trial derived from an amendment of the EsPhALL2004 protocol which was approved by the ethics committee of each participating institution in ten national study groups (AIEOP, BFM-G/CH, COALL, French ALL-group, NOPHO, MRC, DCOG, CPH, PINDA, and HKPHOSG). Eligible patients were children aged 1-17 years, recruited into national frontline trials for ALL, with t(9;22)(q34;q11) detected by conventional cytogenetics and/or BCR/ABL1 fusion transcript identified by reverse transcriptase (RT) PCR or by fluorescence *in-situ* hybridization (FISH). Patients with abnormal renal or hepatic function (grade ≥ 2 toxic effects according to the National Cancer Institute Common Toxicity Criteria [NCI-CTC] Version 2.0) or active systemic infection at day 15 of induction treatment were ineligible. Written, informed consent was obtained from parents or legal guardians.

Procedures

All patients received the Induction phase according to national/group study treatment protocol. The definition of risk subgroups was based on both early response to treatment and achievement of CR (cytology) at the end of induction, with GR patients being those who achieved both. Early response was defined as blast cells count $<1,000$ cells/ μL in peripheral blood (PB) after 7 days of treatment with prednisone and a single intrathecal dose of methotrexate, or $\leq 25\%$ bone marrow (BM) blast cells at day 15, or $<5\%$ BM blast cells at day 21, depending on national induction protocols. Complete remission was defined as $<5\%$ blast cells in BM aspirate (with absolute neutrophil count $\geq 1.5 \times 10^9/\text{L}$ and platelets $\geq 100 \times 10^9/\text{L}$) and absence of leukemia in other organs.

EsPhALL2010 used the same chemotherapy regimen of the EsPhALL2004 study, but introduced imatinib earlier, i.e. from day 15 from diagnosis, and continuously along with chemotherapy until the end of treatment for all patients. Cranial radiotherapy was given to all patients not transplanted in the Interim Maintenance phase, i.e. after Protocol II, first delayed intensification (appendix p 1). Assessment of treatment response during the first 6 months of treatment was based on BM cytology and molecular evaluation of MRD at end of IA, phase IB and of each HR block (appendix p 1). Imatinib during the first year post-HSCT was only recommended and given at an initial dose of $200 \text{ mg}/\text{m}^2$ daily, provided that satisfactory PTL and WBC counts with stable neutrophils engraftment were observed (PTL $>50 \times 10^9/\text{L}$; WBC $>1.5 \times 10^9/\text{L}$; neutrophils $>0.5 \times 10^9/\text{L}$ for at least 15 days) and increased thereafter to $300 \text{ mg}/\text{m}^2$ daily.

Potential total exposure to imatinib for patients not transplanted was 24 months, while in the EsPhALL2004 it was either 3 or 5 months depending on whether patients underwent HSCT or not. Treatment modulation for imatinib was the following: in case of grade 3 or 4 non-hematological toxicity, imatinib had to be withheld until the toxicity had resolved to grade 2 or lower and then reintroduced at $240 \text{ mg}/\text{m}^2$ daily. For grade 3 or 4 hematologic toxicity, not accompanied by clinical manifestations, potential causes of the cytopenia were considered; transfusion support and continuation of drug administration were suggested if the thrombocytopenia or neutropenia were not attributed to imatinib, otherwise imatinib was reduced to $240 \text{ mg}/\text{m}^2$ daily. If Grade 3 or 4 thrombocytopenia or neutropenia were accompanied by clinically significant bleeding or evidence of infection, imatinib was interrupted until toxicity had resolved to grade 2 or lower and the clinical situation had stabilized; imatinib was then restarted at $240 \text{ mg}/\text{m}^2$ daily, to be later increased tot the dose of $300 \text{ mg}/\text{m}^2$, if tolerated. Data collection on actual imatinib delivery was not planned in the protocol.

All patients were screened for the search of an HLA-identical family or unrelated donor. Until 2012, GR patients with a genotype-HLA matched donor (9/10 or 10/10) were eligible to HSCT,

while PR patients were all eligible with any type of available donor. Given the general consensus that Ph+ ALL patients with a favorable early response to the combination of chemotherapy and TKI can be spared HSCT in CR1, indications for HSCT were restricted in 2012. Patients were eligible for HSCT if IG/TR MRD at the end of phase IB was $\geq 5 \times 10^{-4}$ or if it was positive $< 5 \times 10^{-4}$ and remained positive at any detectable level at the end of Block HR3. Transplant procedures were not defined in the protocol and were applied according to the group policy.

MRD was assessed by Real-time Quantitative (RQ)-PCR of rearranged Immunoglobulin/T-cell receptor genes (IG/TR) of mononuclear BM cells collected at specified time points. The MRD analyses were performed in central laboratories (one for each country), according to the Guidelines of the EuroMRD Network.¹³ Genetic investigations on ABL mutations or IKZF1 deletions were not part of the protocol.

After relapse, the only information collected was survival (no indications for salvage therapy were included in the protocol).

Serious Adverse Events (SAEs) were reported for each phase in the trial database with the same criteria of EsPhALL2004 study. The following were considered SAEs: all fatal events, life-threatening events and severe complications, excluding the commonly expected toxic effects of chemotherapy, such as mucositis or neutropenic fever. Treatment adjustments, including dasatinib substitution for imatinib due to previous SAEs (N=6, 2 of them after transplant) or to persistent high MRD levels (N=1), or nilotinib substitution for imatinib (N=1) were not considered exclusion criteria for this study.

Outcomes

The co-primary endpoints were the EFS (calculated as the time from diagnosis to first failure, including resistance, relapse, death from any cause, or second malignant neoplasm [SMN]) and overall survival (OS, defined as the time from diagnosis to death from any cause). Observation periods were censored at the date of last contact if no event was observed. Secondary endpoints included EFS and OS within risk group, DFS by treatment performed (i.e. chemotherapy vs. HSCT, defined as the time from CR to relapse, SMN or death, whichever occurred first), the proportion of patients with negative and positive MRD levels at various time-points.

Statistical analysis

The final follow-up was updated at Dec 31, 2016 and the median follow-up time was 57.4 months (IRQ 41.0-69.5). EFS, DFS and OS curves were estimated according to Kaplan-Meier (with Greenwood standard error) and compared with the log-rank test. Cumulative incidence of relapse (CIR) and death in remission (CID) was estimated accounting for competing risks and compared with the Gray test.¹⁴ Kaplan-Meier plots that compared HSCT with chemotherapy alone were adjusted to account for the waiting time to transplantation and the test was performed at a predefined time point (of 3 or 5 years from remission) based on log-log transformation¹⁵, due to the lack of proportional hazards. The basic Cox model was used for multivariate analysis of prognostic factors. To evaluate the role of HSCT compared to chemotherapy, a Cox model was applied with a time-dependent covariate for treatment, age at diagnosis and WBC count, risk group and an interaction term of this latter with treatment. A separate Cox model by risk group

included an interaction term between treatment and log(time) in order to account for non-proportional hazards (p-values were based on the likelihood ratio test). Analyses included all patients enrolled (intention-to-treat population) and were performed using SAS 9.2. The trial is registered with the European Clinical Trials Database (EudraCT 2004-001647-30) and with ClinicalTrials.gov, number NCT00287105.

Role of the funding source

The sponsors had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The authors had a role in data interpretation and writing and reviewing of the report. AB, MGV, and PDL had full access to all the raw data. The corresponding author had full access to all the data and had final responsibility to submit for publication.

Results

158 patients were registered in the EsPhALL2010 trial from Jan 1, 2010 to Dec 31, 2014 (appendix p 2). Three patients were not eligible because of: age <365 days (N=1), induction treatment differed from the national front-line protocol (N=1) or no information on eligibility criteria (N=1). 155 patients were enrolled in the study. Patient characteristics and early response are shown in Table 1; 62 patients (40.0%) were ≥ 10 years old, 45 patients (29.0%) had $\geq 100 \times 10^9/L$ WBC count at diagnosis. RT-PCR analysis, available in 122 patients, confirmed p190 in 103 (84.4%) and p210 transcript in 19 (15.6%). A good early response was seen in 103 (66.5%, 73.8% of them in the PB and 26.2% in the BM) and a poor early response in 52 (33.5%, 71.2% of them evaluated in the PB and 28.8% in the BM). Morphological CR was achieved in 151 patients (97.4%) after IA and in the remaining 4 patients after phase IB (only 1 of them had WBC count $\geq 100 \times 10^9/L$). One patient with early good response did not achieve CR at the end of IA. Thus 102 (65.8%) met the definition of GR and 53 (34.2%) of PR EsPhALL risk stratification criteria. Enrollment by national study group is reported in the appendix, p 3.

Overall 5-year EFS and OS were 57.0% (SE 4.1) and 71.8% (SE 3.8), respectively. The 5-year cumulative incidence of death in remission (CID) and of relapse (CIR) were 16.1% (SE 3.0) and 26.9% (SE 3.7), respectively (Figure 1, panels A and B). The 5-year EFS and OS were 62.7% (5.0) and 75.7% (4.7) in GR and 46.3% (7.0) and 63.6% (6.7) in PR, as shown in the appendix (p 4), together with CID and CIR curves.

Events are reported in Table 2. Of the 25 deaths in continuous CR (CCR, 16.1%), 14 (14.6%) occurred among 96 patients treated with chemotherapy only (all except one due to infection); 5 deaths occurred in 58 (8.6%) patients aged <10 years at diagnosis and 9 in the 38 (23.7%) patients aged ≥ 10 years. One patient died during phase IB, 6 during HR blocks, 6 during the first delayed intensification (DI), and 1 in the second DI. 78 patients started continuation therapy and 28 (35.9%) of them relapsed. Forty relapses occurred overall; 32 in patients treated with chemotherapy only (of them 20 after discontinuation of chemotherapy and imatinib) and 8 after HSCT (3 while on imatinib, 3 soon after imatinib discontinuation [within 4 months] and 2 in patients who did not receive imatinib post-transplant). Information on follow-up after relapse was available on 37/40 patients: survival at 2-years after relapse was 56.5 (SE 9.0).

MRD measurements were available only for some national groups and reported for 82 patients at end of IA and 74 at end of IB. The distribution of MRD levels at end IA and end IB is shown and

compared with the results of EsPhALL2004 in Figure 2 (panels A and B). At end IA, 27 were MRD-negative, and 55 MRD-positive ($<5 \times 10^{-4}$ in 34 and $\geq 5 \times 10^{-4}$ in 21, see Table 1). After phase IB, 47 were MRD-negative and 27 positive ($<5 \times 10^{-4}$ in 20 and $\geq 5 \times 10^{-4}$ in 7). Of the 47 patients MRD-negative at end of IB, 9 relapsed and 6 died in CCR. Out of 20 patients who had MRD positive not quantifiable at end of IB, 7 relapsed and 3 died in CCR. Finally, of the 7 patients with MRD $\geq 5 \times 10^{-4}$ at end of IB, 2 relapsed and 5 remained in CCR. Outcome curves by MRD at the end of IA and IB (Figure 2, panels C and D) show that early MRD negativity is associated with a better outcome but also with late relapses, indicating the absence of a plateau. MRD at end of block HR3 was available on 45 patients, 41 were MRD-negative and 4 MRD-positive but not quantifiable.

Overall, 59 (41%) patients underwent HSCT with 11 (18.6%) toxic deaths (9 within 1 year from HSCT and 2 afterwards). 23/59 were recruited in the first year after study opening and 8 of them died of toxicity (34.8%), while 36 patients were transplanted thereafter with 3 toxic deaths (8.3%). Eight patients relapsed (2 in the first year after HSCT) and 40 remained in CR1. The proportion of transplanted patients decreased gradually from approximately 50% in the first 2 years to 25% in the last 3 years of recruitment, due to the changing attitude toward transplant in CR1 (versus 81% in the EsPhALL2004 study). Out of 59 transplanted patients, there were 11 early events (9 deaths in CCR and 2 relapses, at 3 and 8 months from HSCT). 39 of the 48 patients (81.3%) who qualified for long-term exposure to imatinib post HSCT received the drug for a median (range) of 7 months (0.5–34), including 6 patients who received it for more than one year, based on the decision by treating physicians (up to 20 months, only 1 for 34 months).

Out of 155 patients, 9 died within 6 months from CR1 (median waiting time to transplant, Table 1). In GR patients, after the waiting time to transplant, there were 4 deaths in CCR and 21 relapses in patients treated with chemotherapy only (N=62) and 3 deaths and 2 relapses in the 33 patients transplanted. In PR, there were 1 death in CCR and 11 relapses in patients treated with chemotherapy only (N=27), and 8 deaths and 6 relapses in the 26 patients transplanted. The outcome of patients by performed treatment, adjusted by waiting time to transplant (5.6 months from CR1), is shown in Figure 3, panel A and B: the 5-year DFS was 52.6% (SE 6.2) and 66.7% (SE 6.2), for chemotherapy and HSCT in CR1, respectively ($p=0.12$). The 5-year OS was 77.3% (SE 5.1) and 73.6% (SE 5.8), respectively ($p=0.63$). Of note, adjustment with landmark at waiting time to transplant, excludes 9 deaths which occurred before that time. The analysis within risk group shows that, for GR patients, the 5-year DFS was 53.4% (SE 7.5) versus 77.1% (SE 6.5, $p=0.009$ at 5 years) and the OS was 84.5% (SE 6.4) versus 87.5% (SE 5.8, $p=0.26$ at 5 years), in patients treated with chemotherapy only versus HSCT (appendix p 5). In PR patients, the 3-year DFS was 50.1% (SE 10.5) versus 44.5% (SE 9.8, $p=0.70$ at 3 years) and the 3-year OS was 75.8% (SE 8.7) versus 55.7% (SE 9.9), in patients treated with chemotherapy only versus HSCT, respectively ($p=0.15$ at 3 years, appendix p 5).

The comparison of results in patients treated with chemotherapy or HSCT by MRD level at the end of phase IB is available on 74 patients. As shown in Table 3, MRD-negative patients were more prevalent than positive ones in the GR (39/53, 73.6%) compared to the PR group (8/21, 38.1%). Among 30 patients MRD-negative at the end of phase IB treated with chemotherapy only, 9 relapsed versus none of the 17 patients transplanted. Among patients with positive MRD at end of phase IB, 12 were treated with chemotherapy only with 5 relapses and 15 underwent HSCT with 5 relapses. To estimate the role of HSCT in the entire study cohort, we performed a Cox regression analysis with an interaction term, testing whether the effect on DFS in GR and PR patients was different. As reported in Table 4, the interaction was significant ($p=0.0042$) showing that GR patients treated with HSCT had one third of risk of failure compared to those treated with

chemotherapy only (HR=0.29, 95% CI=0.11–0.76). HSCT was not significantly different from chemotherapy only in PR patients (HR=1.58, 95% CI=0.73–3.39). A separate analysis for GR and PR patients was performed to show the comparison in time from CR: in the GR group, there was an initial disadvantage of transplantation, which was overcome after 1 year (Model 2), while in PR patients HSCT seemed associated with a reduced risk of failure only after 2 years, although not significantly so (Model 3).

Overall, regardless of treatment received, WBC count showed a marked prognostic impact on univariate analysis. The 5-year EFS was 36.1% (SE 7.4), 57.1% (SE 7.0) and 74.4% (SE 6.2), for patients with WBC count $\geq 100 \times 10^9/L$, 20 to $100 \times 10^9/L$ and $< 20 \times 10^9/L$, respectively ($p=0.0002$, Figure 4, panel A), while 5-year OS was 60.1% (SE 7.7), 69.6% (SE 6.5) and 83.8% (SE 5.4), respectively (appendix p 6). The proportion of patients with hyperleukocytosis was higher in PR (43.4%) than in GR patients (21.6%).

In a Cox regression on EFS, aimed at evaluating prognostic factors (risk group stratification, age and WBC), WBC count emerged as the only independent risk factor, with high WBC count $\geq 100 \times 10^9/L$ associated with a poor prognosis (HR 3.47, 95% CI 1.74–6.89, $p=0.0004$), and WBC count of 20 to $100 \times 10^9/L$ similar to WBC count $< 20 \times 10^9/L$ (HR 1.87, 95% CI 0.95–3.69, $p=0.07$). When the analysis was repeated on the subgroup of patients with data on MRD at end phase IB, results did not change for WBC count (HR for count $\geq 100 \times 10^9/L$ was 3.41, 95% CI 1.72–6.78) and MRD was not significant (data not shown).

A Cox regression analysis on DFS including also treatment (HSCT vs. chemotherapy only) confirmed the prognostic role of WBC count, as shown in Table 4. Patients with WBC $\geq 100 \times 10^9/L$ did not benefit from HSCT in CR1; the 5-year DFS (SE) adjusted for waiting time to transplant was 37.4 (11.1) and 42.4 (11.3) for patients who received chemotherapy and transplant, respectively (univariate comparison at 5 years, $p=0.43$; Figure 4, panel B). In particular, in the 22 GR patients with WBC count $\geq 100 \times 10^9/L$, there were 2 and 4 relapses in 10 and 12 patients receiving HSCT and chemotherapy only, respectively.

We also investigated the prognostic value of fusion protein: 19 patients of the 122 with available information presented with p210 fusion protein; 3 (15.8%) presented with WBC $\geq 100 \times 10^9/L$, 18 (94.7%) were GR and all achieved CR at the end of phase IA. In the 103 patients with p190, 35 (34.0%) had WBC $\geq 100 \times 10^9/L$, 63 (61.2%) were GR and all achieved CR at the end of phase IA. Eight p210 patients underwent HSCT and 1 relapsed (at 15 months from diagnosis); 11 patients were treated with chemotherapy only: there was one death in CCR and 2 relapses both at 26 months from diagnosis. At day 33, MRD of 11 patients with available data was negative in 6 (54.5%), positive $< 5 \times 10^{-4}$ in 3 (27.3%) and positive $\geq 5 \times 10^{-4}$ in 2 (18.2%). After phase IB, MRD was available in 11 patients: 10 (90.9%) were MRD-negative, 1 (9.1%) was MRD-positive $< 5 \times 10^{-4}$. The 5-year EFS (SE) and OS (SE) for p210 vs. p190 cohorts was 79.0 (9.4) vs. 48.9 (5.3), $p=0.03$ and 89.5 (7.0) vs 68.4 (5.1), $p=0.09$, respectively (appendix p 7).

Serious adverse events are described in the appendix, pp 8–10. Overall, 154 events were reported in 80 patients. As shown in panel A, the primary toxicity was infection (mostly bacterial); other events included gastrointestinal disorders and osteonecrosis. SAEs occurred mainly during HR Blocks and DIs, including 14 fatal events (in phase IB [N=1], HR Blocks [N=6], first DI [N=6] and second DI [N=1]).

Discussion

The EsPhALL2010 protocol used the same chemotherapy backbone of the EsPhALL2004 trial. Differences were the earlier and protracted exposure to imatinib, given to all patients and the restriction of transplant indications. The earlier exposure to imatinib (since day 15 of IA) markedly modified the response to induction with 97% of patients achieving CR1 at the end of IA as compared to 78% in the previous EsPhALL2004 study.¹² Of note, among PR patients who showed a poor early response, CR rate was as high as 92% in EsPhALL2010 versus 50% in EsPhALL2004. Unfortunately, data on MRD response were available for a limited number of patients, due to the failure to contribute data by some national groups. However, available MRD findings were consistent with morphological response : out of 82 patients with data at end of IA, only 1 (1%) had MRD $\geq 5 \times 10^{-2}$ in EsPhALL2010, versus 32/90 (36%) in the EsPhALL2004 (Figure 2), in which no imatinib was given during induction. This difference is striking, when considering that it is associated to the administration of only 2 or 3 weeks of imatinib on top of the second part of induction IA phase.

Relapses (N=40) occurred mostly in patients treated with chemotherapy only (N=32); interestingly, 12 patients relapsed more than 30 months after diagnosis (the last one more than 5 years after diagnosis) and a 30% rate of relapse was observed also in patients with MRD negativity at the end of phase IB (9 relapses in 30 patients, 7/9 late relapses, Table 4 and Figure 2). Eight of these 9 patients had documented negative MRD also after HR3 Block (for the remaining one, MRD was unknown). These findings show that the addition of imatinib clearly changed the pattern of relapses in Ph+ALL, preventing the achievement of an early plateau in EFS and selecting a group of patients with late relapses who may still have high chances of rescue by second line treatments, including HSCT and/or innovative therapies. An alternative option of long-term exposure to TKI therapy could also be considered, although in pediatric age this strategy may be associated with relevant bone and growth toxicity. Relapses in the 59 patients who received transplant occurred either very early (7) or early (1). Of note, PR patients with positive MRD at the end of phase IB had a poor outcome despite HSCT, with 4 relapses in 9 patients (one died in CCR).

Since the fraction of patients transplanted in the EsPhALL2010 trial was much lower (38% versus 81% in EsPhALL2004), these data suggest that the earlier introduction and the more extensive use of imatinib result in a similar EFS and OS while reducing the use of HSCT.

These findings are overall confirmatory of those recently published for the COG AALL0622 study, which included also young adults, using dasatinib early in the induction phase and HSCT for standard risk subjects with sibling donors and for high risk subjects. In this COG study, a relatively high incidence of CNS relapse (cumulative, isolated and combined) was observed and attributed to the omission of CNS radiotherapy in non-CNS3 patients and to the discontinuous administration of dasatinib in the majority of patients. In EsPhALL2010, there were 6 CNS isolated relapses and 11 combined with BM, out of 40 relapses (see legend of Table2) although they all received cranial radiotherapy or HSCT, raising further concerns on the optimal CNS therapy which could include an increased use of intrathecal chemotherapy and/or of high dose chemotherapy.

The comparison of results obtained with chemo vs. HSCT is difficult to assess in this study since transplanted patients had worse prognostic features. However, DFS and OS curves show that transplanted patients achieved a better DFS and an early plateau, whereas patients treated with chemotherapy only tended to have late relapses with relatively good chances of rescue with second line treatments and thus with a superimposable OS probability (Figure 3).

Overall results in the EsPhALL2010 trial were similar to those obtained in EsPhALL2004.^{12,17} In GR patients, EFS and OS were however slightly inferior due to an excess of deaths in CCR as explained below, while no difference was seen in PR patients. Interestingly, GR patients had a high risk of late relapses when treated with chemotherapy only (plateau not achieved) and a very low risk of relapse if they underwent transplant (with a plateau from 1 year). On the contrary, PR patients, especially those with positive MRD at the end of phase IB or with WBC count $\geq 100 \times 10^9/L$, had a high risk of very early or early relapses, even after transplant. In this scenario, the eligibility to HSCT remains controversial. In fact, patients with favorable prognosis, shown here to benefit from transplant, have good chances of rescue after second-line treatment and could thus be spared from HSCT. On the other side, patients with poorer prognosis did not benefit from HSCT and thus need alternative treatment, possibly guided by MRD, before and/or after HSCT. In this regard, it is to be considered, however, that after the 3 HR Blocks (i.e. the timing indicated for HSCT), 41/45 (91%) patients were already MRD negative and the remaining 4 (9%) had MRD positive but not quantifiable.

The role of imatinib post-HSCT remains difficult to assess¹⁸⁻²². The relapse rate for transplanted patients in this study, where 81% of patients received imatinib after HSCT, was 13.6%, versus 22.7% in the EsPhALL2004 study, in which no indication was given for imatinib post-transplant. Since in EsPhALL2010 patients were selected negatively compared to EsPhALL2004 (where all patients had indication for transplant) these data might suggest that imatinib post-transplant might be of some benefit. Future studies should be aimed to investigate aspects such as MRD monitoring and impact, and feasibility of TKI administration after transplant, as well as the biological mechanism of imatinib resistance, and clinical course after relapse, which could not be fully assessed in this study.

A major problem in the EsPhALL2010 study was toxicity. As shown in Table 2, there were 14 (14.6%) deaths occurring in CCR in 96 patients treated with chemotherapy only (13 of them due to infectious complications) and 11 (18.6%) in 59 HSCT patients which had a very important impact on outcome. It is of concern that many deaths occurred during HR blocks and the subsequent DI (which could be influenced by the previous HR blocks therapy), especially in patients aged more than 10 years. Of note, a cluster of events for HSCT occurred at the beginning of the study in one country and a cluster of events was observed for patients treated with chemotherapy in another country. However, a sensitivity analysis, excluding these 2 countries, showed that the rate of deaths in CCR would still be 12.2% and 12.1% in HSCT and chemotherapy, respectively. This rate (12.1%) is higher than that observed in high risk patients in the AIEOP-BFM ALL2000 trial^{23,24} as well as in the EsPhALL2004 trial, where imatinib was given intermittently for a shorter period.¹⁷ These data suggest thus that imatinib given early and continuously with intensive chemotherapy may be associated with very severe toxicity. This is of concern also in the context of the increasing use of imatinib or other TKI in addition to chemotherapy in newly identified ALL subgroups with ABL class fusion; the potential benefit with respect to relapse reduction must thus be carefully balanced to the risk of an increase of severe and life-threatening toxicity.

Pre-TKI studies on Ph+ALL showed that NCI criteria had a relevant prognostic value. In this study, patients with NCI HR criteria have a markedly higher incidence of treatment related death, a slower disease clearance and a higher incidence of relapses, with a significant difference both in EFS and OS. Most interestingly, age was associated with minor non-significant differences. On the contrary, WBC count $\geq 100 \times 10^9/L$ regardless of age was found to be the most important prognostic factor in these patients, thus confirming the recent report on the EsPhALL2004 study.¹⁷ This finding raises controversial issues on the optimal treatment strategy for these patients,

considering that HSCT was not successful. Interestingly, despite the high incidence of relapses in patients with WBC count $\geq 100 \times 10^9/L$, 5-year OS remains of 60% suggesting that some patients also in this subgroup can be rescued with second line therapy. No other prognostic factor was significantly associated with outcome in our study. Of note, relapse rate in the small subgroup of patients who had p210 was lower (3/19) than that in patients with p190 fusion protein (34/103); the number of patients, however, is too small to draw any conclusions.

Several data from various study groups have clearly demonstrated that MRD load plays a crucial role in both adult and childhood Ph+ ALL. Of the 32 transplanted patients for whom MRD data were available (Table 3), 5 had documented high MRD levels at end of phase IB ($\geq 5 \times 10^{-4}$) or MRD positivity after block HR3; 4 of them remained in CR1. Although these results seem favorable, the number is too low to draw any conclusion. At the moment, considering the high rate of rescue after relapse of patients treated with chemotherapy only, in our opinion, it may be reasonable to restrict indications to patients with high MRD at end of consolidation ($\geq 5 \times 10^{-4}$) or persistent positivity at subsequent time-points. Indications for HSCT remain an intriguing issue and require long-term investigations.

In our opinion, toxicity observed in this study supports further investigations on the efficacy of less intensive chemotherapy associated with early and protracted exposure to imatinib and on the role of transplant. The ongoing EsPhALL2017/COGAALL1631 study tests the hypothesis of non-inferiority of a treatment without intensive HR blocks (replaced by 4 cycles of HD-MTX [5gr/m²] given as CNS-directed therapy) and with less intensive DI in respect of the EsPhALL2010 treatment strategy, and restricts transplant indications to patients with poor MRD response. Our findings may also justify investigations on immunological approaches (e.g. BiTE, or CAR T-cell therapy) for patients at higher risk of relapse (such as those with high WBC count), for whom transplant did not prove to be effective, regardless of MRD response.

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Data Sharing Statement. Individual participant data that underlie the results reported in this article can be made available, after de-identification, beginning 9 months and ending 36 months after article publication, for individual participant data meta-analysis.

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	Good Risk N=102		Poor Risk N=53		total N=155	
	N	%	N	%	N	%
Sex						
Male	61	59.8	38	71.7	99	63.9
Female	41	40.2	15	28.3	56	36.1
Age at diagnosis (years)						
1-9	66	64.7	27	50.9	93	60.0
≥ 10	36	35.3	26	49.1	62	40.0
WBC at diagnosis (x10⁹/L)						
< 20	42	41.2	11	20.7	53	34.2
20-100	38	37.2	19	35.9	57	36.8
≥ 100	22	21.6	23	43.4	45	29.0
Immunophenotype						
Common	53	52.0	37	69.8	90	58.1
Pre-B	39	38.2	8	15.1	47	30.3
Pro-B	1	1.0	1	1.9	2	1.3
B-lineage Not Classified	8	7.8	6	11.3	14	9.0
T-lineage	1	1.0	1	1.9	2	1.3
CNS-Involvement						
Yes	5	4.9	3	5.7	8	5.2
No	97	95.1	49	92.4	146	94.2
Not known	0	0.0	1	1.9	1	0.6
t(9;22)(q34;q11)						
FISH only	16	15.7	11	20.7	27	17.4
RTQ-PCR only	36	35.3	11	20.7	47	30.3
Both	50	49.0	31	58.6	81	52.3
If RTQ-PCR, transcript detected						
Patients with available data*	81	79.4	41	77.4	122	78.7
p190	63	77.8	40	97.6	103	84.4
p210	18	22.2	1	2.4	19	15.6
NCI criteria						
Standard Risk	36	35.3	9	17.0	45	29.0
High Risk	66	64.7	44	83.0	110	71.0
Early response during induction						
Yes	102	100.0	1	1.9	103	66.5
Peripheral blood	75	73.5	1	100.0	76	73.8
BM	27	26.5	0	0.0	27	26.2
No	-	-	52	98.1	52	33.5
Peripheral blood	-	-	37	71.2	37	71.2
BM	-	-	15	28.8	15	28.8
Complete remission at end of induction (CR1)						
Yes	102	100.0	49	92.5	151	97.4
No	-	-	4 [§]	7.5	4	2.6

MRD results [#]						
MRD at end of phase IA						
patients with available data	59	100.0	23	100.0	82	100.0
negative	25	30.5	2	8.7	27	32.9
<5x10 ⁻⁴	25	30.5	9	39.1	34	41.5
≥5x10 ⁻⁴	9	11.0	12 [^]	52.2	21	25.6
MRD at end of phase IB						
patients with available data	53	100.0	21	100.0	74	100.0
negative	39	73.6	8	38.1	47	63.5
<5x10 ⁻⁴	11	20.7	9	42.9	20	27.0
≥5x10 ⁻⁴	3	5.7	4 ^{^^}	19.0	7	9.5
MRD at end of Block HR3						
patients with available data	33	100.0	12	100.0	45	100.0
negative	32	97.0	9	75.0	41	91.1
<5x10 ⁻⁴	1	3.0	3	25.0	4	8.9
≥5x10 ⁻⁴	0	0.0	0	0.0	0	0.0

Table 1 Baseline characteristics, morphological response and minimal residual disease by risk group. N= number of patients; WBC= white blood cells; RTQ PCR= real-time quantitative PCR; FISH= fluorescence in-situ hybridization; MRD=minimal residual disease; * 27 out of 33 patients, whose transcript was unknown, were analyzed with FISH only. [§] 1 patient with early good response and 3 with early poor response did not achieve CR at the end of Induction. [#] PINDA and HKPHOSG did not contribute data. [^] only 1/12 PR patient had MRD ≥5x10⁻² after IA. ^{^^} none of these 4 PR patients had MRD ≥5x10⁻² after IB.

	Good Risk N=102		Poor Risk N=53		Overall N=155		Overall N=155
	Chemo N=69	HSCT CR1 N=33	Chemo N=27	HSCT CR1 N=26	Chemo N=96	HSCT CR1 N=59	
Deaths in CCR	11 (15.9%)	3 (9.1%)	3 (11.1%)	8 (30.8%)	14 (14.6%)	11 (18.6%)	25 (16.1%)
Infections	10	0	3	0	13	0	13
MOF	1	0	0	0	1	0	1
SCT-related	-	3	-	8	-	11	11
Time from diagnosis to death in CCR							
≤ 5 months	7	0	2	0	9	0	9
6-12 months	4	1	1	5	5	6	11
>12 months	0	2	0	3	0	5	5
Relapses [deaths after relapse]	21 [7]	2 [1]	11 [5]	6 [3]	32 [12]	8 [4]	40 (25.8%) [16]
BM isolated	12	1	7	2	19	3	22
BM combined*	6	0	3	3	9	3	12
CNS	3	1	1	1	4	2	6
Time from diagnosis to relapse[#]							
very early	4	2	5	5	9	7	16
early	6	0	5	1	11	1	12
late	11	0	1	0	12	0	12
CCR	37	28	13	12	50	40	90 (58.1%)

Table 2. Outcome by risk group and treatment performed. Chemo= chemotherapy only; HSCT CR1=hematopoietic stem cells transplantation in first complete remission; BM= bone marrow; CNS= central nervous system; * BM combined relapses are all BM+CNS, except for 1 “late” relapse in BM+eye which occurred in a Good Risk patient who received chemotherapy only. [#] time from diagnosis to relapse is defined as very early (if

relapse occurred <18 months), early (18 to 30 months) and late (≥ 30 months); none of “very early” relapses occurred within 6 months from diagnosis (i.e. median time to HSCT). “Very early” relapses were BM isolated (N=8), BM+CNS (N=4) and CNS isolated (N=4); “early” relapses were BM isolated (N=7), BM+CNS (N=4) and CNS isolated (N=1); “late” relapses were BM isolated (N=7), BM+CNS (N=3), BM+eye (N=1) and CNS isolated (N=1); N=6 of the 12 CNS relapses in patients not transplanted occurred >24 months after the diagnosis.

		MRD at end of phase IB						total
		negative			positive			
		chemo	HSCT	total	chemo	HSCT	total [#]	
Good Risk	deaths in CCR	3	1	4	2	-	2	6
	relapses	8*	-	8	4	1	5	13
	Alive CCR	15	12	27	2	5	7	34
Poor Risk	deaths in CCR	-	2	2	-	1	1	3
	relapses	1	-	1	1	4	5	6
	Alive CCR	3	2	5	3	4	7	12
total		30	17	47	12	15	27	74

Table 3. Outcome by risk group, MRD at end of phase IB and treatment performed. Numbers are number of patients; CCR= continuous complete remission; MRD=minimal residual disease; HSCT= hematopoietic stem cells transplantation. ^{*} 7 of these 8 patients had MRD negative after Block HR3 (for the remaining 1, MRD data were not available). [#] Among 14 Good Risk patients who were positive at end of phase IB, 3 had MRD $\geq 5 \times 10^{-4}$; these 3 patients (eligible to HSCT) were negative at end of Block HR3. Two were transplanted and remained in CCR; one received chemotherapy only relapsed at 2.7 years after diagnosis. Among 13 Poor Risk patients who were positive at end of phase IB, 4 had MRD $\geq 5 \times 10^{-4}$. Three received HSCT in CR1 (1 relapse at 1.3 years after diagnosis) and 1 received chemotherapy only and is in CCR at 4.3 years after diagnosis.

	HR	95% CI	p-value
Model 1			
<i>EsPhALL stratification</i>			
Good Risk	1		
Poor Risk	0.75	0.38 – 1.48	0.3948
<i>Age at diagnosis (years)</i>			
1-9	1		
≥ 10	1.41	0.84 – 2.36	0.1922
<i>WBC at diagnosis (x10⁹/L)</i>			
<20	1		
20-100	1.72	0.87 – 3.40	0.1134
≥ 100	3.89	1.91 – 7.90	0.0001
<i>HSCT vs. Chemotherapy</i>			0.0079
in Good Risk	0.29	0.11 – 0.76	
in Poor Risk	1.58	0.73 – 3.39	
Model 2 – Good Risk			
<i>HSCT vs. Chemotherapy</i>			0.0011
Chemotherapy	1		
HSCT			
at 6 months	1.17	0.30 – 4.66	
at 1 year	0.39	0.14 – 1.08	
at 2 years	0.13	0.03 – 0.57	
at 3 years	0.07	0.01 – 0.48	
Model 3 – Poor Risk			
<i>HSCT vs. Chemotherapy</i>			0.1561
Chemotherapy	1		
HSCT			
at 6 months	5.90	0.87 – 40.10	
at 1 year	1.86	0.75 – 4.61	
at 2 years	0.59	0.15 – 2.25	
at 3 years	0.30	0.04 – 2.30	

Table 4. Effect of HSCT compared with chemotherapy only on disease-free survival, adjusted by prognostic factors. In Model 1, the two estimates of treatment effect are obtained after including an interaction term between treatment and risk group. The coefficient of interaction was 1.70 (SE 0.63) and was significant (p-value 0.0042). Model 2 in Good Risk and Model 3 in Poor Risk patients included a term for interaction of treatment with time from CR and were adjusted by age and WBC counts (estimated HRs for these variables were very similar to those in Model 1, data not shown).

Figures

Figure 1. Event-free survival and overall survival (panel A), cumulative incidence of relapse and of death (panel B) of 155 Ph+ALL patients. EFS=event-free survival; OS= overall survival; CCR= continuous complete remission.

Figure 2: Minimal Residual Disease (MRD) distribution at end IA (panel A) and IB (panel B) in EsPhALL2010 and EsPhALL 2004 study and outcome by MRD levels at end IA (panel C) and IB (panel D) in EsPhALL2010 (MRD data in the EsPhALL2004 study were described in a previous publication [16]). Numbers in panels C and D are 5-year event-free survival estimates and, in parenthesis, standard errors. NEG= negative; low POS= low positive ($<5 \times 10^{-4}$); high POS= high positive ($\geq 5 \times 10^{-4}$).

Figure 3. Disease-free survival (DFS, panel A) and overall survival (OS, panel B) of 155 Ph+ALL patients by treatment performed, adjusted by waiting time to HSCT. The curves originate at a landmark (i.e. the median time to transplantation, 5.6 months after first complete remission) and thus do not include patients who had events or whose follow-up data were censored before that time; the curves account for patients who underwent transplantation after the landmark by delayed entry.

Figure 4: Event-free survival (EFS, panel A) by WBC count and disease-free survival (DFS, panel B) of patients with WBC count $\geq 100 \times 10^9/L$ by treatment performed, adjusted by waiting time to HSCT. The curves in panel B originate at a landmark (i.e. the median time to transplantation, 5.6 months after first complete remission) and thus do not include patients who had events or whose follow-up data were censored before that time; the curves account for patients who underwent transplantation after the landmark by delayed entry.

~~A Treatment of Protocol with imatinib and Intensive Chemotherapy for Pediatric Philadelphia Positive Acute Lymphoblastic Leukemia with imatinib: an Intergroup, Open-label, Single-Arm Clinical Trial, Intergroup Study (EsPhALL2010)~~

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Research in context

Evidence before this study.

We searched PubMed for journal articles to July 20, 2018 without date limitations and with the terms “Ph+ALL” and “children” and “TKI or imatinib”. We found 32 publications in childhood Ph+ALL, but many of these were case reports or based on retrospective small-scale series. We therefore considered three relevant contemporary studies which evaluated with different strategies imatinib combined with front-line chemotherapy and HSCT. In the COG AALL0031 study, continuous imatinib exposure (340 mg/m²/day) improved outcome in cohort 5 patients with a 3-year event-free survival (EFS) of 80% markedly better than historical controls treated without TKI (35%), with no significant toxicities. Three-year EFS was similar for patients in cohort 5 treated with chemotherapy plus imatinib (88%) or sibling donor HSCT (57%). These results were confirmed by a successive report with longer-term follow-up. The Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) reported on the Ph+ALL04 study in which patients received 2 weeks of imatinib (340 mg/m²/day) monotherapy phase between the 3-phase high risk chemotherapy and HSCT. All patients were eligible to HSCT in CR1 and the 4-year event-free survival was 78%. The randomized EsPhALL2004 study showed a 4-year disease-free survival of 73% for patients exposed to short, discontinuous use of imatinib (300 mg/m²/day) after induction and receiving BFM high risk chemotherapy and HSCT, with 10% advantage with respect to outcome of patients who did not receive imatinib. Imatinib was well tolerated. These results suggest that a long and continuous use of imatinib concurrently with chemotherapy may challenge the indications to transplant.

Added value of this study.

This prospective trial introduces the early exposure to continuous imatinib administration (since day 15 of induction) in a protocol with BFM chemotherapy backbone and transplantation. This study allows to evaluate the impact on early response and event-free survival and the role of MRD and transplant in this context. Another relevant aspect is to assess the associated toxicity in comparison with the previous experience of a discontinuous imatinib administration. This study conducted by a large consortium allows to recruit a considerable number of patients in a very rare subgroup of pediatric ALL.

Implications of all the available evidence.

The early exposure to imatinib (since day 15 of IA) markedly improved the response to induction therapy. Continuous, protracted exposure to imatinib clearly delayed the time of relapses in Ph+ALL, yet a plateau in EFS for untransplanted patients was not achieved. The risk of relapse was very low in patients with early MRD negativity who underwent HSCT. High WBC count was found to be the most important prognostic factor either in patients treated with chemotherapy only or transplant. Imatinib given early and continuously with intensive chemotherapy was associated with very severe toxicity. Toxicity observed in this study justifies further investigations on the efficacy of less intensive chemotherapy associated with early and protracted exposure to imatinib, on the role of transplant and on immunological innovative approaches for patients at higher risk of relapse.

Abstract

Background. EsPhALL2004 study showed a 10% advantage in disease-free survival for short, discontinuous use of imatinib after induction in Philadelphia-chromosome-positive acute lymphoblastic leukaemia (ALL) ~~Ph+ALL~~ patients receiving BFM therapy and [hematopoietic stem cell transplantation \(HSCT\)](#). Other contemporary studies showed an advantage from continuous protracted exposure to imatinib challenging the indications to transplant. The EsPhALL2010 study was designed to assess if imatinib given from day 15 of induction and continuously throughout chemotherapeutic treatment allowed to maintain or improve the outcome obtained in EsPhALL2004 despite decreasing the number of patients undergoing HSCT.

Methods. Patients aged 1-17 years recruited into national frontline trials for acute lymphoblastic leukaemia and with t(9;22)(q34;q11) were eligible to the trial. Patients with abnormal renal or hepatic function, or an active systemic infection, were ineligible. Patients received imatinib 300/mg/m² continuously from day 15 of induction, during chemotherapy. Minimal residual disease (MRD) was measured by IG/TR. Eligibility to HSCT depended on early morphological response and MRD. Imatinib was recommended throughout the first year after transplant. The co-primary endpoints were event-free survival and overall survival. Analyses included all patients enrolled (intention-to-treat population). The trial is registered with the European Clinical Trials Database (EudraCT 2004-001647-30) and with ClinicalTrials.gov, number NCT00287105 and is completed.

Findings. 155 evaluable patients were registered between Jan 1, 2010 and Dec 31, 2014. 151 (97%) achieved CR1 after induction and 4 after consolidation phase IB. 59 patients (38%) underwent HSCT in CR1. The 5-year event-free survival and overall survival were 57.0 (SE 4.1) and 71.8 (SE 3.8), respectively. 14 (14.6%) deaths occurred in CCR in 96 patients treated with chemotherapy only (mainly during HR blocks and the subsequent DI), and 11 (18.6%) in 59 HSCT patients. The 5-year cumulative incidence of relapse was 26.9% (SE 3.7, 8 relapses in 59 transplanted patients and 32 relapses in 96 patients who received chemotherapy). In patients MRD negative at end of IB, there were no relapses in those transplanted (N=17) and 9 relapses in those (N=30) treated with chemotherapy. In patients with positive MRD at end of IB, 5 relapses each occurred in 15 transplanted and 12 not transplanted patients. WBC count at diagnosis $\geq 100 \times 10^9/L$ was associated with a poor prognosis (HR 3.47, 95% CI 1.74-6.89, p=0.0004). Serious adverse events (N=154) were reported in 80 (51.6%) of 155 patients. The primary toxicity was infection (61 [39.4%] patients of 155, mostly bacterial); gastrointestinal disorders occurred in 10 (6.5%) patients and osteonecrosis in 8 (5.2%). Serious adverse events occurred mainly during HR Blocks and DIs, including 14 fatal events (in phase IB [N=1], HR Blocks [N=6], first DI [N=6] and second DI [N=1]).

Interpretation. Imatinib given since day 15 of induction markedly improved CR rates to 97% from 78% in EsPhALL2004. Though HSCT rates were significantly lower (38% versus 81%), outcomes were similar in the two studies: 5-year EFS and survival were 57.0% and 71.8% versus 60.3% and 71.6%, respectively. Our data suggest that imatinib given early and continuously with intensive chemotherapy may be associated with very severe toxicity. WBC $\geq 100 \times 10^9/L$ was the most relevant independent prognostic factor.

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Introduction

Approximately 3-5% of pediatric acute lymphoblastic leukemia (ALL) patients present with the t(9;22)(q34;q11.2) translocation or Philadelphia chromosome (Ph+). Historically, Ph+ ALL was associated with a poor prognosis, with long-term event-free-survival (EFS) rates of ~30%.¹⁻⁸ Hematopoietic stem cell transplantation (HSCT) was associated with a superior EFS compared with chemo alone (44% vs. 34%, p=0.05) in the pre-tyrosine kinase inhibitor (TKI) era.^{3,4} There is now a general consensus that Ph+ ALL patients with a favorable early response to the combination of chemotherapy and TKI can be spared HSCT in first complete remission (CR). The COG AALL0031 study tested the combination of imatinib (beginning after Induction) along with an intensive chemotherapy backbone; patients with an HLA-matched sibling were allocated to HSCT in first CR. The 3-year EFS rate for all 44 Ph+ ALL patients who received imatinib continuously (340 mg/m²/day), irrespective of HSCT, was 80% (SE 11%), markedly better than historical controls treated without TKI (35% [4%], p<0.0001).⁹ The 5-year disease-free-survival (DFS) of 28 untransplanted patients was 70% (SE 12%), similar to that of patients treated with HSCT, either from a sibling donor (n=21, 5-year DFS 65% [SE 11%]) or from an unrelated donor (n=13, 5-year DFS 59% [SE 15%], p=0.77).¹⁰

The following COG study AALL0622 evaluated dasatinib on a chemotherapy backbone similar to that of AALL0031 with HSCT recommended for high risk patients and for any standard risk patient with a matched family donor. No relevant difference in results was observed, but a negative prognostic role was shown for IKZF1 deletions.¹¹

Contemporarily, the European intergroup study of Ph+ ALL conducted from 2004 to 2009 (EsPhALL2004) investigated the post-induction treatment with imatinib. Patients were risk-stratified on the basis of early response to therapy and Good Risk (GR) patients were randomized to the use of imatinib while poor Risk (PR) subjects were all given imatinib. The BFM ALL high risk (HR) chemotherapy backbone was used as post induction therapy and imatinib discontinued at maintenance or at HSCT. HSCT was recommended for GR patients with a matched related or unrelated donor, and for PR patients from any donor. Overall, 80% of patients received an allogeneic HSCT in CR1. In the analysis by intention-to-treat, imatinib was associated with a trend toward better 4-year DFS in GR patients (73% vs. 62%, p=0.24); the as-treated analysis showed a 4-year DFS of 75% for GR patients receiving imatinib and 56% for those who did not (p=0.06).¹²

In 2010, the EsPhALL trial was amended to give imatinib continuously from day 15 of induction and to decrease the number of patients undergoing HSCT, which was limited to poor MRD responders from 2012. The results of the EsPhALL2010 protocol are here reported and compared with those of EsPhALL2004.

Methods

Study design and participants

EsPhALL2010 is a prospective, single arm trial derived from an amendment of the EsPhALL2004 protocol (~~EudraCT No. 2004-001647-30~~) which was approved by the ethics committee of each participating institution in ten national study groups (AIEOP, BFM-G/CH, COALL, French ALL-group, NOPHO, MRC, DCOG, CPH, PINDA, and HKPHOSG). Eligible patients were children aged 1-17 years, recruited into national frontline trials for ALL, with t(9;22)(q34;q11) detected by conventional cytogenetics and/or BCR/ABL1 fusion transcript identified by reverse transcriptase (RT) PCR or by fluorescence *in-situ* hybridization (FISH), ~~trial~~. Patients with abnormal renal or hepatic function (grade ≥ 2 toxic effects according to the National Cancer Institute Common Toxicity Criteria [NCI-CTC] Version 2.0) or active systemic infection at day 15 of induction treatment were ineligible. Written, informed consent was obtained from parents or legal guardians.

Procedures

All patients received the Induction phase according to national/group study treatment protocol. The definition of risk subgroups was based on both early response to treatment and achievement of CR (cytology) at the end of induction, with GR patients being those who achieved both. Early response was defined as blast cells count $<1,000$ cells/ μL in peripheral blood (PB) after 7 days of treatment with prednisone and a single intrathecal dose of methotrexate, or $\leq 25\%$ bone marrow (BM) blast cells at day 15, or $<5\%$ BM blast cells at day 21, depending on national induction protocols. Complete remission was defined as $<5\%$ blast cells in BM aspirate (with absolute neutrophil count $\geq 1.5 \times 10^9/\text{L}$ and platelets $\geq 100 \times 10^9/\text{L}$) and absence of leukemia in other organs.

EsPhALL2010 used the same chemotherapy regimen of the EsPhALL2004 study, but introduced imatinib earlier, i.e. from day 15 from diagnosis, ~~and given~~ continuously along with chemotherapy until the end of treatment for all patients (~~appendix Figure S1~~). Cranial radiotherapy was given to all patients not transplanted in the Interim Maintenance phase, i.e. after Protocol II, first delayed intensification (appendix p 1). Assessment of treatment response during the first 6 months of treatment was based on BM cytology and molecular evaluation of MRD at end of IA, phase IB and of each HR block (appendix p 1 Figure S1). ~~In addition, imatinib during the first year post-HSCT was only recommended throughout the first year post-HSCT and given~~ at an initial dose of $200 \text{ mg}/\text{m}^2$ daily, provided that satisfactory PTL and WBC counts with stable neutrophils engraftment were observed (PTL $>50 \times 10^9/\text{L}$; WBC $>1.5 \times 10^9/\text{L}$; neutrophils $>0.5 \times 10^9/\text{L}$ for at least 15 days) and increased thereafter to $300 \text{ mg}/\text{m}^2$ daily.

Potential total exposure to imatinib for patients not transplanted was 24 months, while in the EsPhALL2004 it was either 3 or 5 months depending on whether patients underwent HSCT or not. Treatment modulation for imatinib was the following: in case of grade 3 or 4 non-hematological toxicity, imatinib had to be withheld until the toxicity had resolved to grade 2 or lower and then reintroduced at $240 \text{ mg}/\text{m}^2$ daily. For grade 3 or 4 hematologic toxicity, not accompanied by clinical manifestations, potential causes of the cytopenia were considered; transfusion support and continuation of drug administration were suggested if the thrombocytopenia or neutropenia were not attributed to imatinib, otherwise imatinib was reduced to $240 \text{ mg}/\text{m}^2$ daily. If Grade 3 or 4 thrombocytopenia or neutropenia were accompanied by clinically significant bleeding or evidence of infection, imatinib was interrupted until toxicity had resolved to grade 2 or lower and the clinical situation had stabilized; imatinib was then restarted at $240 \text{ mg}/\text{m}^2$ daily, to be later increased tot the dose of $300 \text{ mg}/\text{m}^2$, if tolerated. Data collection on actual imatinib delivery was not planned in the protocol.

All patients were screened for the search of an HLA-identical family or unrelated donor. Until 2012, GR patients with a genotype-HLA matched donor (9/10 or 10/10) were eligible to HSCT, while PR patients were all eligible with any type of available donor. Given the general consensus that Ph+ ALL patients with a favorable early response to the combination of chemotherapy and TKI can be spared HSCT in CR1, indications for HSCT were restricted in 2012. Patients were eligible for HSCT if ~~IGg/TCR~~ MRD at the end of ~~Protocol~~ phase IB was $\geq 5 \times 10^{-4}$ or if it was positive $< 5 \times 10^{-4}$ and remained positive at any detectable level at the end of Block HR3. Transplant procedures were not defined in the protocol and were applied according to the group policy.

MRD was assessed by Real-time Quantitative (RQ)-PCR of rearranged Immunoglobulin/T-cell receptor genes (IG/TR) of mononuclear BM cells collected at specified time points. The MRD analyses were performed in central laboratories (one for each country), according to the Guidelines of the EuroMRD Network.¹³ Genetic investigations on ABL mutations or IKZF1 deletions were not part of the protocol.

After relapse, the only information collected was survival (no indications for salvage therapy were included in the protocol).

Serious Adverse Events (SAEs) were reported for each phase in the trial database with the same criteria of EsPhALL2004 study. The following were considered SAEs: all fatal events, life-threatening events and severe complications, excluding the commonly expected toxic effects of chemotherapy, such as mucositis or neutropenic fever. Treatment adjustments, including dasatinib substitution for imatinib due to previous SAEs (N=6, 2 of them after transplant) or to persistent high MRD levels (N=1), or nilotinib substitution for imatinib (N=1) were not considered exclusion criteria for this study.

Outcomes

The co-primary endpoints were the EFS (calculated as the time from diagnosis to first failure, including resistance, relapse, death from any cause, or second malignant neoplasm [SMN]) and overall survival (OS, defined as the time from diagnosis to death from any cause). Observation periods were censored at the date of last contact if no event was observed. Secondary endpoints included EFS and OS within risk group, DFS by treatment performed (i.e. chemotherapy vs. HSCT, defined as the time from CR to relapse, SMN or death, whichever occurred first), the proportion of patients with negative and positive MRD levels at various time-points.

Statistical analysis

The final follow-up was updated at Dec 31, 2016 and the median follow-up time was 57.4 months (IRQ 41.0-69.5). EFS, DFS and OS curves were estimated according to Kaplan-Meier (with Greenwood standard error) and compared with the log-rank test. Cumulative incidence of relapse (CIR) and death in remission (CID) was estimated accounting for competing risks and compared with the Gray test.¹⁴ Kaplan-Meier plots that compared HSCT with chemotherapy alone were adjusted to account for the waiting time to transplantation and the test was performed at a predefined time point (of 3 or 5 years from remission) based on log-log transformation¹⁵, due to the lack of proportional hazards. The basic Cox model was used for multivariate analysis of prognostic factors. To evaluate the role of HSCT compared to chemotherapy, a Cox model was

applied with a time-dependent covariate for treatment, age at diagnosis and WBC count, risk group and an interaction term of this latter with treatment. A separate Cox model by risk group included an interaction term between treatment and log(time) in order to account for non-proportional hazards (p-values were based on the likelihood ratio test). Analyses included all patients enrolled (intention-to-treat population) and were performed using SAS 9.2. The trial is registered with the European Clinical Trials Database (EudraCT 2004-001647-30) and with ClinicalTrials.gov, number NCT00287105.

Role of the funding source

The sponsors had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The authors had a role in data interpretation and writing and reviewing of the report. AB, MG, and PDL had full access to all the raw data. The corresponding author had full access to all the data and had final responsibility to submit for publication.

Results

158 patients were registered in the EsPhALL2010 trial from Jan 1, 2010 to Dec 31, 2014 (appendix [p 2Figure S2](#)). Three patients were not eligible because of: age <365 days (N=1), induction treatment differed from the national front-line protocol (N=1) or no information on eligibility criteria (N=1). 155 patients were enrolled in the study. Patient characteristics and early response are shown in Table 1; 62 patients (40.0%) were ≥10 years old, 45 patients (29.0%) had ≥100x10⁹/L WBC count at diagnosis. RT-PCR analysis, available in 122 [patients](#), confirmed p190 in 103 (84.4%) and p210 transcript in 19 (15.6%). A good early response was seen in 103 (66.5%, 73.8% of them in the PB and 26.2% in the BM) and a poor early response in 52 (33.5%, 71.2% of them evaluated in the PB and 28.8% in the BM). Morphological CR was achieved in 151 patients (97.4%) after IA and in the remaining 4 patients after phase IB (only 1 of them had WBC count ≥100x10⁹/L). One patient with early good response did not achieve CR at the end of IA. Thus 102 (65.8%) met the definition of GR and 53 (34.2%) of PR EsPhALL risk stratification criteria. [Enrollment by national study group Distribution by country](#) is reported in the appendix, [p 3Table S1](#).

Overall 5-year EFS and OS were 57.0% (SE 4.1) and 71.8% (SE 3.8), respectively. The 5-year cumulative incidence of death in remission (CID) and of relapse (CIR) were 16.1% (SE 3.0) and 26.9% (SE 3.7), respectively (Figure 1, panels A and B). The 5-year EFS and OS were 62.7% (5.0) and 75.7% (4.7) in GR and 46.3% (7.0) and 63.6% (6.7) in PR, as shown in [the appendix \(p 4\)Figure 1 \(panels C and E\)](#), together with CID and CIR curves [\(panels D and F\)](#).

Events are reported in Table 2. Of the 25 deaths in continuous CR (CCR, 16.1%), 14 (14.6%) occurred among 96 patients treated with chemotherapy only (all except one due to infection); 5 deaths occurred in 58 (8.6%) patients aged <10 years at diagnosis and 9 in the 38 (23.7%) patients aged ≥10 years. One patient died during phase IB, 6 during HR blocks, 6 during the first delayed intensification (DI), and 1 in the second DI. 78 patients started continuation therapy and 28 (35.9%) of them relapsed. Forty relapses occurred overall; 32 in patients treated with chemotherapy only (of them 20 after discontinuation of chemotherapy and imatinib) and 8 after HSCT (3 while on imatinib, 3 soon after imatinib discontinuation [within 4 months] and 2 in patients who did not receive imatinib post-transplant). [Information on follow-up after relapse was available on 37/40 patients: survival at 2-years after relapse was 56.5 \(SE 9.0\)](#).

MRD measurements were available only for some national groups and reported for 82 patients at end of IA and 74 at end of IB. The distribution of MRD levels at end IA and end IB is shown and compared with the results of EsPhALL2004 in Figure 2 (panels A and B). At end IA, 27 were MRD-negative, and 55 MRD-positive (34 MRD-positive $<5 \times 10^{-4}$ in 34 and 21 $\geq 5 \times 10^{-4}$ in 21, (see Table 1). After phase IB, 47 were MRD-negative and 27 positive ($<5 \times 10^{-4}$ in 20 and $\geq 5 \times 10^{-4}$ in 7). Of the 47 patients MRD-negative at end of IB, 9 relapsed and 6 died in CCR. Out of 20 patients who had MRD positive not quantifiable at end of IB, 7 relapsed and 3 died in CCR. Finally, of the 7 patients with MRD $\geq 5 \times 10^{-4}$ at end of IB, 2 relapsed and 5 remained in CCR. Outcome curves by MRD at the end of IA and IB (Figure 2, panels C and D) show that early MRD negativity is associated with a better outcome but also with late relapses, indicating the absence of a plateau. MRD at end of block HR3 was available on 45 patients, 41 were MRD-negative and 4 MRD-positive but not quantifiable.

Overall, 59 (41%) patients underwent HSCT with 11 (18.6%) toxic deaths (9 within 1 year from HSCT and 2 afterwards). 23/59 were recruited in the first year after study opening and 8 of them died of toxicity (34.8%), while 36 patients were transplanted thereafter with 3 toxic deaths (8.3%). Eight patients relapsed (2 in the first year after HSCT) and 40 remained in CR1. The proportion of transplanted patients decreased gradually from approximately 50% in the first 2 years to 25% in the last 3 years of recruitment, due to the changing attitude toward transplant in CR1 (versus 81% in the EsPhALL2004 study). Out of 59 transplanted patients, there were 11 early events (9 deaths in CCR and 2 relapses, at 3 and 8 months from HSCT). 39 of the 48 patients (81.3%) who qualified for long-term exposure to imatinib post HSCT received the drug for a median (range) of 7 months (0.5–34), including 6 patients who received it for more than one year, based on the decision by treating physicians (up to 20 months, only 1 for 34 months).

Out of 155 patients, 9 died within 6 months from CR1 (median waiting time to transplant, Table 1). In GR patients, after the waiting time to transplant, there were 4 deaths in CCR and 21 relapses in patients treated with chemotherapy only (N=62) and 3 deaths and 2 relapses in the 33 patients transplanted. In PR, there were 1 death in CCR and 11 relapses in patients treated with chemotherapy only (N=27), and 8 deaths and 6 relapses in the 26 patients transplanted. The outcome of patients by performed treatment, adjusted by waiting time to transplant (5.6 months from CR1), is shown in Figure 3, panel A and B: the 5-year DFS was 52.6% (SE 6.2) and 66.7% (SE 6.2), for chemotherapy and HSCT in CR1, respectively ($p=0.12$). The 5-year OS was 77.3% (SE 5.1) and 73.6% (SE 5.8), respectively ($p=0.63$). Of note, adjustment with landmark at waiting time to transplant, excludes 9 deaths which occurred before that time. The analysis within risk group shows that, for GR patients, the 5-year DFS was 53.4% (SE 7.5) versus 77.4% (SE 6.5, $p=0.009$ at 5 years) and the OS was 84.5% (SE 6.4) versus 87.5% (SE 5.8, $p=0.26$ at 5 years), in patients treated with chemotherapy only versus HSCT (appendix p 5 Figure 3, panels C and D). In PR patients, the 3-year DFS was 50.1% (SE 10.5) versus 44.5% (SE 9.8, $p=0.70$ at 3 years) and the 3-year OS was 75.8% (SE 8.7) versus 55.7% (SE 9.9), in patients treated with chemotherapy only versus HSCT, respectively ($p=0.15$ at 3 years, appendix Figure 3, p 5 panels E and F).

The comparison of results in patients treated with chemotherapy or HSCT by MRD level at the end of phase IB is available on 74 patients. As shown in Table 3, MRD-negative patients were more prevalent than positive ones in the GR (39/53, 73.6%) compared to the PR group (8/21, 38.1%). Among 30 patients MRD-negative at the end of phase IB treated with chemotherapy only, 9 relapsed versus none of the 17 patients transplanted. Among patients with positive MRD at end of phase IB, 12 were treated with chemotherapy only with 5 relapses and 15 underwent HSCT with 5 relapses. To estimate the role of HSCT in the entire study cohort, we performed a Cox regression analysis with an interaction term, testing whether the effect on DFS in GR and PR patients was

different. As ~~reported shown~~ in Table 4, the interaction was significant ($p=0.0042$) showing that GR patients treated with HSCT had one third of risk of failure compared to those treated with chemotherapy only ($HR=0.29$, 95% $CI=0.11-0.76$). HSCT was not significantly different from chemotherapy only in PR patients ($HR=1.58$, 95% $CI=0.73-0.39$). A separate analysis for GR and PR patients was performed to show the comparison in time from CR: in the GR group, there was an initial disadvantage of transplantation, which was overcome after 1 year (Model 2), while in PR patients HSCT seemed associated with a reduced risk of failure only after 2 years, although not significantly so (Model 3).

Overall, regardless of treatment received, WBC count showed a marked prognostic impact on univariate analysis. The 5-year EFS was 36.1% (SE 7.4), 57.1% (SE 7.0) and 74.4% (SE 6.2), for patients with WBC count $\geq 100 \times 10^9/L$, 20 to $100 \times 10^9/L$ and $< 20 \times 10^9/L$, respectively ($p=0.0002$, Figure 4, panel A), while 5-year OS was 60.1% (SE 7.7), 69.6% (SE 6.5) and 83.8% (SE 5.4), respectively (appendix p 6 Figure 4, panels A and B). The proportion of patients with hyperleukocytosis was higher in PR (43.4%) than in GR patients (21.6%).

In a Cox regression on EFS, aimed at evaluating prognostic factors (risk group stratification, age and WBC), WBC count emerged as the only independent risk factor, with high WBC count $\geq 100 \times 10^9/L$ associated with a poor prognosis ($HR=3.47$, 95% $CI=1.74-6.89$, $p=0.0004$), and WBC count of 20 to $100 \times 10^9/L$ similar to WBC count $< 20 \times 10^9/L$ ($HR=1.87$, 95% $CI=0.95-3.69$, $p=0.07$). When the analysis was repeated on the subgroup of patients with data on MRD at end phase IB, results did not change for WBC count (HR for count $\geq 100 \times 10^9/L$ was 3.41, 95% $CI=1.72-6.78$) and MRD was not significant (data not shown).

A Cox regression analysis on DFS including also treatment (HSCT vs. chemotherapy only) confirmed the prognostic role of WBC count, as shown in Table 4. Patients with WBC $\geq 100 \times 10^9/L$ did not benefit from HSCT in CR1; the 5-year DFS (SE) adjusted for waiting time to transplant was 37.4 (11.1) and 42.4 (11.3) for patients who received chemotherapy and transplant, respectively (univariate comparison at 5 years, $p=0.43$; Figure 4, panel B). In particular, in the 22 GR patients with WBC count $\geq 100 \times 10^9/L$, there were 2 and 4 relapses in 10 and 12 patients receiving HSCT and chemotherapy only, respectively.

We also investigated the prognostic value of fusion protein: 19 patients of the 122 with available information presented with p210 fusion protein; and all achieved CR at the end of phase IA. 3 (15.8%) presented with WBC $\geq 100 \times 10^9/L$, 18 (94.7%) were GR and all achieved CR at the end of phase IA. In the 103 patients with p190, 35 (34.0%) had WBC $\geq 100 \times 10^9/L$, 63 (61.2%) were GR and all achieved CR at the end of phase IA. Eight p210 patients underwent HSCT and 1 relapsed (at 15 months from diagnosis); 11 patients were treated with chemotherapy only: there was one death in CCR and 2 relapses both at 26 months from diagnosis. At day 33, MRD of 11 patients with available data was negative in 6 (54.5%), positive $< 5 \times 10^{-4}$ in 3 (27.3%) and positive $\geq 5 \times 10^{-4}$ in 2 (18.2%). After phase IB, MRD was available in 11 patients: 10 (90.9%) were MRD-negative, 1 (9.1%) was MRD-positive $< 5 \times 10^{-4}$. The 5-year EFS (SE) and OS (SE) for p210 vs. p190 cohorts was 79.0 (9.4) vs. 48.9 (5.3), $p=0.03274$ and 89.5 (7.0) vs 68.4 (5.1), $p=0.09$, respectively (appendix p 7 Figure S3).

Serious adverse events are described in the appendix, pp 8-10 Table 5. Overall, 154 events were reported in 80 patients. As shown in panel A, the primary toxicity was infection (mostly bacterial); other events included gastrointestinal disorders and osteonecrosis. SAEs occurred mainly during HR Blocks and DIs, including 14 fatal events (in phase IB [N=1], HR Blocks [N=6], first DI [N=6] and second DI [N=1]).

Discussion

The EsPhALL2010 protocol used the same chemotherapy backbone of the EsPhALL2004 trial. Differences were the earlier and protracted exposure to imatinib, given to all patients and the restriction of transplant indications. The earlier exposure to imatinib (since day 15 of IA) markedly modified the response to induction with 97% of patients achieving CR1 at the end of IA as compared to 78% in the previous EsPhALL2004 study.¹² Of note, among PR patients who showed a poor early response, CR rate was as high as 92% in EsPhALL2010 versus 50% in EsPhALL2004. Unfortunately, data on MRD response were available for a limited number of patients, due to the failure to contribute data by some national groups. However, available MRD findings were consistent with morphological response : out of 82 patients with data at end of IA, only 1 (1%) had MRD $\geq 5 \times 10^{-2}$ in EsPhALL2010, versus 32/90 (36%) in the EsPhALL-2004 (Figure 2), in which where no imatinib was given during induction. This difference is striking, when considering that it is associated to the administration of only 2 or 3 weeks of imatinib on top of the second part of induction IA phase.

Relapses (N=40) occurred mostly in patients treated with chemotherapy only (N=32); interestingly, 12 patients relapsed more than 30 months after diagnosis (the last one more than 5 years after diagnosis) and a 30% rate of relapse was observed also in patients with MRD negativity at the end of phase IB (9 relapses in 30 patients, 7/9 late relapses, Table 4 and Figure 2). Eight of these 9 patients had documented negative MRD also after HR3 Block (for the remaining one, MRD was unknown). These findings show that the addition of imatinib clearly changed the pattern of relapses in Ph+ALL, preventing the achievement of an early plateau in EFS and selecting a group of patients with late relapses who may still have high chances of rescue by second line treatments, including HSCT and/or innovative therapies. An alternative option of long-term exposure to TKI therapy could also be considered, although in pediatric age this strategy may be associated with relevant bone and growth toxicity. Relapses in the 59 patients who received transplant occurred either very early (7) or early (1). Of note, PR patients with positive MRD at the end of phase IB had a poor outcome despite HSCT, with 4 relapses in 9 patients (one died in CCR).

Since the fraction of patients transplanted in the EsPhALL2010 trial was much lower (38% versus 81% in EsPhALL2004), these data suggest that the earlier introduction and the more extensive use of imatinib results in a similar EFS and OS while reducing the use of HSCT.

These findings are overall confirmatory of those recently published for the COG AALL0622 study, which included also young adults, using dasatinib early in the induction phase and HSCT for standard risk subjects with sibling donors or-and for high risk subjects. In this COG study, a relatively high incidence of CNS relapse (cumulative, isolated and combined) was observed and attributed to the omission of CNS radiotherapy in non-CNS3 patients and to the discontinuous administration of dasatinib in the majority of patients. In EsPhALL2010, there were 6 CNS isolated relapses and 11 combined with BM, out of 40 relapses (see legend of Table2) although they all received cranial radiotherapy or HSCT, raising further concerns on the optimal CNS therapy in these patients which- could include an increased use of intrathecal chemotherapy and/or of high dose chemotherapy.

The comparison of results obtained with chemo vs. HSCT is difficult to assess in this study since transplanted patients had worse prognostic features. However, DFS and OS curves show that transplanted patients achieved a better DFS and an early plateau, whereas patients treated with

chemotherapy only tended to have late relapses with relatively good chances of rescue with second line treatments and thus with a superimposable OS probability (Figure 34).

Overall results in the EsPhALL2010 trial were similar to those obtained in EsPhALL2004.^{12,17} In GR patients, EFS and OS were however slightly inferior due to an excess of deaths in CCR as explained below, while no difference was seen in PR patients. Interestingly, GR patients had a high risk of late relapses when treated with chemotherapy only (plateau not achieved) and a very low risk of relapse if they underwent transplant (with a plateau from 1 year). On the contrary, PR patients, especially those with positive MRD at the end of phase IB or with WBC count $\geq 100 \times 10^9/L$, had a high risk of very early or early relapses, even after transplant. In this scenario, the eligibility to HSCT remains controversial. In fact, patients with favorable prognosis, shown here to benefit from transplant, have good chances of rescue after second-line treatment and could thus be spared from HSCT. On the other side, patients with poorer prognosis did not benefit from HSCT and thus need alternative treatment, possibly guided by MRD, before and/or after HSCT. In this regard, it is to be considered, however, that after the 3 HR Blocks (i.e. the timing indicated for HSCT), 41/45 (91%) patients were already MRD negative and the remaining 4 (9%) had MRD positive but not quantifiable.

The role of imatinib post-HSCT remains difficult to assess¹⁸⁻²². The relapse rate for transplanted patients in this study, where 81% of patients received imatinib after HSCT, was 13.6%, versus 22.7% in the EsPhALL2004 study, in which no indication was given for imatinib post-transplant. Since in EsPhALL2010 patients were selected negatively compared to EsPhALL2004 (where all patients had indication for transplant) these data might suggest that imatinib post-transplant might be of some benefit. Future studies should be aimed to investigate aspects such as MRD monitoring and impact, and feasibility of TKI administration after transplant, as well as the biological mechanism of imatinib resistance, and clinical course after relapse, which could not be fully assessed in this study.

A major problem in the EsPhALL2010 study was toxicity. As shown in Table 2, there were 14 (14.6%) deaths occurring in CCR in 96 patients treated with chemotherapy only (13 of them due to infectious complications) and 11 (18.6%) in 59 HSCT patients which had a very important impact on outcome. It is of concern that many deaths occurred during HR blocks and the subsequent DI (which could be influenced by the previous HR blocks therapy), especially in patients aged more than 10 years. Of note, a cluster of events for HSCT occurred at the beginning of the study in one country and a cluster of events was observed for patients treated with chemotherapy in another country. However, a sensitivity analysis, excluding these 2 countries, showed that the rate of deaths in CCR would still be 12.2% and 12.1% in HSCT and chemotherapy, respectively. This rate (12.1%) is higher than that observed in high risk patients in the AIEOP-BFM ALL2000 trial^{23,24} as well as in the EsPhALL2004 trial, where imatinib was given intermittently for a shorter period.¹⁷ These data suggest thus that imatinib given early and continuously with intensive chemotherapy may be associated with very severe toxicity. This is of concern also in the context of the increasing use of imatinib or other TKI in addition to chemotherapy in newly identified ALL subgroups with ABL class fusion; the potential benefit with respect to relapse reduction must thus be carefully balanced to the risk of an increase of severe and life-threatening toxicity.

Pre-TKI studies on Ph+ALL showed that NCI criteria had a relevant prognostic value. In this study, patients with NCI HR criteria have a markedly higher incidence of treatment related death, a slower disease clearance and a higher incidence of relapses, with a significant difference both in EFS and OS. Most interestingly, age was associated with minor non-significant differences. On the

contrary, WBC count $\geq 100 \times 10^9/L$ regardless of age was found to be the most important prognostic factor in these patients, thus confirming the recent report on the EsPhALL2004 study.¹⁷ This finding raises controversial issues on the optimal treatment strategy for these patients, considering that HSCT was not successful. Interestingly, despite the high incidence of relapses in patients with WBC count $\geq 100 \times 10^9/L$, 5-year OS remains of 60% suggesting that some patients also in this subgroup can be rescued with second line therapy. No other prognostic factor was significantly associated with outcome in our study. Of note, relapse rate in the small subgroup of patients who had p210 was lower (3/19) than that in patients with p190 fusion protein (34/103); the number of patients, however, is too small to draw any conclusions.

Several data from various study groups have clearly demonstrated that MRD load plays a crucial role in both adult and childhood Ph+ ALL. Of the 32 transplanted patients for whom MRD data were available (Table 3), 5 had documented high MRD levels at end of phase IB ($\geq 5 \times 10^{-4}$) or MRD positivity after block HR3; 4 of them remained in CR1. Although these results seem favorable, the number is too low to draw any conclusion. At the moment, considering the high rate of rescue after relapse of patients treated with chemotherapy only, in our opinion, it may be reasonable to restrict indications to patients with high MRD at end of consolidation ($\geq 5 \times 10^{-4}$) or persistent positivity at subsequent time-points. Indications for HSCT remain an intriguing issue and require long-term investigations.

In our opinion, toxicity observed in this study supports further investigations on the efficacy of less intensive chemotherapy associated with early and protracted exposure to imatinib and on the role of transplant. The ongoing EsPhALL2017/COGAALL1631 study tests the hypothesis of non-inferiority of a treatment without intensive HR blocks (replaced by 4 cycles of HD-MTX [5gr/m²] given as CNS-directed therapy) and with less intensive DI in respect of the EsPhALL2010 treatment strategy, and restricts transplant indications to patients with poor MRD response. Our findings may also justify investigations on immunological approaches (e.g. BiTE, or CAR T-cell therapy) for patients at higher risk of relapse, (such as those with high WBC count), for whom transplant did not prove to be effective, regardless of MRD response.

Authorship Contributions. AB, VG, MS, and MGV designed and planned the study. AB, MGV, VC, PDL and VL wrote the report. PDL oversaw data pooling, data checking, reporting, and analyses. MGV was the study statistician. AB, VG, GC, MC, AC, RP, AB, AV, JS, GE, CKL, VS, MS coordinated the clinical study in their own countries and were responsible for data in their group. GC, HC, JB were responsible for the laboratory data in their group. All authors have revised and approved the final version of the report.

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Data Sharing Statement. Individual participant data that underlie the results reported in this article can be made available, after de-identification, beginning 9 months and ending 36 months after article publication, for individual participant data meta-analysis.

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	Good Risk N=102		Poor Risk N=53		total N=155	
	N	%	N	%	N	%
Sex						
Male	61	59.8	38	71.7	99	63.9
Female	41	40.2	15	28.3	56	36.1
Age at diagnosis (years)						
1-9	66	64.7	27	50.9	93	60.0
≥ 10	36	35.3	26	49.1	62	40.0
WBC at diagnosis (x10⁹/L)						
< 20	42	41.2	11	20.7	53	34.2
20-100	38	37.2	19	35.9	57	36.8
≥ 100	22	21.6	23	43.4	45	29.0
Immunophenotype						
Common	53	52.0	37	69.8	90	58.1
Pre-B	39	38.2	8	15.1	47	30.3
Pro-B	1	1.0	1	1.9	2	1.3
B-lineage Not Classified	8	7.8	6	11.3	14	9.0
T-lineage	1	1.0	1	1.9	2	1.3
CNS-Involvement						
Yes	5	4.9	3	5.7	8	5.2
No	97	95.1	49	92.4	146	94.2
Not known	0	0.0	1	1.9	1	0.6
t(9;22)(q34;q11)						
FISH only	16	15.7	11	20.7	27	17.4
RTQ-PCR only	36	35.3	11	20.7	47	30.3
Both	50	49.0	31	58.6	81	52.3
If RTQ-PCR, transcript detected						
Patients with available data *	81	79.4	41	77.4	122	78.7
p190	63	77.8	40	97.6	103	84.4
p210	18	22.2	1	2.4	19	15.6
NCI criteria						
Standard Risk	36	35.3	9	17.0	45	29.0
High Risk	66	64.7	44	83.0	110	71.0
Early response during induction						
Yes	102	100.0	1	1.9	103	66.5
Peripheral blood	75	73.5	1	100.0	76	73.8
BM	27	26.5	0	0.0	27	26.2
No	-	-	52	98.1	52	33.5
Peripheral blood	-	-	37	71.2	37	71.2
BM	-	-	15	28.8	15	28.8
Complete remission at end of induction (CR1)						
Yes	102	100.0	49	92.5	151	97.4
No	-	-	4 [§]	7.5	4	2.6

MRD results [#]						
MRD at end of phase IA^Δ						
patients with available data	59	100.0	23	100.0	82	100.0
negative	25	30.5	2	8.7	27	32.9
<5x10 ⁻⁴	25	30.5	9	39.1	34	41.5
≥5x10 ⁻⁴	9	11.0	12 ^Δ	52.2	21	25.6
MRD at end of phase IB^Δ						
patients with available data	53	100.0	21	100.0	74	100.0
negative	39	73.6	8	38.1	47	63.5
<5x10 ⁻⁴	11	20.7	9	42.9	20	27.0
≥5x10 ⁻⁴	3	5.7	4 ^{ΔΔ}	19.0	7	9.5
MRD at end of Block HR3						
patients with available data	33	100.0	12	100.0	45	100.0
negative	32	97.0	9	75.0	41	91.1
<5x10 ⁻⁴	1	3.0	3	25.0	4	8.9
≥5x10 ⁻⁴	0	0.0	0	0.0	0	0.0

Table 1 Baseline characteristics, morphological response and minimal residual disease by risk group. N= number of patients; WBC= white blood cells; RTQ PCR= real-time quantitative PCR; FISH= fluorescence in-situ hybridization; MRD=minimal residual disease; * 27 out of 33 patients, whose transcript was unknown, were analyzed with FISH only. [§] 1 patient with early good response and 3 with early poor response did not achieve CR at the end of Induction. [#] PINDA and HKPHOSG did not contribute data. ^Δ only 1/12 PR patient had MRD ≥5x10⁻² after IA. ~~and~~ ^{ΔΔ} none of these 4 PR patients had MRD ≥5x10⁻² after IB.

	Good Risk N=102		Poor Risk N=53		Overall N=155		Overall N=155
	Chemo N=69	HSCT CR1 N=33	Chemo N=27	HSCT CR1 N=26	Chemo N=96	HSCT CR1 N=59	
Deaths in CCR	11 (15.9%)	3 (9.1%)	3 (11.1%)	8 (30.8%)	14 (14.6%)	11 (18.6%)	25 (16.1%)
Infections	10	0	3	0	13	0	13
MOF	1	0	0	0	1	0	1
SCT-related	-	3	-	8	-	11	11
Time from diagnosis to death in CCR							
≤ 5 months	7	0	2	0	9	0	9
6-12 months	4	1	1	5	5	6	11
>12 months	0	2	0	3	0	5	5
Relapses [deaths after relapse]	21 [7]	2 [1]	11 [5]	6 [3]	32 [12]	8 [4]	40 (25.8%) [16]
BM isolated	12	1	7	2	19	3	22
BM combined*	6	0	3	3	9	3	12
CNS	3	1	1	1	4	2	6
Time from diagnosis to relapse[#]							
very early	4	2	5	5	9	7	16
early	6	0	5	1	11	1	12
late	11	0	1	0	12	0	12
CCR	37	28	13	12	50	40	90 (58.1%)

Table 2. Outcome by risk group and treatment performed. Chemo= chemotherapy only; HSCT CR1=hematopoietic stem cells transplantation in first complete remission; BM= bone marrow; CNS= central nervous system; *BM combined relapses are all BM+CNS, except for 1 “late” relapse in BM+eye which occurred in a Good Risk patient who received chemotherapy only. [#] time from diagnosis to relapse is defined as very early (if

relapse occurred <18 months), early (18 to 30 months) and late (≥ 30 months); none of “very early” relapses occurred within 6 months from diagnosis (i.e. median time to HSCT). “Very early” relapses were BM isolated (N=8), BM+CNS (N=4) and CNS isolated (N=4); “early” relapses were BM isolated (N=7), BM+CNS (N=4) and CNS isolated (N=1); “late” relapses were BM isolated (N=7), BM+CNS (N=3), BM+eye (N=1) and CNS isolated (N=1); N=6 of the 12 CNS relapses in patients not transplanted occurred >24 months after the diagnosis.

		MRD at end of phase IB						total
		negative			positive			
		chemo	HSCT	total	chemo	HSCT	total [#]	
Good Risk	deaths in CCR	3	1	4	2	-	2	6
	relapses	8*	-	8	4	1	5	13
	Alive CCR	15	12	27	2	5	7	34
Poor Risk	deaths in CCR	-	2	2	-	1	1	3
	relapses	1	-	1	1	4	5	6
	Alive CCR	3	2	5	3	4	7	12
total		30	17	47	12	15	27	74

Table 3. Outcome by risk group, MRD at end of phase IB and treatment performed. Numbers are number of patients; CCR= continuous complete remission; MRD=minimal residual disease; HSCT= hematopoietic stem cells transplantation. * 7 of these 8 patients had MRD negative after Block HR3 (for the remaining 1, MRD data were not available). [#] Among 14 Good Risk patients who were positive at end of phase IB, 3 had MRD $\geq 5 \times 10^{-4}$; ~~these 3 patients (eligible to HSCT)~~ All of them were found negative at end of Block HR3. ~~Two were transplanted; the 2 receiving HSCT in CR1 and~~ remained in CCR; ~~one, while the one~~ receiving chemotherapy only relapsed at 2.7 years after diagnosis. Among 13 Poor Risk patients who were positive at end of phase IB, 4 had MRD $\geq 5 \times 10^{-4}$. Three received HSCT in CR1 (1 relapse at 1.3 years after diagnosis) and 1 received chemotherapy only and is in CCR at 4.3 years after diagnosis.

	HR	95% CI	p-value
Model 1			
<i>EsPhALL stratification</i>			
Good Risk	1		
Poor Risk	0.75	0.38 – 1.48	0.3948
<i>Age at diagnosis (years)</i>			
1-9	1		
≥ 10	1.41	0.84 – 2.36	0.1922
<i>WBC at diagnosis ($\times 10^9/L$)</i>			
<20	1		
20-100	1.72	0.87 – 3.40	0.1134
≥ 100	3.89	1.91 – 7.90	0.0001
<i>HSCT vs. Chemotherapy*</i>			0.0079
in Good Risk	0.29	0.11 – 0.76	
in Poor Risk	1.58	0.73 – 3.39	
Model 2 – Good Risk			
<i>HSCT vs. Chemotherapy</i>			0.0011
Chemotherapy	1		
HSCT			
at 6 months	1.17	0.30 – 4.66	
at 1 year	0.39	0.14 – 1.08	
at 2 years	0.13	0.03 – 0.57	
at 3 years	0.07	0.01 – 0.48	
Model 3 – Poor Risk			
<i>HSCT vs. Chemotherapy</i>			0.1561
Chemotherapy	1		
HSCT			
at 6 months	5.90	0.87 – 40.10	
at 1 year	1.86	0.75 – 4.61	
at 2 years	0.59	0.15 – 2.25	
at 3 years	0.30	0.04 – 2.30	

Table 4. Effect of HSCT compared with chemotherapy only on disease-free survival, adjusted by prognostic factors. In Model 1, the two estimates of treatment effect are obtained after including an interaction term between treatment and risk group. The coefficient of interaction was 1.70 (SE 0.63) and was significant (p-value 0.0042). Model 2 in Good Risk and Model 3 in Poor Risk patients included a term for interaction of treatment with time from CR and were adjusted by age and WBC counts (estimated HRs for these variables were very similar to those in Model 1, data not shown).

A			
	Good Risk n (%)	Poor Risk n (%)	overall n (%)
On-study patients	102	53	155
Any primary system organ class	51 (50.0)	29 (54.7)	80 (51.6)
Infections and Infestations	40 (39.2)	21 (39.6)	61 (39.4)
Fungal Infection	6	4	10
Localised Infection	14	8	22
Infection	29	13	42
Encephalopathy	0	3	3
Vascular Disorders	3 (2.9)	2 (3.8)	5 (3.2)
Deep Vein Thrombosis	0	2	2
Veno-occlusive Disease	3	0	3
Nervous System Disorders	2 (2.0)	1 (1.9)	3 (1.9)
Convulsion	2	1	3
Gastrointestinal Disorders	6 (5.9)	4 (7.5)	10 (6.5)
Pancreatitis	6	3	9
Gastrointestinal Haemorrhage	0	1	1
Psychiatric Disorders	2 (2.0)	0	2 (1.3)
Psychotic Disorder	2	0	2
Musculoskeletal and Connective Tissue Disorders	6 (5.9)	2 (3.8)	8 (5.2)
Osteonecrosis	6	2	8
Hepatobiliary Disorders	2 (2.0)	0	2 (1.3)
Hepatic Failure	2	0	2
Renal and Urinary Disorders	3 (2.9)	3 (5.7)	6 (3.9)
Renal Impairment	3	3	6
Other	10 (9.8)	10 (18.9)	20 (12.9)

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B			
	Good Risk n (%)	Poor Risk n (%)	overall n (%)
Pts. on study	102	53	155
Pts. with at least one SAE (%)	51 (50.0)	29 (54.7)	80 (51.6)
Induction since d+15			
—— Pts. at risk	98	52	150
—— Pts. with at least one SAE (%)	12 (12.2)	6 (11.5)	18 (12.0)
—— No. of SAEs	15	7	22
Phase IB			
—— Pts. at risk	98	52	150
—— Pts. with at least one SAE (%)	11 (11.2)	6 (11.5)	17 (11.3)
—— No. of SAEs	11	6	17
HR1 Block			
—— Pts. at risk	96	50	146
—— Pts. with at least one SAE (%)	8 (8.3)	7 (14.0)	15 (10.3)
—— No. of SAEs	9	7	16
HR2 Block			
—— Pts. at risk	90	46	136
—— Pts. with at least one SAE (%)	5 (5.6)	5 (10.9)	10 (7.4)
—— No. of SAEs	5	5	10
HR3 Block			
—— Pts. at risk	90	45	135
—— Pts. with at least one SAE (%)	9 (10.0)	4 (8.9)	13 (9.6)
—— No. of SAEs	10	7	17
Protocol II, first delayed intensification			
—— Pts. at risk	64	26	90
—— Pts. with at least one SAE (%)	16 (25.0)	8 (30.8)	24 (26.7)
—— No. of SAEs	17	13	30
Interim Maintenance			
—— Pts. at risk	51	21	72
—— Pts. with at least one SAE (%)	5 (9.8)	1 (4.8)	6 (8.3)
—— No. of SAEs	6	1	7
Protocol II, second delayed intensification			
—— Pts. at risk	47	18	65
—— Pts. with at least one SAE (%)	9 (19.1)	3 (16.7)	12 (18.5)
—— No. of SAEs	9	4	13
Continuation			
—— Pts. at risk	42	18	60
—— Pts. with at least one SAE (%)	3 (7.1)	3 (16.7)	6 (10.0)
—— No. of SAEs	3	3	6
HSCT in CR1			
—— Pts. at risk	33	26	59
—— Pts. with at least one SAE (%)	5 (15.2)	10 (38.5)	15 (25.4)
—— No. of SAEs	5	10	15

Table 5. Serious adverse events. A Serious Adverse Event (SAE) was defined as an undesirable sign, symptom or medical condition which was fatal, or life threatening, or required (or prolonged) in-patient hospitalization, or resulted in persistent or significant disability/incapacity, or was a congenital anomaly/birth defect or was medically significant. Panel A: SAEs by system organ class (SOC) and preferred term. A patient with more than one SAE in a preferred term is counted only once under this preferred term. A patient with more than one SAE in different preferred terms under the same SOC is counted only once under this SOC. A patient with more than one SAE during treatment is counted once in the row 'Any primary system organ class'. Panel B: SAEs by treatment phase. A patient with more than one SAE in any treatment phase is counted only once under the 'Pts. at risk' row for this phase. Percentages are on the number of patients on study or on the number at risk for each treatment phase, as appropriate. Deaths in CCR occurred during phase IB (N=1), HR blocks (N=6), the first delayed intensification (N=6), and the second delayed intensification (N=1). One SAE (sepsis) that occurred in a GR patient is not listed here because treatment phase during SAE occurrence was not known.

Figures

Figure 1. Event-free survival ~~and (EFS)~~, overall survival (OS ~~panel A~~), cumulative incidence of relapse and ~~of death (panel B)~~ of 155 Ph+ALL patients, ~~overall (Panels A and B) and by risk group (Good Risk in Panels C and D, Poor Risk in Panels E and F).~~ EFS=event-free survival; OS= overall survival; CCR= continuous complete remission.

Figure 2: Minimal Residual Disease (MRD) distribution at end IA (panel A) and IB (panel B) in EsPhALL2010 and EsPhALL 2004 study and outcome by MRD levels at end IA (panel C) and IB (panel D) in EsPhALL2010 (MRD data in the EsPhALL2004 study were described in a previous publication [16]). Numbers in panels C and D are 5-year ~~EFS~~event-free survival estimates and, in parenthesis, standard errors. NEG= negative; low POS= low positive ($<5 \times 10^{-4}$); high POS= high positive ($\geq 5 \times 10^{-4}$).

Figure 3. Disease-free survival (DFS, ~~panel A~~) and overall survival (OS, ~~panel B~~) of 155 Ph+ALL patients by ~~risk group and~~ treatment performed, adjusted by waiting time to HSCT. The curves originate at a landmark (~~i.e. the~~ median time to transplantation, 5.6 months after first complete remission) and thus do not include patients who had events or whose follow-up data were censored before that time; the curves account for patients who underwent transplantation after the landmark by delayed entry.

Figure 4: Event-free survival (EFS, panel A), ~~and overall survival (OS, panel B),~~ by WBC count and disease-free survival (DFS, panel ~~BC~~) of patients with WBC count $\geq 100 \times 10^9/L$ and overall survival (OS, panel D) by treatment performed, adjusted by waiting time to HSCT, ~~in patients with WBC count $\geq 100 \times 10^9/L$.~~ The curves in panels ~~BC and D~~ originate at a landmark (~~i.e. the~~ median time to transplantation, 5.6 months after first complete remission) and thus do not include patients who had events or whose follow-up data were censored before that time; the curves account for patients who underwent transplantation after the landmark by delayed entry.

Appendix

	Good Risk	Poor Risk	Overall
	N=102	N=53	N=155
AIEOP	16	5	21
BFM-A	0	1	1
BFM-G	9	9	18
COALL	1	4	5
CPH	4	1	5
DCOG	7	2	9
French-ALL-Group	26	12	38
MRC	12	10	22
NOPHO	14	1	15
HKPHOSG	4	0	4
PINDA	9	8	17

Table S1. AIEOP=Associazione Italiana Ematologia Oncologia Pediatrica (Italy). BFM-A=Berlin Frankfurt Münster Austria (Austria). BFM-G=Berlin Frankfurt Münster Germany (Germany, Switzerland). COALL=Cooperative study group for treatment of ALL (Germany). CPH=Czech Paediatric Haematology (Czech Republic). DCOG=Dutch Childhood Oncology Group (Netherlands). NOPHO=Nordic Society of Paediatric Haematology and Oncology (Sweden, Denmark, Norway, Finland). HKPHOSG= Hong Kong Paediatric Haematology & Oncology Group. PINDA=Programa Infantil Nacional de Drogas Antineoplásicas (Chile).

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Figure S1. EsPhALL2010-protocol-outline

Figure S2 CONSORT flow-chart

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Necessary Additional Data

[Click here to download Necessary Additional Data: EsPhALL2010_Appendix.docx](#)

Reviewers' comments:

Reviewer #1: None

Reviewer #3: Critique of Revised Manuscript D-18-00319R1

The authors have provided responses to detailed comments/queries submitted by the reviewers; however, in some instances the ensuing modifications to the manuscript should be more detailed/explicit, to provide readers with upfront information rather than the potential questions which may remain after their perusal with answers only provided to the reviewers, as summarized below.

Although the manuscript has significant value, there are several unfortunate shortcomings which would have significantly impacted the analyses of outcomes owing to missing data. As mentioned by several of the reviewers, the lack of fairly basic data available in most clinical trials of Philadelphia positive acute lymphoblastic leukemia (Ph-ALL), irrespective of pediatric or adult setting, weakens the study. This includes the lack of data with respect to: (1) additional chromosomal aberrancies (unclear why routine karyotyping not available), (2) PCR for bcr-abl (p210 vs p190 missing in 20% of the subjects) at baseline and at MRD timepoints - since rates of MRD clearance by IgH/PCR, bcr-abl, or multiparameter flow cytometry (MFC) may differ, (3) MRD by PCR for IgH/TCR rearrangements at all MRD timepoints in more subjects (e.g., only available in 51% of subjects at end of induction 1A) especially owing to use as criteria for allogeneic SCT since 2012, and (4) two assessments with expected challenges in a multinational study but for which residual material could have been used in a protocol modification: IKZF1 deletions at baseline and ABL mutation status at the time of relapse. In my estimation, the most glaring issue is the concept of allocation to allogeneic stem cell transplantation (SCT) by MRD response (as per protocol modification in 2012) without having MRD data available (according to authors MRD in at least 13 subjects may have been assessed by other parameters such as PCR for bcr-abl or MFC but there is not a definitive answer). Finally, another issue remains with respect to delivery of imatinib as per protocol - no information has been provided with respect frequency of dose interruptions or dose reductions or even with respect to the various chemotherapeutic components/blocks particularly owing the high rate of infectious mortality observed in the study - the dose delivery is not mentioned anywhere in the manuscript. The authors should be more definitive upfront about these shortcomings or at least expand the discussion even further to emphasize these points rather than just fairly generalizable statements indicating future studies should include these investigations.

Reply: we fully agree with the above comments which are all well taken. These limitations are due to the type of study which involved cooperative groups of many different countries. Some difficulties are related to incomplete reporting. Other issues, such as the lack of data on IKZF1 deletions, or on actual imatinib delivery, are due to the fact that they were unfortunately not planned as study investigations. We have taken these points into account as explained below, point by point.

The responses provided by the authors have allowed better comprehension of the analysis and the following clarifications are requested:

On page 5, the imatinib post allogeneic SCT is listed as "recommended" but authors need to further emphasize this somehow as in the response to Reviewer #1, since it is indicated in Figure S1 as part of the treatment schema.

Reply. We have emphasized that imatinib post-HSCT was “only recommended” in the procedure Section of the Methods.

The response to Reviewer #5 that allogeneic SCT procedures were not defined in the protocol and were applied per group policy should be inserted in this section.

Reply. We added to the procedure Section of the Methods the following sentence: “Transplant procedures were not defined in the protocol and were applied according to the group policy”.

On page 6, in the Procedures section, where MRD assessments (PCR for IgH/TCR) are discussed, a notation should be made of the assessments that were not performed, such as ABL mutations or IKZF1 deletions, indicating not part of protocol design. If alternative MRD methods (e.g., PCR for bcr-abl or multiparameter flow cytometry) were used to allocate to allogeneic SCT then a sentence should be provided indicating the methodologies used.

Reply. We added to the Procedure section of the Methods the following sentence: “Genetic investigations on ABL mutations or IKZF1 deletions were not planned in the protocol”.

On page 7 where the sentence on relapses was added, information on the CR rate with salvage therapy and the reply to Reviewer #5 "Patients in CR after second line therapy survived for a median of 1.9 years (range: 1 month - 5.5 years)" should be added (also allude to the fact that specific salvage therapy was not directed by the protocol).

Reply. We have added in the Procedure section the sentence: “After relapse, the only information collected was survival (no indications for salvage therapy were included in the protocol).” and, in the Results, the sentence “Information on follow-up after relapse was available on 37/40 patients: survival at 2-years after relapse was 56.5 (SE 9.0).”

On page 7 where MRD results are provided, assessments were apparently available in 82 subjects at the end of 1A, but data on negativity/positivity is only provided on 61 subjects, with the 21 subjects with high MRD positivity (according to Table 1) not listed. Please correct.

Reply. We understand that, as written, the sentence “At end 1A, 27 were MRD-negative, 34 MRD-positive $<5 \times 10^{-4}$ and 21 $\geq 5 \times 10^{-4}$ (Table 1)” was misleading. We have re-phased it as follows: “At end 1A, 27 were MRD-negative, and 55 MRD-positive ($<5 \times 10^{-4}$ in 34 and $\geq 5 \times 10^{-4}$ in 21, see Table 1).”

On page 8, where chemotherapy is compared with allogeneic SCT, the CIR and TRM rates should be provided for the GR and PR groups within these subsets, to provide perspective on the differences in DFS and OS.

Reply. We added the description of these data to the Results, in the following sentences: “In GR patients, after the waiting time to transplant, there were 4 deaths in CCR and 21 relapses in patients treated with chemotherapy only (N=62) and 3 deaths and 2 relapses in the 33 patients transplanted. In PR, there were 1 death in CCR and 11 relapses in patients treated with chemotherapy only (N=27), and 8 deaths and 6 relapses in the 26 patients transplanted.”

On page 9, where the p210 subset is discussed, it should be clarified that 19 out of 122 subjects presented with this fusion protein, since data was not available on the entire cohort of 155 subjects. In addition, the reply to Reviewer #5 regarding comparison of p210 vs p190 should be added to this section.

Reply: We added this information in the Results as follows: “We also investigated the prognostic value of fusion protein: 19 patients of the 122 with available information presented with p210 fusion protein; 3 (15.8%) presented with $WBC \geq 100 \times 10^9/L$, 18 (94.7%) were GR and all achieved CR at the end of phase IA. In the 103 patients with p190, 35 (34.0%) had $WBC \geq 100 \times 10^9/L$, 63 (61.2%) were GR and all achieved CR at the end of phase IA.”

In the discussion, several points should be further expanded. For example, the identification of late relapses and the notable predominance of relapses off imatinib therapy suggest one possible solution would be to administer imatinib indefinitely (as done in some adult Ph-ALL regimens where single agent TKI is continued beyond combination maintenance chemoimmunotherapy). This is not offered as a possibility.

Reply: the following statement has been added to the Discussion: “An alternative option of long-term exposure to TKI therapy could also be considered, although in pediatric age this strategy may be associated with relevant bone and growth toxicity.”

Also, the section on CNS relapses does not provide commentary on the role of intrathecal (IT) chemotherapy and the potential for increasing the intensity of administration as one option for averting CNS relapse. It is unclear from the supplemental figure provided when IT treatments or CNS radiation were administered and if there were differences in allocation by GR/PR stratification and/or WBC at presentation.

Reply: this comment is correct. The chemotherapy was described in the previous paper in the Lancet Oncology (reference No. 12). The following statement has been added to the Procedures section: “Cranial radiotherapy was given to all patients not transplanted in the Interim Maintenance phase, i.e. after Protocol II, first delayed intensification (appendix Figure S1). We have also added the following section to the relevant part of the Discussion: “...raising further concerns on the optimal CNS therapy which could include an increased use of intrathecal chemotherapy and/or of high dose chemotherapy.”

In the ensuing section a statement is made that the subset that underwent allogeneic SCT had negative prognostic features, but the GR group with MRD negativity did extremely well with allogeneic SCT. Also the statement indicating 91% were MRD negative prior to allogeneic SCT is misleading since data were not available in all subjects (as per the authors' response to my query indicating only 15 subjects had MRD data available immediately prior to allogeneic SCT), so the proportion for whom this data were available should be indicated. One concern would be the potential for increasing MRD levels or development of MRD positivity with dose interruption of imatinib prior to allogeneic SCT as per usual practice (usually 4 weeks or more but not stated in the Procedures section).

Reply: we agree with the comments. We have re-phrased the relevant sentence in the Discussion, adding the number of patients with available MRD data at the end of HR3 Block. The sentence now reads as follows: "In this regard, it is to be considered, however, that after the 3 HR Blocks (i.e. the timing indicated for HSCT), 41/45 (91%) patients were already MRD negative and the remaining 4 (9%) had MRD positive but not quantifiable."

The section reviewing the deaths in CCR related to infectious complications could address the role of chemotherapy reductions (as in ongoing protocol - and impact of removal of HR intensifications on potential for CNS relapse), although it is unclear whether all the subjects experiencing deaths in CCR were on full doses of imatinib since no information is provided on treatment delivery of imatinib (% with full dose delivery, % requiring dose attenuations or % with interruptions). This is a limitation of the study which should be mentioned in the discussion since the new protocol design involves dose reductions of the chemotherapy components.

Reply: In the ongoing protocol, the patients assigned to the treatment without HR Blocks receive 4 cycles of HD-MTX (5gr/m²); it is thus not expected to see any increase in the rate of CNS relapses. We have added this information in the relevant sentence of the Discussion, as follows: "The ongoing EsPhALL2017/COGAALL1631 study tests the hypothesis of non-inferiority of a treatment without intensive HR blocks (replaced by 4 cycles of HD-MTX [5gr/m²] given as CNS-directed therapy) ...".

As previously requested, the last sentence of the discussion where "immunological" approaches is mentioned should be further clarified as to whether authors are referring to monoclonal antibodies (e.g. anti-CD20), BiTE therapy, or CAR T-cell therapy or all of these as potential options for those with poor MRD response prior to allogeneic SCT or even the PR group regardless of MRD response (recognizing that the kinetics of MRD response by IgH/TCR and bcr-abl may differ) since no benefit to allogeneic SCT was observed.

Reply: Thanks, we have added this information and the sentence now reads as follows: "Our findings may also justify investigations on immunological approaches (e.g. BiTE, or CAR T-cell therapy) for patients at higher risk of relapse, (such as those with high WBC count), for whom transplant did not prove to be effective, regardless of MRD response."

Tables/Figures:

In Table 1, it is notable (and expected) that a higher proportion of patients with PR had higher WBC compared with the GR subset. This should be mentioned in the text where prognostic value of WBC is first delineated.

Reply: We have added the following sentence to the Results: “The proportion of patients with hyperleukocytosis was higher in PR (43.4%) than in GR patients (21.6%).”

The footnote states “^ only 1 PR patient had MRD $\geq 5 \times 10^{-2}$ after IA and none after 1B” is confusing and the exponent needs to be corrected to -4.

Reply: Actually, the MRD level for the mentioned patients was $\geq 5 \times 10^{-2}$. We understand that, as written, it may be misleading. Therefore, we have split and modified the legend as follows: “^ only 1/12 PR patient had MRD $\geq 5 \times 10^{-2}$ after IA. ^^ none of these 4 PR patients had MRD $\geq 5 \times 10^{-2}$ after IB.”

In Table 3, it appears that 8 GR and 4 PR subjects did not undergo allogeneic SCT as per protocol related to poor MRD response, presumably owing to lack of donor or SCT ineligibility? Please add comment to footnote to clarify.

Reply: Actually, among patients for whom transplant was indicated on the basis of MRD ($\geq 5 \times 10^{-4}$) at the end of Phase IB, only 1/3 in GR and 1/4 in PR did not undergo HSCT. We have clarified this in the legend as follows: “#Among 14 Good Risk patients who were positive at end of phase IB, 3 had MRD $\geq 5 \times 10^{-4}$; these 3 patients (eligible to HSCT) were negative at end of Block HR3. Two were transplanted and remained in CCR; one received chemotherapy only relapsed at 2.7 years after diagnosis. Among 13 Poor Risk patients who were positive at end of phase IB, 4 had MRD $\geq 5 \times 10^{-4}$. Three received HSCT in CR1 (1 relapse at 1.3 years after diagnosis) and 1 received chemotherapy only and is in CCR at 4.3 years after diagnosis.”

In Table 4, there is an asterisk by HSCT vs chemotherapy in Model 1 but nothing in footnote for the asterisk.

Reply: Thank you for pointing this out. The asterisk was a typo and was deleted.

In Table 5, definition of serious adverse event should be provided in footnote. The sentence on sepsis in GR patient is duplicated. The text provided on page 7 regarding the 14 deaths in CCR in the chemotherapy only group should be added to the footnote “one patient died during phase IB, 6 during HR blocks, 6 during the first delayed intensification (DI), and 1 in the second DI”.

Reply: We have modified the footnote as suggested and have thus:

- added the definition of serious adverse events
- deleted the duplicate sentence
- added the sentence on the 14 deaths in CCR

Please note that, to comply with request from the editor, this Table is now included in the Appendix, pp 8-10.

Figures 3 and 4 should explicitly state the median time to transplantation was 5.9 months in their legends.

Reply: Figure 3, panels C-F and Figure 4, panels B and D have been moved to the Appendix, to comply with request from the editor (see appendix p 5 and 6, respectively). We have modified the legends of Figure 3, Figure 4, Figure in appendix p 5 and Figure in appendix p 6 as follows: “The curves originate at a landmark (i.e. the median time to transplantation, 5·6 months after first complete remission) and ...”.

Figures 3 and S3 should have p-values added to graphs.

Reply: We have added the p-values as requested. Please note that, to comply with request from the editor, Figure 3, panels C-F are included as a separate Figure in the Appendix, p 5. Figure S3 remains in the Appendix, p 7.

Reviewer #4: None.

Reviewer #5: None

Reviewer #6: None.

Editorial comments (*Numbers correspond to the ones in my previous editorial decision letter.)

#1. Please make sure your response to the reviewers' concerns are appropriately incorporated in the manuscript.

- Please make sure your response to the remaining concerns above is fully incorporated in the manuscript, especially regarding the missing MRD data and the other limitation discussion points.

Reply: We have taken the reviewer's comments into account and added further information in the Methods, Results or Discussion section, as appropriate (see our reply to reviewer #3).

#2. Please double check that the study title has a descriptor—ie, randomised trial, case-control study, prospective analysis, population-based study etc... —and must be non-declamatory (ie, not state the findings of the paper).

- The paper is an open-label, single-arm phase 2 clinical trial, rather than a protocol. Please revise the title accordingly.

Reply: we have modified the title as follows: "Treatment of Pediatric Philadelphia Positive Acute Lymphoblastic Leukemia with imatinib: an Intergroup, Open-label, Single-Arm Clinical Trial (EsPhALL 2010)". We have not included 'phase 2', since the study was not only aimed at activity (early response), but also at long-term endpoints (EFS and OS).

#7. Please note that when applicable, if new authors are added to the manuscript, we require each of the original authors to provide signed, written consent to approve this. The following wording can be used:

"We agree to the addition of X, X, and X to the authorship of this manuscript, and have seen and approve the new order of authorship".

"In this case, please scan and upload these statements when submitting your revised manuscript."

Reply: see Point 4 above. Do we need anyway to collect these signed consents?

- We do need signed consents in this case. Thank you for your corporation.

Reply: the signed consents have been provided with the new revision

#12. Summary: Your abstract should conform to the CONSORT guidelines for abstracts (CONSORT for Abstracts: Lancet 2008; 371: 281-83), and must include:

e) Methods: An explicit description of the actual primary endpoint.

- It is somewhat confusing regarding the primary endpoint(s) based on the information in the protocol amendment. It seems DFS was stated as the primary endpoint on page 8, but was also listed as a secondary objectives on page 20. Nonetheless, in the paper, EFS and OS were both listed as the primary endpoints. Could you clarify and revise to make sure only primary endpoint(s) are described in this section?

i) Findings: data for the primary outcome only.

- Similar to point #e above, please clarify and revise if needed.

Reply: apologies for the confusion. There is actually a typo in the protocol synopsis on page 8 of the protocol. Instead, Section 10.2 on page 46 correctly states that EFS and OS are both evaluated for the primary analysis, therefore we indicated them as co-primary endpoints and reported findings on both of them.

13. Please confirm that your study conforms to the appropriate reporting guidelines by completing and returning the relevant checklist (see below).

CONSORT - for RCTs - <http://download.thelancet.com/flatcontentassets/authors/tlo-consort-checklist.pdf>

Reply: STROBE checklist was completed

- Sorry for the confusion. Since this is a clinical trial report, rather than an observational study, please complete the CONSORT checklist, rather than STROBE, and submit the filled CONSORT form in the revision. You can ignore the part relevant only to randomization.

Reply: we have completed and submitted the CONSORT form

41. We require completed author contribution forms from all authors listed (that they agree with the submission and content and to being listed), declaring their contribution to the article, and stating the role of the funding source. The form can be downloaded at <https://thelancet.com/pb/assets/raw/Lancet/authors/tlhae-author-signatures.pdf>

Reply: collection is ongoing

- Please provide the author contribution forms as soon as possible. We cannot publish the paper before the forms are all submitted.

Reply: we have submitted the author contribution forms

#42. We require completed ICMJE declaration forms from all authors listed declaring any potential conflicts of interest. The form can be found at <https://thelancet.com/for-authors/forms?code=lancet-site#icmje-coi>

Reply: done

- Sorry for the confusion, but we did not see the ICMJE forms submitted in the last version. Could you kindly make sure all the ICMJE forms are submitted at this round?

Reply: we have submitted all the ICMJE forms

#49. All abstracts and references in press should be updated with DOIs or page numbers as appropriate. For papers listed in references that are "in press" we need to see a galley proof and/or letter from the publisher stating that it is "in press" as well as (where known) the full expected citation (ie, publication date/volume/issue etc). References that are "submitted" should be removed and citations in the text replaced with "(unpublished data; authors)". I prefer not to have any unpublished data cited (hasn't been peer-reviewed).

Reply: done except for reference 17 which is "provisionally accepted" by Haematologica and which we would like to keep as a reference. We are rather confident that this paper will be definitely accepted very soon.

- We can keep the reference for now, but please be noted that this paper needs to be in press at the copy-editing stage, and updated accordingly. Otherwise, we need to remove this reference at that point.

Reply: we have updated the reference 17 which is now "Epub ahead of print" and have asked Haematologica a final statement on publication.

1. Biondi A, Cario G, De Lorenzo P, et al. Long-term follow-up of Pediatric Philadelphia Positive Acute Lymphoblastic Leukemia treated with the EsPhALL2004 study: high white blood cell count at diagnosis is the strongest prognostic factor. Haematologica, ~~submitted~~ 2018 Sep 13. pii: haematol.2018.199422. doi: 10.3324/haematol.2018.199422. [Epub ahead of print].

52. We are not able to refer to specific tables and figures in the appendix. Please ensure all your supplementary materials are compiled into a single separate webappendix document and paginated, and then please refer to the page on which the item appears in the manuscript to reference that item.

Reply: the supplementary material remains now included in the appendix at the bottom of the file and a separate document will be produced after final revision following your instructions.

- Please address this point at this round.

Reply: the supplementary material was included in a separate file and referenced in the text as requested (with page numbers).

#57. Please include a Data Sharing Statement at the end of the manuscript.

Reply: we are contacting each nation group representative on this issue and will be back to you as soon as possible.

- Please address this point at this round.

Reply: the Data Sharing Statement was added at the end of the manuscript.

* Additional editorial point: There are currently 20 non-text items (including panels within each main figure and all main tables) in the paper, and we generally allow 8 non-text items. I'd suggest that you move the items representing secondary outcomes to the supplemental materials.

Reply: as requested, we moved items representing secondary outcomes (e.g. analyses by risk groups) to the Appendix. In particular:

- Figure 1, panels C-F are now in appendix, p 4
- Figure 3, panels C-F are now in appendix, p 5
- Figure 4, panels B and D are now in appendix, p 6
- Table 5, panels A-B are now in appendix, pp 8-10

- End of editorial points

Figure1

Figure2

Figure3

Figure4

Necessary Additional Data

[Click here to download Necessary Additional Data: EsPhALL_CONSORT 2010 Checklist.doc](#)

EsPhALL

EUDRACT-n° 2004-001647-30

**An open-label study to evaluate the safety and efficacy
of IMATINIB with chemotherapy in pediatric patients with Ph+/BCR-ABL+
acute lymphoblastic leukemia (Ph+ALL)**

Amendment n°1/2009 version 01.12.2009

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List of abbreviations

AE	adverse event
ALL	acute lymphoblastic leukemia
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
AUC	area under the curve
BCR	breakpoint cluster region
BSA	body surface area
BUN	blood urea nitrogen
CML	chronic myeloid leukemia
CRF	case report/record form
ECG	Electrocardiogram
HSCT	hematopoietic stem cell transplantation
IRB/EC	Institutional Review Board/Ethics Committee
LDH	lactic dehydrogenase
Ph chromosome	Philadelphia chromosome
PK	Pharmacokinetic
PLT	platelet count
RT-PCR	reverse-transcriptase polymerase-chain-reaction
SAE	serious adverse event
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
TdT	terminal deoxynucleotidyl transferase
ULN	upper limit of normal range
WBC	white blood cell count

PROTOCOL SYNOPSIS

Title	An open-label study to evaluate the safety and efficacy of IMATINIB with chemotherapy in pediatric patients with Ph+/BCR-ABL+ Acute Lymphoblastic Leukemia (Ph+ALL).
Study phase	Phase II
Patients and Stratification	<p>Pediatric patients with Philadelphia-Positive ALL (Ph+ALL) will be stratified as <u>Good-Risk Ph+ALL</u> and <u>Poor-Risk Ph+ALL</u>.</p> <p>a. <u>Poor-Risk group</u>: for protocols which adopt a steroid prephase patients who are Prednisone-poor responder (i.e. blast cell count $\geq 1000/\mu\text{l}$ in peripheral blood after 7 days of Prednisone given in combination with intrathecal Methotrexate), for protocols which do not adopt steroid prephase patients who have M3 BM at day 15 or M2/M3 BM at day 21; for all protocols patients who do not achieve CR after the induction course.</p> <p>b. <u>Good-Risk group</u>: for protocols which adopt a steroid prephase patients who are Prednisone-good responder (i.e. blast cell count $< 1000/\mu\text{l}$ in peripheral blood after 7 days of Prednisone given in combination with intrathecal Methotrexate) and achieve CR after the induction course; for protocols which do not adopt steroid prephase patients who have M1/M2 BM at day 15 or M1 BM at day 21 and achieve CR after the induction course.</p> <p>The expected stratification of the Ph+ALL population is as follows: 25-30% in the Poor-Risk group and 70-75% in the Good-Risk group.</p>
Primary objective	To evaluate in patients with Ph+ALL the efficacy and safety of IMATINIB continuous exposure on top of intensive, BFM-type chemotherapy. The endpoint for response will be the evaluation on the long-term clinical outcome.
Secondary objectives	<p>A. To compare the outcome with historical controls of patients treated with BFM oriented protocols (including patients treated with Imatinib in the original EsPhALL protocol) and with recent results from the COGAALL0031 (Children Oncology Group-USA) study, which adopts a more intensive chemotherapy approach than BFM.</p> <p>B. To evaluate the overall EFS, DFS and survival in both risk groups.</p> <p>C. To assess the antileukemic potential of IMATINIB given to patients with Ph+ALL. The pattern of the molecular response will be analyzed on the basis of 5 measurements taken at different time points from the beginning of phase IB to the beginning of the reinduction phase or HSCT. The findings will be compared with historical controls.</p> <p>D. To evaluate the impact of HSCT on prognosis (EFS/DFS).</p>

	<p>E. To evaluate the impact of MRD on prognosis (EFS/DFS).</p> <p>F. To evaluate possible delays in chemotherapy administration under Imatinib continuous exposure</p>
Treatment groups	The target population includes patients aged more than 365 days and less than 18 years with documented Ph+ALL enrolled in the national study groups of AIEOP, BFM-G/CH and BFM-A, COALL, DCOG, EORTC, FRALLE, UKALL, NOPHO,CPH, PINDA and HONG KONG.
Study design	International, intergroup, multicenter, open-label, phase II study
Study size	Overall, these groups are expected to contribute 55 Ph+ALL patients per year.
Study duration	Start of patient enrollment in January 2010. Duration of enrollement 2 years.
Eligibility Criteria – Inclusion criteria	Children and adolescents aged 1-17 years at diagnosis, with Ph+ALL documented by either cytogenetics , PCR or FISH for bcr-abl, who are eligible for the current local prospective therapeutic study of childhood ALL and for whom informed consent was given by the parents or by legal guardian.
Eligibility Criteria – Exclusion criteria On day 15 at the start of The first course of IMATINIB	<p>1.Abnormal hepatic function (ALAT/ASAT > 10 times the upper limit of the normal range);</p> <p>2.Abnormal renal function (creatinine > 1.5 times the upper limit of the normal range or a calculated creatinine clearance of 80ml/min or less, adjusted to a body surface area of 1.73 sqm);</p> <p>3.Active systemic bacterial, fungal or viral infection as documented by positive cultures, radiological imaging techniques, septic shock symptoms.</p>
Primary endpoint	Long term clinical outcome in Ph+ALL, will be evaluated with Disease free survival (DFS). DFS will be calculated as the time from accrual to either one of the following events: relapse, death in CCR, second malignancies.
Secondary endpoints	<p>1. Feasibility and safety of the addition of IMATINIB to conventional chemotherapy schedule.</p> <p>2. Long term clinical outcome (EFS, survival)</p> <p>3. Pattern of molecular response at the 5 scheduled time points for MRD measurement.</p> <p>4. Conversion rate to CR in patients resistant to the first part of the induction phase of chemotherapy included in the Poor-risk group.</p>

1. Introduction

1.1 Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL)

Clinical features and results of chemotherapy and stem cell transplantation.

Recent advances in treatment have increased the cure of childhood ALL to 75 percent or better (1,2). However attempts to improve results for resistant subtypes of ALL, such as Ph+ ALL, have been largely unsuccessful. Overall Ph+ALL, which accounts only for 3-5% of children with ALL have a dire prognosis (rates of EFS are 25-30% in children and even less in adults) (3). Some investigators suggest that Ph+ALL in childhood is heterogeneous with regard to sensitivity to treatment. Good initial response to steroids (which are given in combination with intrathecal methotrexate before induction chemotherapy is instituted) as well as age and leukocyte count at diagnosis, have been shown to correlate with a good clinical outcome in children treated only with chemotherapy (3,4). The heterogeneity of Ph+ALL with respect to clinical outcome has been confirmed by the analysis of the largest series of pediatric ALL treated by 10 European and United States study groups or large single institutions from 1986 to 1996 (3). Among patients who presented with WBC higher than 100,000 per cubic millimeter, 85% did not have long-term EFS at five years. The inadequacy of current therapy for such patients, most of whom can be readily identified by their initial response to prednisone, indicates a need for new treatments. Patients who are younger than 10 years old and have a WBC less than 50,000 per cubic millimeter at the time of diagnosis have about a 50 percent chance of long term DFS whereas the remaining patients (those with WBC of 50,000 to 100,000 per cubic millimeter and those with less than 50.000 leukocytes per cubic millimeter who are older than 10 years of age) have an intermediate prognosis (estimate of five-year DFS, 30 %) (3, 5). Stem cell transplantation from HLA-matched related donor yielded till recent updates a significant better outcome than chemotherapy alone (3,6). The absence of any significant superiority to chemotherapy in patients undergoing SCT from a mismatched donor or matched unrelated donor (MUD), could be explained by the high number of transplantation-related deaths, reported in this study (6). In most recent years better results have been obtained with unrelated donor HSCT, in series which include either children and adults (7). The leukemic cell burden present before HSCT influences the rate of relapse-free survival: patients with detectable BCR-ABL-expression prior to HSCT have a significantly worse prognosis. Overall these findings underline the need of large prospective cooperative studies worldwide to generate and test relevant hypothesis to gain better clinical results in a dismal subset of ALL in children. For these studies it has been evaluated that initial response to therapy identified patients with different risk (4). This is well documented by the study on the role of response to prednisone in

the “Berlin-Frankfurt-Munster” (BFM) protocols (Fig.1)(4) and it has been confirmed, as far as early morphological bone-marrow evaluation is concerned, by an analysis of the BFM data, as shown in Fig.2 (M.Shrappe, personal communication ,2002)

Fig. 1: EFS according to Prednisone Response in Childhood Ph+ ALL
Results from BFM and AIEOP (1986-95)

sti_new.ppt [M.S.]

Schrappé M, Arico M, Harbott J, et al. BLOOD 92 (1998): 2730

Pathogenetic role of the BCR-ABL translocation

Ph+ALL is characterised by a reciprocal translocation between chromosomes 9 and 22 (Philadelphia chromosome) that fuses genetic sequences of the *bcr* gene on chromosome 22 with *c-abl* sequences translocated from chromosome 9. The chimeric *bcr-abl* gene generates several types of fusion proteins, of which the p185^{BCR-ABL} form is detectable in 85% of patients with Ph+ALL, whereas the p210^{BCR-ABL} chimeric protein typical of CML is observed in approximately 10% of patients. The BCR-ABL fusion proteins are characterised by a constitutive protein tyrosine kinase (PTK) activity that is absent in the normal ABL protein. This dysregulated PTK activity, which results in changes of multiple signal transduction pathways, is crucial to the transforming activity of the BCR-ABL fusion proteins and their ability to cause leukemias *in vivo*. Therefore, inhibition of the PTK activity of this oncoprotein is a rational therapeutic approach for BCR-ABL expressing leukemia (15).

The ABL tyrosine kinase inhibitor IMATINIB

IMATINIB is an inhibitor of the protein-tyrosine kinases associated with Bcr-Abl, the platelet-derived growth factor (PDGF) receptor and c-Kit, but not of other members of the Type III receptor kinase family, such as Flt-3 and Fms (reviewed in Ref. 16). IMATINIB shows selectivity for the Abl protein-tyrosine kinase at the *in vitro*, cellular and *in vivo* level (17). The compound

specifically inhibits proliferation of Bcr-Abl expressing cells. In colony forming assays using *ex vivo* peripheral blood and bone marrow samples, IMATINIB shows selective inhibition of Bcr-Abl positive colonies from CML patients (18). In animal models, the compound shows potent anti-tumor activity against Bcr-Abl and v-Abl expressing cells at tolerated doses. Studies in bcr-abl CML patients showed a hematological response in 95% of patients in the chronic phase, 60% in accelerated phase and 30% in blast crisis. The cytogenetic response rates in these 3 groups were respectively 60%, 24% and 16% (19-22).

Inhibition of the Bcr-Abl kinase has been shown to have anti-leukemic effects not only in chronic myeloid leukemia but also the subset of B-precursor ALL characterised by the translocation (9;22) which leads to formation of the Philadelphia chromosome. In 2001 IMATINIB has been registered for CML treatment and it is currently being tested in several phase II and III trials covering most Ph+ALL patients (ALL0031, EsPhALL).

Results of IMATINIB in Ph⁺ALL

Philadelphia chromosome positive acute lymphoblastic leukemia (Ph⁺ALL) represents a suitable disease target for therapy with IMATINIB given that the Bcr-Abl kinase is believed to play a dominant leukemogenic role in this disease. The activity of single-agent imatinib was initially investigated in patients with relapsed or refractory Ph+ ALL. A phase 1 clinical trial of imatinib at doses of 300 to 1000 mg daily led to a 70% hematologic response rate with a 20% CR rate (19). A phase 2 trial of intermediate-dose imatinib yielded a CR rate of 29% (19,23,24). Disease recurrence was usually observed within a median of 2 months, and responses were durable only in a minority of patients. Relapse in the central nervous system (CNS) was not uncommon, as imatinib concentrations in the cerebrospinal fluid only reach 1% to 2% of detectable serum levels, emphasizing the need for concurrent CNS prophylaxis (25). However, despite its activity in this disease, treatment of patients with advanced, i.e. relapsed or refractory Ph+ALL with IMATINIB, has in most cases had only temporary effects, with frequently rapid occurrence of resistance (23, 24). Resistance to IMATINIB is established via an increase in BCR/ABL mRNA, amplification of the oncogene, altered drug metabolism/transport, point mutations of the ABL catalytic domain (26) or compensatory mutations in genes other than BCR/ABL.

Imatinib was thus incorporated into combination chemotherapy regimens typically used for *de novo* Ph+ ALL, either concurrently (simultaneous imatinib and chemotherapy) or sequentially (alternating imatinib with chemotherapy). The first report of a clinical trial of this nature included 20 patients with *de novo* or minimally treated Ph+ ALL (no age restrictions) (27). Imatinib was given concurrently with the hyper-CVAD regimen (fractionated cyclophosphamide, vincristine,

doxorubicin, and dexamethasone alternating with cycles of high-dose methotrexate and cytarabine); CR rate was 96%, with a 2-year DFS rate of 85%. The molecular remission rate or negativity for *BCR-ABL* transcripts by RT-PCR and nested PCR approached 60%. The addition of imatinib to the chemotherapy improved outcome. Lee et al also reported favorable outcomes after incorporating imatinib into a conventional L-asparaginase-based ALL regimen for newly diagnosed Ph+ ALL (28).

Yanada et al observed a CR rate of 96% and molecular remission rate of 71% in *de novo* patients with Ph+ ALL aged less than 65 years after concurrent imatinib and induction chemotherapy followed by alternating blocks of imatinib and consolidation chemotherapy (29). Long-term DFS and overall survival rates were significantly superior to the historical experience in these studies. In a subsequent report of outcome with imatinib-based frontline chemotherapy, two sequential cohorts of patients with *de novo* Ph+ ALL were treated according to German Multi-Centre Acute Lymphoblastic Leukemia (GMALL) protocols. First, a treatment regimen consisting of alternating blocks of chemotherapy and single-agent imatinib was designed because of concerns of potential toxicity. Once the feasibility and tolerance of concurrent imatinib and chemotherapy was demonstrated by other investigators, a concurrent regimen was implemented. The superiority of the latter approach was evidenced by a higher rate of molecular remission (52% vs 19%; $P > .01$), although the greater antileukemia efficacy did not translate into significant improvements in DFS or overall survival compared with the alternating regimen (30).

Tolerability of IMATINIB

In over 3000 patients treated, IMATINIB has been generally well tolerated, enabling chronic once daily oral dosing. There has been one death due to hepatic toxicity in a 58-year-old Indian patient in CML accelerated phase treated at the 600 mg/daily in which the causality assessment to trial drug was suspected. As this patient was taking acetaminophen 0.5 gm 6-8 times/daily, a drug interaction between IMATINIB and acetaminophen has to be considered. This death occurred against a background of Grade 2/3 elevations in liver transaminases in seven patients, without a clear dose-relationship. These elevations were often attributed to progression of the underlying disease. When suspected to be related to study drug, they were managed successfully by temporary discontinuation of drug, whereupon transaminase values promptly decreased. Modest dose reductions upon re-initiation of therapy were performed for Grade 3 toxicity, and no patient has permanently discontinued therapy due to hepatic toxicity.

The most commonly reported adverse event related to imatinib administration was mild nausea, which has been observed in more than 40% of patients. It is now established that nausea may be

prevented or mitigated by the prior ingestion of a small snack. Arthralgias, myalgias and periorbital edema have each been observed in 10% of patients. Interstitial edema and weight gain have been seen in some patients treated with 600 mg and 750 mg/daily; however, these findings were not associated with capillary leak syndrome and no patient has experienced congestive heart failure or other adverse events as a result of fluid retention.

Several patients have experienced episodes of gastrointestinal bleeding associated with erosions at the gastroesophageal junction and presumed due to the irritant effect of the drug.

In a phase II study published by Ottmann in 2002 (24), Imatinib toxicity profile was similar to that observed in the trials of single agent imatinib for Ph+ CML, and included transient myelosuppression, fluid retention syndrome, nausea, muscle cramps, rash, and transient elevations in hepatic transaminases. When incorporated into combination chemotherapy regimens the safety profile of the drug still proved to be tolerable. Not surprisingly, the use of concurrent imatinib and L-asparaginase often resulted in hyperbilirubinemia, requiring dose interruptions or modifications of therapy.

Experience of IMATINIB in pediatric patients

A Phase I study of Imatinib, POG-P9973, was conducted by POG to estimate the maximum tolerated dose (MTD) of Imatinib administered orally once daily, without interruption to children with recurrent Ph+ leukemia.

No MTD was observed up to 570 mg/m², equivalent to the adult phase 2 and 3 dose. Grade 3-4 neutropenia was seen in the 23 of 93 courses, thrombocytopenia in 16 of 93 courses. Some of the hematologic toxicity may have been secondary to underlying leukemia as they were present in 25-30% during the first course of treatment. Gr 3-4 diarrhea was observed in 15/ 93, nausea in 16/93 vomiting in 13/93, abdominal cramping 6/ 93. Grade 3 liver enzyme elevation occurred in 1/93. One intracranial hemorrhage occurred in a patient who was thrombocytopenic and was receiving enoxaparin.

The most common toxicity was grade 1 nausea, seen in 7 of 14 patients (50%). Diarrhea, vomiting, abdominal pain and headaches were each reported in 3 patients (21%). Other toxicities reported in 2 patients each included fatigue, stomatitis, bone pain, and various metabolic alterations (increased alkaline phosphatase, increased AST, hyperglycemia). Patients accrued on the first 3 treatment levels cumulate a total of 43 courses of treatment (median 3; range 2-8).

The recently completed COG AALL0031 trial established the safety and tested the efficacy of an intensive chemotherapy backbone plus imatinib in treating children with Ph+ ALL. To date, addition of imatinib has been safe and associated with relatively minor additional toxicity (mild

asymptomatic transaminitis requiring intermittent rather than continuous dosing during Maintenance therapy).

Among pediatric patients with Ph+ ALL, there have been limited reports evaluating the impact of imatinib on either induction CR rates and/or overall outcomes. In a short report by Fuster et al.

(31), the authors describe four pediatric patients with Ph+ ALL (ages 2–8 years) treated with imatinib in combination with chemotherapy prior to proceeding to allogeneic HCT. All patients achieved a molecular remission (undetectable bcr/abl transcript by RT-PCR) by 16 weeks (range, 2–16 weeks) from the start of combination therapy and were alive in hematologic remission after a median time of 24 months (range, 11–53 months) from initiating imatinib.

Burke et al reported 37 children diagnosed with Ph+ B-precursor ALL receiving a myeloablative allogeneic HCT (32). Patients received similar pre-HCT chemotherapy with the majority receiving Berlin–Frankfurt–Munster (BFM) based regimens according to Children’s Cancer Group (CCG) or COG protocols. Thirteen patients received imatinib therapy either pre- and/or post-HCT comprising the imatinib group. The remaining 24 patients either never received imatinib or received it only at time of relapse post-HCT. IMATINIB doses ranged from 240 to 340 mg/m²/day. Patients treated with IMATINIB pre-HCT received therapy for a median of 3 months (range, 1–27 months). When IMATINIB therapy was used postallogeneic HCT, it was started, on average, on day 120 (range, 80–180) and continued for a median of 8 (range, 2–10) months. OS and DFS at 3 years was 59% and 62% for the IMATINIB group compared to 58% and 53% for the non-IMATINIB group (P = 0.80 and 0.9 respectively)

Main Study COG AALL0031 (33).

This study was a phase III open, non-controlled, dose-escalation study conducted by the Children’s Oncology Group (COG), a NCI supported clinical co-operative group, in the US. Study Participants were male and female patients younger than 21 years old with Very High Risk leukemias including 93 patients with Ph+ leukaemia.

The chemotherapy regimen was based on previous strategies in which patients first received 4 weeks of standard induction chemotherapy and were entered onto AALL0031, which included an intensive consolidation phase followed by a continuation regimen. All patients received a minimum of two consolidation chemotherapy blocks. Patients with an HLA-matched related donor entered the BMT arm following these blocks. Total duration of chemotherapy for those not receiving BMT was approximately 27 months. For patients with Ph+ALL, imatinib 340 mg/m²/d was introduced into the chemotherapy regimen in a stepwise fashion, with toxicity assessed for each cohort before progression to the next cohort. Each cohort had 12 subjects except for cohort 1 (n=7), which was discontinued early on the basis of published data demonstrating acceptable imatinib toxicity with

high-dose methotrexate. Cohort 5 was expanded to accrue a total of 50 patients to provide a more precise estimate of outcome. The total imatinib exposure (before maintenance) was 42 days in cohort 1, 63 days in cohort 2 (n=17), 84 days in cohort 3, 126 days in cohort 4 (n=22), and 280 days in cohort 5 (n=44). All groups received an additional 336 days of imatinib exposure in maintenance cycles 1 through 12. For all patients receiving BMT on protocol, imatinib was started between week 16 and week 24 after BMT when the absolute neutrophil count was ≥ 750 and the platelet count was $\geq 75,000$ given for a total of 24 weeks. Dosing started at $230\text{mg}/\text{m}^2/\text{d}$ and increased after 28 days to $340\text{mg}/\text{m}^2/\text{d}$ if no grade 3 or 4 toxicity was observed.

This study aimed to determine the feasibility in terms of patient accrual and toxicity of an intensified chemotherapeutic regimen incorporating novel agents for treatment of children and adolescents with very high risk ALL.

The 3-year EFS of patients in cohort 5 receiving continuous imatinib was $80.5\% \pm 11.2\%$ (95% CI, 64.5% to 89.8%), including those assigned to a sibling BMT. This is significantly higher than historical controls, after excluding induction failures from previous POG studies (N=120; 3-year EFS, $35.0\% \pm 4.4\%$; $P < .0001$).

Twenty-one patients had matched sibling transplants (8 of 39 in cohorts 1-4 and 13 of 44 in cohort 5). There was no significant difference in 3-year EFS between patients (n = 25) treated with cohort 5 chemotherapy ($87.7\% \pm 10.9\%$; 95% CI, 66.4% to 95.8%), patients (n=21) receiving BMT from a sibling donor ($56.6\% \pm 21.5\%$; 95% CI, 30.4% to 76.1%), and patients (n = 11) receiving BMT from an alternative donor ($71.6\% \pm 19.0\%$; 95% CI, 35.0% to 89.9%; $P = .14$).

Role of Hematopoietic Stem Cell Transplantation (HSCT) in Ph positive ALL

Because of the universally dismal prognosis of *de novo* Ph+ ALL in the pre-imatinib era, all patients who achieved CR were recommended to undergo allogeneic SCT as feasible, inclusive of all stem cell sources such as matched unrelated marrow and umbilical cord blood. The benefits of SCT in first CR were attributed to the intense myeloablative therapy and graft-versus-leukemia effect; the high risk of transplantation-related mortality was accepted given the alternative of poor outcomes with chemotherapy alone. Two large multicenter trials confirmed the benefit of allogeneic SCT in the pre-imatinib era. In the Ph+ subset of patients with ALL (n=167) enrolled in the UKALL XII/ECOG E2993 trial, the 5-year relapse risk was decreased from 81% with either chemotherapy alone or autologous SCT to 32% with allogeneic SCT (8). Five-year event-free survival (EFS) and overall survival rates improved from 17% to 36% and 19% to 42%, respectively. Furthermore, the prospective multicenter French, Belgian, Swiss and Australian LALA-94 trial of

154 patients with Ph+ ALL showed that achievement of negativity for BCR-ABL by RT-PCR and undergoing allogeneic SCT predicted for improved DFS and overall survival (9).

The role of allogeneic SCT for *de novo* Ph+ ALL in the imatinib era continues to be refined, with feasibility of this approach still limited by the availability of an appropriate donor, absence of significant comorbidities, and ability to sustain a complete remission. Advances in SCT such as the application of nonmyeloablative reduced intensity conditioning regimens to patients with comorbidities prohibiting traditional myeloablative regimens, in addition to increased availability of umbilical cord blood as a source of stem cells, have allowed this modality to be applied in a more systematic fashion (10).

Several studies have reported an improvement in the rate of allogeneic SCT in first CR after imatinib-based therapy compared with the prior experience (27,29,30,38). This success is in part related to (1) an increase in the proportion of sustained remissions, offering additional time for identification of a suitable donor, and to (2) an improvement in the quality of the remissions (e.g., lower levels of *BCR-ABL* transcripts after imatinib-based therapy), resulting in a lower pretransplantation tumor burden. Two of the early nonrandomized studies of imatinib-based chemotherapy for *de novo* Ph+ ALL applied allogeneic SCT in first CR as standard of care when feasible. Similar survival outcomes were observed with or without allogeneic SCT, despite the selection biases favoring SCT (27,29,38). Additional experience and longer follow-up is needed to clarify whether allogeneic SCT can be deferred in a select group of patients with Ph+ ALL otherwise eligible for this modality. To this regard, the Gandemer series identified two groups of patients with marked differences in five-year outcome: children with age<10, leukocyte count <100,000/mm³ and day-21 M1 marrow had a more favorable prognosis (14 pts: 100% CR, event free survival [EFS]: 57%, overall survival [OS]: 79%), than the high-risk group (22 patients: 55% CR, EFS: 18%, OS: 27%) ($p < 0.005$) (41). The combination of available tools such as minimal residual disease assessment with determination of these simple factors could be useful for refining indications for BMT in the current era of tyrosine-kinase inhibitor-based treatment.

Postallogeneic SCT maintenance strategies are also being explored, particularly as the detection of minimal residual disease (MRD) following SCT predicts imminent relapse in the absence of intervention (40). Wassmann et al (11) investigated the use of single-agent imatinib in the post transplantation setting after detection of MRD by quantitative RT-PCR for BCR-ABL. Standard-dose (400 mg) imatinib resulted in eradication of molecular disease in 52% of the 27 patients treated. Notably, failure to achieve molecular remission within the first 6 weeks of therapy heralded overt leukemia relapse despite other additional manipulations (e.g., donor lymphocyte infusions). Using imatinib in the post-transplantation setting in a prophylactic manner, immediately after

engraftment and prior to the detection of MRD, may further improve outcome by preventing resurgence of the leukemia clone. Two small series have shown that this approach is feasible, with transient elevations in hepatic transaminases usually responding to dose interruptions or modifications (12, 13). Additional experience will be required to determine whether imatinib monotherapy after transplantation would eventually lead to development of resistance.

2. Study population

2.1 Patient population

The target population includes children and adolescents with newly diagnosed and documented Ph+ALL enrolled in the treatment front-line protocols of the national study groups of AIEOP, BFM-G/CH and BFM-A, COALL, DCOG, EORTC, FRALLE, UKALL, NOPHO, CZECH REPUBLIC, PINDA and HONG KONG.

2.2 Inclusion and exclusion criteria

2.2.1 Inclusion criteria

The criteria for the patients with newly diagnosed Ph+ALL to enter the study are:

1. Age greater than 1 year (365 days) and less than 18 years (17 years and 365 days) at diagnosis.
2. Documented presence of t(9;22)(q34;q11) determined by institutional cytogenetics or FISH and/or of the presence of BCR-ABL fusion transcript identified by RT-PCR or FISH.
3. Eligibility for the current local prospective therapeutic study of childhood ALL.
4. Informed consent given by the parents or by the legal guardian.

It is important that all children with Ph+ALL, including those who are eligible but are not treated according to the present protocol will be registered so that any selection bias can be monitored.

2.2.2 Exclusion criteria (on day 15 at the time to start IMATINIB)

1. Abnormal hepatic function (ALAT/ASAT > 10 times the upper limit of the normal range);
2. Abnormal renal function (creatinine > 1.5 times the upper limit of the normal range or a calculated creatinine clearance of 80 ml/min or less, adjusted to a body surface area of 1.73 m²);
3. Active systemic bacterial, fungal or viral infection as documented by positive cultures, radiological imaging techniques, septic shock symptoms.

2.3 Stratification

All patients will receive the induction phase according to national/group study treatment protocol. Either early response to treatment parameters (PB blast/count after 7 days of prednisone given in combination with intrathecal methotrexate before induction chemotherapy is instituted; or evaluation of BM at day 15 and 21) or no achievement of CR (cytology) at the end of induction,

will allow the definition of Ph+ALL risk- subgroups in the different study groups. More precisely, the following definitions hold:

- c. Poor-Risk group: for protocols which adopt a steroid prephase patients who are Prednisone-poor responder (i.e. blast cell count $\geq 1000/\mu\text{l}$ in peripheral blood after 7 days of Prednisone given in combination with intrathecal Methotrexate), for protocols which do not adopt steroid prephase patients who have M3 BM at day 15 or M2/M3 BM at day 21; for all protocols patients who do not achieve CR after the induction course.
- d. Good-Risk group: for protocols which adopt a steroid prephase patients who are Prednisone-good responder (i.e. blast cell count $< 1000/\mu\text{l}$ in peripheral blood after 7 days of Prednisone given in combination with intrathecal Methotrexate) and achieve CR after the induction course; for protocols which do not adopt steroid prephase patients who have M1/M2 BM at day 15 or M1 BM at day 21 and achieve CR after the induction course.

3. Study objectives

3.1 Primary objective

To evaluate in patients with Ph+ALL the efficacy and safety of IMATINIB continuous exposure on top of intensive, BFM-type chemotherapy. The endpoint will be the evaluation on the long-term clinical outcome.

3.2 Secondary objectives

- ◆ To compare the outcome with historical controls of patients treated with BFM oriented protocols (including patients treated with Imatinib in the original EsPhALL protocol) and with recent results from the COGAALL0031 (Children Oncology Group-USA) study, which adopts a more intensive chemotherapy approach than BFM.
- ◆ To evaluate the overall EFS,DFS and survival.
- ◆ To assess the antileukemic potential of IMATINIB by analyzing the pattern of molecular response on the basis of 5 MRD measurements taken at different time points from the beginning of phase IB to the beginning of protocol II or HSCT at week 22.
- ◆ To evaluate the impact of MRD on prognosis (EFS/DFS).
- ◆ To evaluate the impact of HSCT on prognosis (EFS/DFS).

- ♦ To evaluate possible delays in chemotherapy administration under Imatinib continuous exposure

Fig. 3 European intergroup study on treatment of Ph+ALL with IMATINIB

4. Overall study design

In the context of Ph+ALL most of the data produced so far have shown only a transient effect in blast cell reduction. These observation prompted several investigators to consider the use of IMATINIB in the context of multi-agent approach. The clinical heterogeneity of Ph+ALL will offer the opportunity to test the impact of IMATINIB in two subgroups of patients as defined by early response to treatment (Poor and Good Risk Ph+ALL).

While we allow for differences in induction therapies applied by different groups, it is very important that all subsequent therapy plan is uniform. For this purpose, the current AIEOP-BFM-ALL 2000 strategy for High-risk patients has been considered appropriate. Treatment starts with approximately 4 weeks of induction therapy according to the national protocol. During this, the Ph+ status of the patient has to be determined. After this first induction therapy, further treatment will be according to the international schedule. This will include a phase IB of induction, followed by three blocks of treatment as shown in detailed in Fig.4 - 7. With respect to the different proposed arms for reinduction therapy, the AIEOP variant which included a modified- BFM protocol II repeated twice, will be used, as shown in Fig.8 , 9.

Good-risk and Poor Risk Ph+ALL patients will receive IMATINIB starting on day 15 during Induction phase and continuously throughout the consolidation and reinduction phase of chemotherapy

All patients will be screened for an HLA-identical family or unrelated donor.

Good-risk patients: patients with a genotype-matched donor (9/10 or 10/10), will receive HSCT, while the others will continue on chemotherapy, thus receiving IMATINIB in combination with the standard chemotherapy.

Poor-risk patients: patients will be eligible for any type of donor (matched or mismatched family donors, unrelated or haploidentical donors). Patients not transplanted will continue on chemotherapy, thus receiving IMATINIB in combination with the standard chemotherapy.

5. Treatment plan

5.1 Frontline induction

All patients will receive the first induction according to national/group study treatment protocol.

Imatinib 300 mg/m² daily will be introduced on day + 15 from diagnosis in all patients on top of ongoing chemotherapy till day + 33.

5.2 Phase IB

Figure 4.

IMATINIB : 300 mg/m²/day orally days 1-28 (total 28 days).

All Good and Poor Risk Patients will receive IMATINIB. To allow close patient monitoring, patient will attend outpatient clinics at least weekly because of ARA-C injections.

Requirements for beginning of phase IB:

1. Good general condition without serious infections;
2. Creatinine level within normal limits according to age;
3. WBC > 2000/μl, ANC > 500/μl; platelets > 50,000/μl

CYCLOPHOSPHAMIDE (CPM): 1000 mg/m²/dose i.v. (1 hour) days 1,28.

- Associate:
- * hyperhydratation 3.000 ml/m² over 24 hours : G 5%+
NaCl 0,45%+ 90 mEq/m²KCl
 - * MESNA (1/3 of CPM dose, hours 0, 4, 8 from CPM start).
 - * FUROSEMIDE 0.5-1 mg/kg i.v if input > output +400ml/ m²/12 h.

Please consider adequate anti-emetic supportive therapy.

6-MERCAPTOPURINE (6-MP): 60 mg/m²/day p.o., to be taken in the evening on an empty stomach (1 hour before or after dinner), not together with milk, days 1-28(total 28 days).

CYTOSINE ARABINOSIDE (ARA-C): 75 mg²/day s.c. o i.v. in one daily dose, days 3-6, 10-13, 17-20,24-27.

Each 4-day cycle should be started when WBC >500 μ l and platelets >30.000/ μ l; each cycle when started should not be stopped unless for acute infection (please consider that fever might be also induced by the drug).

INTRATECHAL METHOTREXATE : days 3, 17 (together with ARA-C cycles 1 and 3), age-dosed:

AGE	MTX
≥1 year <2 years	8 mg
≥2 years <3 years	10 mg
≥3 years	12 mg

5.3 Consolidation Block 1 (HR1')

Figure 5.

Consolidation therapy will start on day 78 of therapy, provided the patient is in good general condition and adequate ANC ($\geq 500/\mu\text{l}$) and platelet counts ($\geq 50.000/\mu\text{l}$) are documented (rising counts). Imatinib administration will not be interrupted between the end of phase IB and the beginning of the first HR block. **The interval between each block element should be 21 days (counting from day 1 of HR-1 to day 1 of HR-2).**

DEXAMETHASONE (DXM): 20 mg/m²/day p.o. or i.v. in 3 doses, days 1-5 (no tapering).

VINCRIStINE (VCR): 1.5 mg/m²/day i.v.(max dose: 2mg), day 1 and 6.

HIGH-DOSE METHOTREXATE (HD-MTX): 5 g/m²/dose i.v. over 24 hours on day 1 (1/10 in 30 minutes, the remaining 9/10 in 23.5 hour-infusion). Hyperhydration: 3.000 ml/m² over 24 hours : Gluc. 5%+ NaCl 0,45%+ 90 mEq/m²KCl+NaHCO₃ 90 mEq/m². Urine pH>7.0 over the time of infusion.

Serum levels of MTX must be determined at hours 24, 42, 48 from start of MTX infusion. For monitoring of MTX serum levels and intensification of LCV rescue, see Appendix I.

CITROVORUM FACTOR (Folinic acid): 7.5 mg/m² i.v (Levo form) or 15 mg/m² i.v (Racemic form) at hours 42, 48, 54 from start of infusion. For monitoring of MTX serum levels and intensification of LCV rescue, see Appendix I.

Starting from hour 60, Citrovorum Factor is needed only if serum levels at hour 48 exceed 0.5 $\mu\text{mol/l}$. In this case, see nomogram for therapeutic adjustments.

HIGH-DOSE ARA-C (HD-ARA-C): 2 gr/m²/iv in 3-hour infusion, repeated after 12 hours, day 5. The use of prednisolone eye drops is suggested.

HIGH-DOSE L-ASPARAGINASE (HD-L-ASP): E. coli (medac or kidrolase): 25.000 IU/m²/dose, over 2 h i.v., 3 hours after completion of the infusion of the second dose of HD-ARA-C. In case of an allergic reaction , PEG-ASP(ONCASPARG) may be used in a single dose of 1000 I.E./m² over 1 h i.v.

CYCLOPHOSPHAMIDE (CPM): 200 mg/m² i.v. in 1 hour q 12 hours, 5 doses, days 2-4. Start immediately after the completion of HD-MTX infusion. MESNA 70 mg/m² hours 0, 4 and 8 from start of CPM.

INTRATHECAL THERAPY: day 1, 2 hours after start of HD-MTX, dose according to age:

AGE	MTX	ARA-C	PRED
≥1 year <2 years	8 mg	20 mg	6 mg
≥2 years <3 years	10 mg	26 mg	8 mg
≥3 years	12 mg	30 mg	10 mg

G-CSF: 5 µg/kg/day s.c. starting from the 5th day after completion of the block, until the WBC count is >20.000/µl

IMATINIB : 300 mg/m²/day orally starting from the first day of chemotherapy block and given for a total of 21 days. IMATINIB should not be given longer than 21 days if next chemotherapy block has to be postponed.

5.4 Consolidation Block 2 (HR2')

Figure 6.

#only if initially CNS positive

DEXAMETHASONE (DXM): 20 mg/m²/day p.o. o i.v. in 3 doses, days 1-5 (no tapering).

VINDESINE (VDS): 3 mg/m²/day i.v.(max 5 mg), days 1 and 6.

HIGH-DOSE METHOTREXATE (HD-MTX): 5 g/m²/dose i.v. on day 1 over 24 hours (1/10 in 30 minutes, the remaining 9/10 over a 23.5 hour-infusion). Hyperhydration: 3.000 ml/m² over 24 hours : Gluc. 5%+ NaCl 0,45%+ 90 mEq/m²KCl+NaHCO₃ 90 mEq/m². Urine pH>7.0 over the time of infusion.

Serum levels of MTX must be determined at hours 24, 42, 48 from infusion start. For monitoring of MTX serum levels and intensification of LCV rescue, see Appendix I.

CITROVORUM FACTOR (Folinic acid): 7.5 mg/m² i.v (Levo form) or 15 mg/m² i.v (Racemic form) at hours 42, 48, 54 from start of infusion. For monitoring of MTX serum levels and intensification of LCV rescue, see Appendix I.

Starting from hour 60, CF is needed only if serum levels at hour 48 exceed 0.5 µmol/l. In this case, see nomogram for therapeutic adjustments.

IFOSPHAMIDE (IFO): 800 mg/m² i.v. over 1-hour infusion, q 12 hours, 5 doses, days 2-4. Start immediately after completion of HD-MTX infusion. MESNA 300 mg/m² i.v. hour 0, 4 and 8 from start of infusion.

HIGH-DOSE L-ASPARAGINASE (HD-L-ASP): E. coli (medac or kidrolase): 25.000 IU/m²/dose, over 2 h i.v. In case of an allergic reaction, PEG-ASP(ONCASPAR) may be used in a single dose of 1000 I.E./m² over 1 h i.v., on day 5

DAUNORUBICIN (DNR): 30 mg/m²m over 24-hour infusion on day 5

INTRATHECAL THERAPY: day 1, 2 hours after start of HD-MTX, according to age:

AGE	MTX	ARA-C	PRED
≥1 year <2 years	8 mg	20 mg	6 mg
≥2 years <3 years	10 mg	26 mg	8 mg
≥3 years	12 mg	30 mg	10 mg

G-CSF: 5 µg/kg/day s.c. starting from the 5th day after completion of the block, until the WBC count is >20x10⁹/l.

IMATINIB : 300 mg/m²/day orally starting from the first day of chemotherapy block and given for a total of 21 days. IMATINIB should not be given longer than 21 days if next chemotherapy block has to be postponed.

5.5 Consolidation Block 3 (HR 3')

Figure 7.

DEXAMETHASONE (DXM): 20 mg/m²/day p.o. o i.v. in 3 doses, days 1-5 (no tapering).

HIGH-DOSE ARA-C (HD-ARA-C): 2 g/m² i.v. in 3-hour infusion, q 12 hours, 4 total doses, days 1-2.

VP-16: 100 mg/m² i.v. in 1 hour , q 12 hours, 5 total doses, days 3-5.

HIGH-DOSE L-ASPARAGINASE (HD-L-ASP): E. coli (medac or kidrolase): 25.000 IU/m²/dose, over 2 h i.v. In case of an allergic reaction ,PEG-ASP(ONCASPAR) may be used in a single dose of 1000 I.E./m² over 1 h i.v. on day 5

INTRATHECAL THERAPY: day 5, dose according to age:

AGE	MTX	ARA-C	PRED
≥1 year <2 years	8 mg	20 mg	6 mg
≥2 years <3 years	10 mg	26 mg	8 mg
≥3 years	12 mg	30 mg	10 mg

G-CSF: 5 µg/kg/day s.c. starting from the 5th day after completion of the block, until the WBC count is >20x10⁹/l.

IMATINIB : 300 mg/m²/day orally starting from the first day of chemotherapy block and given for a total of 20 days. IMATINIB should not be given longer than 20 days if next chemotherapy block has to be postponed.

5.6 Reinduction (Protocol II)

Reinduction with protocol II starts 14 days after completion of consolidation block-therapy (intended as day 5 of HR block 3) provided the patient is in good general condition and adequate ANC ($\geq 500/\mu\text{l}$) and platelet counts ($\geq 50.000/\mu\text{l}$) are documented. Protocol II comprises two phases, IIa and IIb.

Figure 8.

#only if initially CNS positive

5.6.1 Phase IIa

DEXAMETHASONE (DXM) : 10mg/m²/day p.o. in 3 doses, day 1-21, then tapered over 9 days by reducing the dose by 50% q 3 days.

VINCRIStINE (VCR): 1.5 mg/m²/dose i.v. (maximum dose 2.0 mg/dose), day 8, 15, 22, 29.

DOXORUBICIN (DOX)/ADRIAMYCIN (ADR): 25 mg/m²/dose i.v. to be infused over 1h on days 8, 15, 22, 29.

L-ASPARAGINASE (L-ASP) E. coli (medac or kidrolase): 10.000 IU/m²/dose, over 1 h i.v., days 8,11,15 and 18. In case of an allergic reaction , PEG-ASP(ONCASPARG) may be used in a single dose of 1000 I.E./m² over 1 h i.v.

IMATINIB : 300 mg/m²/day orally, days 1-35 (total 35 days)

5.6.2 Phase IIb

It starts on day 36 of protocol II provided the patient is in good general condition and adequate ANC ($\geq 500/\mu\text{l}$) and platelet counts ($\geq 50.000/\mu\text{l}$) are documented.

CYCLOPHOSPHAMIDE (CPM): 1000 mg/m²/dose i.v. (1 hour) day 36.

Associate: * hyperhydration 3.000 ml/m² over 24 hours : Gluc. 5%+ NaCl 0.45%+ 90 mEq/m² KCl .

* MESNA (1/3 of CPM dose, hours 0, 4, 8 from CPM start).

* FUROSEMIDE 0.5-1 mg/kg i.v if input>output +400 ml/m²/12h.

Please consider adequate anti-emetic supportive therapy.

6-THIOGUANINE (6-TG): 60 mg/m²/day p.o., taken in the evening on the empty stomach without milk (1 hour before or after dinner), days 36-49 (total 14 days).

CYTOSINE ARABINOSIDE (ARA-C): 75 mg/m²/day s.c. o i.v. in one daily dose, days 38-41, 45-48.

Each 4-day cycle should be started when ANC >200/ μl and platelets >50.000/ μl ; each cycle when started should not be stopped unless for acute infection (please consider that fever might be also induced by the drug).

INTRATHECAL METHOTREXATE : days 38, 45 (together with ARA-C cycles),dosage according to age:

AGE	MTX
≥ 1 year <2 years	8 mg
≥ 2 years <3 years	10 mg
≥ 3 years	12 mg

IMATINIB : 300 mg/m²/day orally, days 36-63 (total 28 days) for all patients

5.7 Interim maintenance

This short phase is aimed to allow administration of cranial irradiation during antimetabolite-based non-intensive chemotherapy. It will start 2 weeks after completion of the previous phase (day 49 of protocol II) and will last 4 weeks i.e. the time comprised between the first and the second administration of protocol II. Imatinib administration will not be interrupted between the end of protocol II and the beginning of interim maintenance.

6-MERCAPTOPURINE (6-MP): 50 mg/m²/day p.o., taken in the evening on the empty stomach without milk (1 hour before or after dinner), days 1-28.

METHOTREXATE (MTX): 20 mg/m²/dose p.o. once a week days 8,15,22,29.

IMATINIB : 300 mg /m²/day, days 1-29

In selected cases it could be necessary to start the two drugs with increasing doses according to the patient's compliance and/or haematological reconstitution.

CRANIAL IRRADIATION: During interim maintenance cranial irradiation will be administered to all patients at the dose of 18 Gy (single dose: 1.4-1.7 Gy).

Dose modifications:

- Patients younger than 2 years will receive 12-Gy.
- Patients with CNS-disease will receive 24 Gy.

5.8 Second administration of Protocol II

It will start immediately after the interim maintenance provided the patient is in good general condition and adequate ANC ($\geq 500/\mu\text{l}$) and platelet counts ($\geq 50.000/\mu\text{l}$) are documented.

Figure 9.

5.8.1 Phase IIa

DEXAMETHASONE (DXM) : 10 mg/m²/day p.o. in 3 doses, day 1-21, then tapered over 10 days by reducing the dose by 50% q 3 days.

VINCRIStINE (VCR): 1.5 mg/m²/dose i.v. (maximum dose 2.0 mg/dose), day 8, 15, 22, 29.

DOXORUBICIN (DOX)/ADRIAMYCIN (ADR): 25 mg/m²/dose i.v. to be infused over 1 h on days 8, 15, 22, 29.

L-ASPARAGINASE (L-ASP) E. coli (medac or kidrolase): 10.000 IU/m²/dose, over 1 h i.v. In case of an allergic reaction, PEG-ASP(ONCASPARG) may be used in a single dose of 1000 I.E./m² over 1 h i.v.

IMATINIB : 300 mg/m²/day orally, days 1-35 (total 35 days)

5.8.2 Phase IIb

It starts on day 36 of protocol II provided the patient is in good general condition and adequate ANC ($\geq 500/\mu\text{l}$) platelet counts ($\geq 50.000/\mu\text{l}$) are documented.

CYCLOPHOSPHAMIDE (CPM): 1000 mg/m²/dose i.v. (1 hour) days 36.

Associate: * hyperhydration 3.000 ml/m² over 24 hours : Gluc. 5%+ NaCl 0.45%+ 90 mEq/m² KCl.

* MESNA (1/3 of CPM dose, hours 0, 4, 8 from CPM start).

* FUROSEMIDE 0.5-1 mg/kg i.v if input>output +400 ml/m²/12h.

Please consider adequate anti-emetic supportive therapy.

6-THIOGUANINE (6-TG): 60 mg/m²/day p.o., taken in the evening on the empty stomach without milk (1 hour before or after dinner), days 36-49 (total 14 days).

CYTOSINE ARABINOSIDE (ARA-C): 75 mg/m²/day s.c. o i.v. in one daily dose, days 38-41, 45-48.

Each 4-day cycle should be started when WBC>500/ μl and platelets >30.000/ μl ; each cycle when started should not be stopped unless for acute infection (please consider that fever might be also induced by the drug.

IMATINIB : 300 mg/m²/day orally, days 36-63 (total 28 days) for all patients.

5.9 Continuation therapy

Continuation therapy will start 2 weeks after completion of delayed intensification and is based on antimetabolite drugs. Imatinib administration will not be interrupted between the end of phase IB and the beginning of continuation therapy.

6-MERCAPTOPURINE (6-MP): 50 mg/m²/day p.o., taken in the evening on the empty stomach without milk (1 hour before or after dinner).

METHOTREXATE (MTX): 20mg/m²/weekly p.o.

IMATINIB : 300 mg/m²/day p.o.

Chemotherapy will be modulated to keep WBC count between 1,000 and 3,000/μl. The dosage of the two drugs will be adjusted accordingly:

Leukocyte count /μl	Dose of 6-MP/MTX
<1.000	0%
1.000-2000	50%
2000-3000	100%
>3.000	consider to increase the dosage to achieve a WBC count between 2000 and 3000/μl
ANC < 500/μl	0%
Lymphocytes<300/μl	50%

COMPLETION OF TREATMENT

Total duration of chemotherapy will not exceed 24 months. At the time of treatment completion confirm CR by bone marrow aspirate and CSF examination.

6.0 Investigational drug

Glivec® (IMATINIB) will be supplied by Novartis as 100 mg capsules packaged in polyethylene bottles. Medication labels will comply with the legal requirements of each country and will be printed in the local language. They will supply no information about the patient, just the patient identification number. The storage conditions for the study drug will be described on the medication label. Bottles must be stored in a safe, secure location.

Glivec® should be administered each morning together with breakfast. Glivec® is a local irritant and must be taken in a sitting position with a large glass of water (250 ml/8oz; at least 100 ml/4 oz for children ≤ 3 years of age).

If the child/patient can not swallow the capsules the drug should be administered according to the following guidelines: pour the contents of a capsule by small portions into 20 ml of water, milk or apple juice. Stir with a spoon and administer the suspension immediately afterwards. Do not use any other beverage like Coca-Cola or orange juice. Note: the excipients used in the capsule will not dissolve. However, they are white whilst the active substance is yellow. Thus if a white solid residue remains in the glass, it does not matter as long as the capsule has been slowly added and well dispersed to allow the active substance to dissolve during stirring. If a yellow residue is observed, it means that the active substance was not completely dissolved and only a fraction of the dose has been swallowed.

Medications, which interfere with P-450 cytochrom metabolism (see Appendix III), should be avoided and recommended doses of acetaminophen, should not be exceeded. **The following medications and foods can interfere with P-450 metabolism:** grapefruit juice, erythromycin, azithromycin, clarithromycin, rifampin and its analogs, fluconazole, ketoconazole, itraconazole, cimetidine, cannabinoids (marijuana or dronabinol) and the leukotriene inhibitors used in asthma such as zafirleukast and zileuton. **In addition, drug interactions in patients receiving prochlorperazine (Compazine) and coumadin are possible. Patients who require prochlorperazine during therapy should be monitored for extrapyramidal symptoms and those on coumadin should have weekly prothrombin times while on therapy. These medications should not be used during IMATINIB administration unless there is unavoidable medical need and no other appropriate alternative agents are available. Also, acetaminophen should not be taken in greater than the recommended dose.**

7.0 Stem cell transplantation: All patients will be screened for an HLA-identical family or unrelated donor.

Good-risk patients: patients with a genotype-matched donor (9/10 or 10/10), will receive HSCT, while the others will continue on chemotherapy, thus receiving IMATINIB in combination with the standard chemotherapy.

Poor-risk patients: patients will be eligible for any type of donors (matched or mismatched family donors, unrelated or haploidentical donors). Patients not transplanted will continue on chemotherapy, thus receiving IMATINIB in combination with the standard chemotherapy.

7.1 Use of Imatinib after stem cell transplantation:

The use of IMATINIB is recommended in all HSCT recipient.

An initial dose of 200 mg/m² daily will be administered to all HSCT recipient provided they have a satisfactory PTL and WBC count with stable neutrophils engraftment (PTL > 50 x 10⁹/L; WBC > 1.5 x 10⁹/L; neutrophils > 0.5 x 10⁹/L for at least 15 days).

Imatinib dosage can be increased to a maximum of 300 mg/m² daily if well tolerated by the patient.

Imatinib administration is suggested throughout the first year post-transplantation, till day +365 from HSCT.

It is recommended that a bone marrow aspirate with morphologic evaluation and PCR detection of BCR/ABL is done before the beginning of post-transplant Imatinib and every 3 months for the first year post-transplantation.

During Imatinib post-transplant administration weekly assessment of hepatic function and complete blood count is required.

8.0 Therapy modifications for toxicity (all phases)

8.1 Treatment toxicity during chemotherapy of Phase 1B, consolidation and re-induction by using protocol II

Based on the closed treatment protocol AIEOP and BFM-ALL 95, patients enrolled in high-risk schedule, require careful monitoring to prevent fatal or life-threatening complications. On average, these patients spent approximately 100 days in the hospital during the five intensive treatment phases covering the first nine months from diagnosis (27). However, the death rate in first CR was only 2%, justifying wider application of this therapy in patients with high-risk ALL. Although this treatment regimen included a high cumulative dose of anthracyclines (daunomycin and doxorubicin) of 410 mg/m², the cardiac function was not reported as a clinical problem after

treatment completion in this study. Some relevant parameters related with the treatment burden are summarized in the following table.

	Prot. Ia	Prot. Ib	Consolidation blocks**	Prot. II (1st)	Prot. II (2nd)
Number of patients analysed	182	157	124	109	31
Red blood cells					
Patients receiving ≥ 1 unit (%)	85	96	90	69	65
No. of units (mean*)	2.9	3.1	3.7	2.4	2.1
Platelets					
Patients receiving ≥ 1 unit (%)	51	36	70	16	23
No. of units (mean*)	4.3	3.6	5.1	3.6	3.4
IV antibiotic therapy					
Patients (%)	46	37	82	46	74
Duration in days (mean*)	11	7	14	9	14
Central venous line ***					
Patients (%)	67	75	83	80	74
No. days (mean*)	35	50	68	74	78
Hospitalization					
Patients (%)	97	85	99	85	87
No. days (mean*)	22	14	32	11	19
Phase duration (days)					
Scheduled by protocol	42	42	63	64	64
Observed (mean*)	46	59	73	81	89

* Mean values estimated including only patients who actually required the specific support.

** Data presented pertain to the entire phase (3 blocks)

*** Central line data are cumulative

8.2 Therapy modifications for IMATINIB toxicity

Non-Hematologic Toxicity

Grade 3/4

If a patient experiences unexpected non hematological Grade 3-4 toxicity, study drug must be withheld until the toxicity has resolved to < Grade 2 or lower. An assessment of possible drug interactions should be performed, and any drug that is suspected as contributing to the toxicity should be reevaluated regarding its dosage and/or necessity of administration. When toxicity is grade 2 or lower, IMATINIB should be reintroduced at 20% less dose.

Hepatic Toxicity:

Patients with a total serum bilirubin < 1.5 x ULN at baseline who experience Grade 3-4 elevations should be managed using the criteria detailed above for non-hematological toxicity

Hematologic Toxicity

Patients developing anemia are transfused at the discretion of the investigator. No dose reductions are foreseen for any grade of anemia, except for Grade 3 and 4 anemia resulting from an acute cause considered to be related to administration of IMATINIB (e.g. severe gastrointestinal hemorrhage).

In the event of Grade 3 thrombocytopenia (platelets 10.000-<50.000/ μ l) or neutropenia (500<1000/ μ l) not accompanied by clinical manifestations, i.e. significant bleeding or neutropenic fever, potential causes of the cytopenias should be considered (e.g. leukemic progression, infection); if the thrombocytopenia or neutropenia are attributed to the study drug, the dose will be reduced at 20% less dose.

With the occurrence of Grade 4 thrombocytopenia (platelets <10.000/ μ l) ,not accompanied by clinical manifestations, platelets support and continuation of the drug are suggested.

G-CSF is permitted in the case of neutropenic fever or suspected drug induced neutropenia. If Grade 3 or 4 thrombocytopenia or neutropenia are accompanied by clinically significant bleeding or evidence of an infection, IMATINIB will be interrupted until toxicity has resolved to grade 2 or lower and the clinical situation has stabilized; IMATINIB will then be restarted at 240 mg/m² daily.

9.0 Visit schedule and assessments

Visit schedule

Patients must be followed at the study centers according to the visit schedule and assessments according to national study-group policy. Provided there are no safety concerns, patients may be evaluated by the referring physician after the first month of treatment. The referring physician must agree to perform all required evaluations as requested by the protocol for those specific visits, and agree to forward copies to the study center regularly. Patients must be evaluated at the study centers for all bone marrow assessments.

Efficacy assessments

Assessment of efficacy during the first 6 months of treatment is based on bone marrow cytology (histology only in case of a dry aspirate) and molecular evaluation of MRD according to the time points in Fig 3 (at the beginning of Phase IB, of each blocks and before the reinduction). Analysis of residual disease by flow cytometry is optional.

Minimal residual disease

MRD will be assessed by quantitative real-time PCR of mononuclear bone marrow and peripheral blood cells collected at specified time points (see Fig 3). To guarantee comparability of data, it is essential that the analyses are performed in central laboratories (one for each country) that agree on standard procedures. MRD evaluation will be performed by quantitative PCR amplification of the BCR-ABL fusion gene, according to the Guidelines of the European Against Cancer Project on “STANDARDIZATION AND QUALITY CONTROL STUDIES OF REAL TIME RT-PCR FOR MINIMAL RESIDUAL DISEASE DETECTION OF FUSION GENE TRANSCRIPTS IN LEUKEMIA - A EUROPE AGAINST CANCER PROGRAM” (28). Comparative data will be obtained by using Ig and TcR gene amplification as previously reported (29).

Cytogenetic and FISH analysis

It is recommended that a cytogenetic and FISH analysis will be performed on bone marrow samples obtained prior to start of IMATINIB and in the event of relapse or disease progression as determined by substantially increasing MRD levels. A minimum of 20 metaphases should be examined whenever possible.

Hematological remission

For the purpose of this study, a hematological response includes any of the following:

- 1) complete hematological remission,
- 2) no evidence of leukemia in peripheral blood and bone marrow, without full peripheral blood recovery,

Each of these response categories is described in detail below. Hematological response must be confirmed after ≥ 4 weeks.

- 1) Complete hematological remission requires that all of the following are present:
 - a) Adequate bone marrow cellularity with a blast count $< 5\%$
 - b) No peripheral blood blasts
 - c) ANC $\geq 1500/\mu\text{l}$
 - d) Platelet count $\geq 100.000/\mu\text{l}$
 - e) No evidence of extramedullary involvement
- 2) No evidence of leukemia in peripheral blood and bone marrow, without full peripheral blood recovery requires that the following are present:
 - a) Blast count $< 5\%$ in bone marrow
 - b) No peripheral blood blasts
 - c) Platelet count $\geq 20.000/\mu\text{l}$ platelet transfusion independent and no evidence of bleeding)
 - d) No evidence of extramedullary involvement

For the purpose of assessing hematological response, peripheral blood samples and bone marrow aspirates will be performed, and extramedullary leukemic involvement will be assessed by physical examination.

Resistance to treatment (only for Poor-risk group)

$\geq 5\%$ of blasts in the bone marrow aspirate after the three blocks of consolidation in patients who enter the poor-risk group because of no-CR at the end of induction

Safety assessments

Safety assessments will consist of monitoring and recording all toxicity and serious adverse events. Hematology, liver function tests (SGOT [AST], SGPT [ALT], LDH, alkaline phosphatase and bilirubin) and other biochemistries (BUN [urea], creatinine and uric acid) will be monitored weekly during the time of concomitant IMATINIB administration. Thereafter, hematology and liver function tests will be monitored according to national study protocols.

Toxicity

Information about all toxic events, whether volunteered by the patient, discovered by the responsible investigator, or detected through physical examination, laboratory test, or other means, will be collected and recorded on the Toxicity Form. An adverse event is any undesirable sign, symptom or medical condition occurring after starting treatment , in any arm.

As far as possible, each toxic event will be reported by the NCI/NIH Common Toxicity Criteria severity grades 1 – 4.

Serious adverse events (SAE)

Each Serious Adverse Event (SAE) must be reported by the clinical center to the clinician responsible (contact person) of its own national study group, within 24 hours of learning of its occurrence, even if is not felt to be treatment-related. After ensuring that the forms be accurately and fully completed, the clinician who is the group contact person must send the SAE form immediately to the local Novartis representative and to the Coordination Unit. Follow-up information about a previously reported SAE must also be reported within 24 hours with the same modalities.

Information about all serious adverse events will be collected and recorded on the SAE Form, according to Novartis standard.

A Serious Adverse Event is an undesirable sign, symptom or medical condition which:

1. is fatal or life-threatening
2. required or prolonged hospitalization
3. results in persistent or significant disability/incapacity
4. constitutes a congenital anomaly or a birth defect
5. are medically significant, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Events **not** considered to be SAE are hospitalizations occurring under the following circumstances:

- planned before entry into the clinical study
- for elective treatment of a pre-existing condition
- occur on an emergency, outpatient basis and do not result in overnight hospitalization (unless fulfilling the criteria above)
- routine treatment or monitoring of the study indication and not associated with any deterioration in condition.

For instructions about returning Serious Adverse Event Report Forms to the study secretariat refer to Novartis standards, see Appendix V.

Laboratory evaluations

Hematology

Peripheral blood will be drawn weekly during the time of concomitant IMATINIB administration. Thereafter, hematology and liver function tests will be monitored according to national study protocols. Hematology includes assessment of hemoglobin, total WBC count, ANC, platelet count, and a differential count including neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils, early forms, and blast percentage.

The ANC used by the center to make dose adjustments is to be recorded on the CRF. For analysis purposes however, the ANC will be calculated automatically from $WBC \times \text{the total segmented neutrophils plus bands} \times 10^9/L$.

Biochemistry

Liver function tests - SGOT (AST), SGPT (ALT), LDH, alkaline phosphatase and bilirubin - will be performed weekly during the time on concomitant IMATINIB administration. During chemotherapy alone, biochemistries will follow the national study treatment policy.

Physical examination

A physical examination, including vital signs, will be performed during screening. The physical examination will be repeated monthly throughout the study, including assessment of vital signs. Information about the physical examination and vital signs must be present in the source documentation at the study site. Clinically significant findings present prior to the start of study drug must be included in the Relevant Medical History/Current Medical Conditions CRF. Clinically significant findings made after the start of study drug which meet the definition of an adverse event must be recorded on the Adverse Event Case Report Form

Performance Status

Performance status will be measured at baseline using the Lansky Performance Status Score.

10 Statistical considerations

10.1 Randomization

The randomized part of the study was stopped based on external evidence (COG AALL0031) and on DSMC consensus. Thus this present part of the protocol must be neglected.

Each child who is alive and in CR after induction in the Good-Risk group is eligible for randomization. The request for randomization should be made, after consent from parents or guardians is obtained, when the patient is known to be Ph+ and the parameter of the early response (Prednisone response or BM at day 15, 21) is known. This will allow a timely start of phase Ib. There might be very few cases, randomized in the Good-Risk group thanks to the achievement of an early response, who are not in CR at the end of induction. In these cases there will be a shift to the Poor-Risk group treatment, whatever the assigned arm. Randomization will be performed by the data center of each group, so that treatment arms will be balanced within each group. Patients will be randomized by telephoning to the data center which will perform a check on the eligibility criteria before assignment. Therefore, at the time of randomization, at least the registration, diagnosis and induction/response data should be available at the data center in order to verify eligibility. The random assignment will be produced by an automatic procedure based on random permuted blocks.

If parents do not give consent, the center should not ask for randomization and the treatment arm should be decided case by case.

10.2 Analysis

The **primary analysis** will be the evaluation of the outcome for all Ph+ALL patients in terms of EFS by considering the time from beginning of phase Ib to the following endpoints: resistance, relapse, death in CCR, second malignancy. The evaluation of outcome will also be performed in terms of survival time from beginning of phase Ib (endpoint is death from any cause).

Secondary analyses will include:

1. Evaluation of EFS within each risk group
2. The evaluation of MRD as molecular indicator of disease burden at the end of consolidation (i.e. end of blocks, time point 5, TP5) in all patients: the proportion of patients with negative MRD levels and alive in CCR at TP5 will be the measure of response.

3. The description and analysis of the MRD pattern (slope) based on 5 measurements taken at subsequent time points from the start of phase IB to the beginning of the re-induction phase.
4. The evaluation of the role of the molecular indicator as surrogate for DFS.

An analysis censoring HSCT will also be performed.

10.3 Sample size

This study asks the question whether the continuous exposure to IMATINIB, on top of a BFM oriented protocol affects the prognosis of children with Ph+ALL. As Ph+ALL is estimated to account for 2.5% of the childhood ALL population, the participating groups will have the following expected recruitment per year:

AIEOP (Italy)	8
BFM (Germany, Switzerland, Austria)	11
COALL (Germany)	3
CPH (Czech Republic)	1
DCOG (the Netherlands)	3
EORTC-CLCG (France, Belgium)	6
FRALLE (France)	4
NOPHO (Scandinavian countries)	4
UKCCSG (United Kingdom)	9
HONG KONG	2
PINDA (Chile)	4
Total:	55 Ph+ALL cases per year

With a 2 year recruitment and accounting for approximately 15% of patients who do not enter this protocol for lack of consensus, clinical considerations or logistical reasons, a total of 90 patients are expected to be enrolled and evaluable. In keeping with data from COG AALL0031 study, these patients are expected to have better EFS than patients treated with imatinib according to the original EsPhALL protocol. With 90 patients recruited, the study will have 90% power to show an advantage of 20% EFS over the historical EsPhALL figure of 60% 3-year EFS and 80% power to show a 17% advantage ($\alpha=0.05$, logrank test). A 20% advantage would correspond to the 80.5% 3-year EFS figure reported by COG ALL0031 on cohort 5.

10.4 Interim analysis

The randomized part of the study was stopped based on external evidence (COG) and on DSMC consensus. Thus this present part of the protocol must be neglected.

Interim analysis evaluates, while the trial is still in progress, the randomized question on treatment effect. The aim is to avoid prolongation of the study beyond a time when clear superiority can be demonstrated for one of the randomized treatment schedules. One intermediate analysis is planned to be performed after the first two years of randomization on the primary end-point. The significance level of the interim test, adjusted for the multiplicity of looks according to O'Brien and Fleming (31), is calculated with a total type I error $\alpha=0.05$ and a power of 80% (two-tailed test) and is shown below:

Significance levels for interim analysis

Years	First	Final
p-level	0.003051	0.05
Patients	70	140
Years from 1st randomization	2	4

10.5 Secondary analysis on response

The randomized part of the study was stopped based on external evidence (COG) and on DSMC consensus. Thus this present part of the protocol must be neglected.

Specifically, for the evaluation of response in term of MRD, past experience suggests that a reasonable estimate of the proportion of MRD negative patients, at the end of consolidation, i.e. after blocks (identified in the figure below as TP3), is about 40% as shown by the following data derived from a prospective study on MRD in Ph+ ALL patients (30).

This experience is based on a qualitative, end-stage PCR evaluation of MRD, whereas the protocol will analyze MRD by quantitative-PCR. A negative result for MRD (identified by the green dot) was referred to a “nested-PCR” approach with a sensitivity of at least 10^{-4} (30).

For sample size calculation and analysis based on the secondary end-point, i.e. the negativity of MRD at TP5, either one of the following events would be considered as treatment failure, i.e. non negativity at TP5:

- Positive MRD at TP5
- Death in CR within TP5
- Relapse within TP5.

In the available data used above, none of the 18 patients who were good responders to PDN and in CR at the end of induction Ia, either relapsed or died during consolidation.

We considered a baseline value for response of 35% in order to take into account and additional 5% possibility of occurrence of death in CR or relapse before TP5. Different hypotheses on possible differences in the proportions of MRD negative patients between the two arms were also considered. The table reports the power calculations under each scenario for the final two-sided test on the difference in MRD negative proportions (null hypothesis: no difference) assuming the first type error $\alpha=0.05$.

Sample size: 140 subjects
35% baseline proportion of MRD negative patients

Proportion of MRD negative patients in experimental group	Absolute difference in proportions as compared to baseline	Power
50%	15%	37
55%	20%	60
60%	25%	80

The power calculations show that, with a 4-year recruitment, in the presence of a marked difference in the proportion of MRD negative patients between the two treatment arms, i.e. 25% absolute difference or more, the study could have sufficient power (80%).

This sample size calculation could be reviewed in the light of the accumulating quantitative data on MRD by considering the comparison of the mean levels of MRD in the two groups at TP5.

10.6 Methods of analysis

The EFS in the overall group of Ph+ patients and in the two risk groups will be calculated using the Kaplan-Meier estimator and the 3 year figure with 95% CI reported (based on Greenwood variance). For historical comparison with the original EsPhALL protocol, a Cox model will be fitted to the data, adjusting for sex, age at diagnosis, WBC count at diagnosis and treatment (HSCT would be censored or, in alternative, included as a time-dependent variable), after check of the model adequacy, in order to compare the two study cohorts.

As for the secondary objectives of the study on response, the proportions of MRD negative patients in CCR at TP5 will be evaluated and compared between poor and good risk patients.

Also, the different longitudinal profiles in MRD response up to TP5 will be evaluated, if possible, in order to study their impact on prognosis, i.e. on the clinical end point (EFS).

The same methods of analysis will be adopted for the set of Poor Risk patients (observational study).

10.7 Early stopping guidelines for treatment related mortality

These guidelines are necessary given the new strategy of the protocol, which introduces IMATINIB into the standard BFM-like chemotherapy for the treatment of childhood leukemia. They are designed to ensure that the treatment would be stopped as early as possible if its application is associated with a treatment related mortality higher than acceptable in standard treatments.

The table below shows the minimum number of treatment related deaths (excluding deaths after HSCT) at which experimenters and DMC should carefully evaluate the possibility of stopping the application of the treatment.

Guidelines for early stopping due to treatment related mortality

No. of events	No. of subjects in study arm
2	11-15
3	16-25
4	26-35
5	36-45
6	46-56
7	57-67
8	68-78

The method applied for developing these guidelines follows a Bayesian approach (32), extending that of Metha and Caine (33). In these guidelines, the maximum acceptable level of $p_1=7\%$ probability of treatment related deaths was considered. The number of failures is assumed to be taken from a Binomial distribution. The prior distribution for the probability of the endpoint of interest was taken as a Beta (1,1), corresponding to an uninformative Uniform distribution. The stopping bounds reported in the table are the experimental results that give a posterior probability of 90% or more, of observing $p_1 \geq 7\%$.

11.0 Organizational aspects and data management

Each participating group will refer to the contact person of the group and to its own usual network of clinical centers, data center and experts (statistician, laboratories, etc) for:

- ♦ The implementation of this protocol
- ♦ The monitoring of the group's data and of the quality of the data

Pag.2-4 report names and addresses of contact persons of each participating group. The international study coordinators, and the trial data center will act as a co-ordination unit for the exchange of information among groups and for the evaluation of their data.

11.1 Data Collection

The trial data center designed a specific web-based system for data collection which is used by each participating centre.

The participants have agreed upon:

- registration of each new child or adolescent newly diagnosed with Ph+ ALL;
- collection by web of a selected set of data on each patient who enters the protocol;

The set of data to be collected and evaluated by the participants are listed in Appendix IV. Each group will:

- use the same form for data collection that include the data items listed in Appendix IV;
- centralize the forms for input and quality checks in its own data center, according to the approach routinely used in the group;
- input of the data in the same WEB database provided by the trial operation centre;
- provide yearly the data file to the coordination unit.

The major requirements that each group will have to ask to the clinical center are:

1. to register at the group data center each new patient diagnosed with Ph+ ALL, regardless of whether he/she will subsequently enter the present protocol. This is necessary in order to know which percentage of eligible patients is treated according to the protocol. Registration should be done as soon as the presence of t(9;22)(q34;q11) in newly diagnosed childhood ALL has been documented;
2. to report immediately (within 24 hours recognition) by fax specific events (death, relevant toxicity) to the responsible clinician of each group, to the local Novartis and to the coordination unit, with the appropriate WEB forms. This is strictly necessary for the international standards in phase II studies on IMATINIB;
3. to send to the group data center on a regular basis the forms with information on diagnosis, response, randomization, treatment, and toxicity as soon as they can be completed.
4. to up-date follow-up at the end of each calendar year.

For eligible patients registered but not included in the present protocol, follow-up data only will be routinely requested.

11.2 Data evaluation and analysis (34)

The trial data center, in collaboration with the study chairman and the contact persons and the statisticians of each group, will be responsible to pool and evaluate the data reported in the WEB system according to the protocol aims.

The data evaluation will be done with the following steps:

- ◆ Follow up will be updated at December of each year
- ◆ The data will be used for the trial aims only;
- ◆ A report will be produced each year on the study progress (recruitment, toxicity and so on) and the interim analysis performed when planned;
- ◆ Reports will be circulated via WEB by the coordination unit to the contact persons of the groups and to the Data Monitoring Committee (DMC);
- ◆ Members of the DMC are experienced researchers (one clinician and one statistician) not involved in the trial who will be responsible of providing the investigators with guidance on the trial conduction and, in case of problems, on whether the trial should be stopped, modified or continued.

The trial database will be used for the trial aims only, under the responsibility of the participating groups. Novartis will be informed of the SAE and eventually of trial results.

11.3 Ethics and Good Clinical Practice

The last revision of the Helsinki Declaration as well as the provisions of the Oviedo Declaration, provide the general framework for the ethical conduct of the study.

The study protocol is designed to ensure adherence to Good Clinical Practice (GCP) principles and procedures.

11.4 Informed consent

Informed consent forms have to be written by each group itself, adapted to the local situation.

12. Definitions

Absolute Neutrophil Count (ANC)

The ANC is defined as the WBC/ μ L times the neutrophil percentage

Absolute Blast Count

The absolute blast count is defined as the WBC/ μ L times the blast percentage

Central Nervous System (CNS) Disease at Diagnosis

CNS leukemia is defined as an elevated CSF WBC (≥ 5 cells/ μ L) and a cytocentrifuge preparation demonstrating lymphoblast cells. CNS leukemia may also be diagnosed when the CSF WBC is normal, but clinical signs of CNS leukemia such as facial nerve palsy or hypothalamic syndrome are present. Patients who have less than 5 cell/ μ L and a positive cytopspin will not be considered to be CNS positive.

Overt Testicular Leukemia at Diagnosis

Unilateral or bilateral testiculomegaly with leukemic infiltration confirmed by biopsy. Equivocal findings on testicular exam warrant biopsy

Bone Marrow Status

M1: $< 5\%$ blasts of all nucleated cells including erythropoiesis. In case of regenerating marrow with a high erythropoietic predominance, at least a total count of 100 non-erythropoietic cells should be counted.

M2 : 5-25% blast all nucleated cells including erythropoiesis. In case of regenerating marrow with a high erythropoietic predominance, at least a total count of 100 non-erythropoietic cells should be counted.

M3: $> 25\%$ blasts in a bone marrow aspirate.

Resistance to “Induction”

$\geq 5\%$ of blasts in the bone marrow aspirate.

Resistance to protocol IB

$\geq 5\%$ of blasts in the bone marrow aspirate at the end of protocol IB (day 78) in patients resistant to “Induction”.

Resistance to treatment

≥ 5% of blasts in the bone marrow aspirate after the first three blocks of consolidation in patients not resistant to protocol IB.

Late response

<5% of blasts in the bone marrow after the phase IB or after the three blocks of consolidation.

Complete remission

< 5% blasts in the bone marrow aspirate regardless of the proportion of mature lymphocytes at the end of the “Induction”.

Continuos complete remission

Persistence of CR status.

Bone marrow relapse

≥25% blasts in the bone marrow aspirate after that of 1st CR has been achieved.

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Appendix I

Table for monitoring of MTX serum levels and intensification of LCV rescue

Appendix II

Notable laboratory value criteria, special methods and scales

Special scale

Lansky Performance Status Scale For Children

Score	Performance
100	fully active, normal
90	minor restrictions in physically strenuous activity
80	active, but tires more quickly
70	both greater restriction of, and less time spent in, active play
60	up and around, but minimal active play, keeps busy with quieter activities
50	gets dressed, but lies around much of the day, no active play, able to participate in all quiet play and activities
40	mostly in bed; participates in quiet activities
30	in bed; needs assistance even for quiet play
20	often sleeping, play entirely limited to very passive activities
10	no play; does not get out of bed
0	unresponsive

Appendix III: Drugs known to be metabolized by CYP450 isoenzymes

CYP450 isoenzyme	Substrates	Inhibitors	Inducers	Markers
CYP2D6	Several antidepressants Neuroleptics Beta-blockers Antiarrhythmics Codeine Dextromethorphan Ethylmorphine Nicotine	Ajmaline Chinidine Fluoxetine Paroxetine Quinidine Ritonavir	None known	Debrisoquine Dextromethorphan
CYP3A4	Acetaminophen Carbamazepine Cyclosporin Digitoxin Diazepam Erythromycin Felodipine Fluoxetine Nifedipine Quinidine Saquinavir Steroids (e.g. cortisol) Terfenadine Triazolam Verapamil Warfarin	Clotrimazole Ketoconazole Ritonavir Troleandomycin	Dexamethasone Phenytoin Rifampin Troleandomycin	Dapsone Erythromycin Ketoconazole Lidocaine

Appendix IV Variables in the database

Study conduct is now organized via web, but with the same requirements listed below.

In this WEB database all patients diagnosed with Ph+ ALL will be evaluated, regardless of whether they entered the present protocol or not. For patients who do not enter the protocol (registered only), possibly provide data on follow-up. For patients who enter the protocol, please provide all data. Each group will provide the data according to the variables listed below and the operation unit will produce, based on them, the database. The type of file would be, in order of preference: SAS, Excel and ASCII. Dates are in the DDMMYYYY format.

Diagnosis-treatment-follow-up file

Registration

	Var Name	Code	Format
Identification number	UPN	use your own UPN	C 7
Initials (family, first name)	INIT		C 3
Group	GROUP	1=AIEOP 2=BFM-Austria 3=BFM-Germany/Switzerland 4=ALL-REZ 5=COALL 6=DCLSG 7=EORTC 8=FRALLE 9=NOPHO 10=MRC 11= CPH 12=PINDA 13=HONG KONG	I 2
Date of birth	DOB_		D 8
Sex	SEX	1=Male 2=Female	I 1
Date of diagnosis	DOD_		D 8
Enters the protocol	ENTER	1=No 2=Yes	I 1
Reason for ENTER=No	COENTER	-1=Not applicable Give your own code	I 2

Diagnosis

	Var Name	Code	Format
% blasts (peripheral blood)	BLA	-2=Not evaluable -1=No data	I 3
WBC at diagnosis (/mmc)	WBC	-1=No data	I 7
Platelets (/mmc)	PLT	-1=No data	I 7
Hb (g/dl)	HB	-1=No data	R 4.1
FAB Morphology	FAB	1=L1 2=L2 3=L1/2 4=L3 5=Not classified 9=No data	I 1
Peroxidase	POX	1=Negative 2=Positive 3=Not done 9=No data	I 1
Immunophenotype	IMMPHEN	1=Pro-T 2=Pre-T 3=Intermediate (cortical) T 4=Mature T 5=T-lineage not classified 6= Pro-B 7= Common 8=Pre-B 9=Mature B 10=B-lineage not classified 11=AUL 99=No data	I 2

	Var Name	Code	Format
<i>Chromosomal abnormalities</i>			
Data on t(9;22)(q34;q11)			
Karyotyping	K_CA		C 20
<i>CNS involvement</i>			
CNS involvement	CNS_INV	1=No 2=Yes 9=No data	I 1
Cells in CSF (/μl)	CSF_CELL	-2=Not evaluable -1=No data	I 3
Blast in CSF (%)	BLA_CSF	-2=Not evaluable -1=No data	I 3
CSF contaminated with blood	CSF_INV	1=No 2=Yes 3=No puncture 9=No data	I 1
<i>Organ involvement</i>			
Hepatomegaly	HEP_INV	1=No 2=Yes 3=Questionable 4=Not done 9=Not known	I 1
Cm below costal margin of liver	MEA_LIV		I 2.1
Splenomegaly	SPL_INV	1=No 2=Yes 3=Questionable 4=Not done 9=Not known	I 1
Cm below costal margin of spleen	MEA_SPL		I 2.1
...			
Other organ involvement	OTH_INV	Please specify	C 20

Response to Prednisone/induction IA

	Var Name	Code	Forma t
Cumulative dose of Prednisone (mg)	PDN_DOSE	-1=Not known	R 4.1
Blast cell count/μl at day 8	COBLA_8	-2=Not evaluable -1=No data	I 5
WBC/μl at day 8	WBC_B	-1=No data	I 7
BM day 15, % blasts	BLA_15	-2=Not evaluable -1=No data	I 3
BM day 21, % blasts	BLA_21	-2=Not evaluable -1=No data	I 3
BM day 33, % blasts	BLA_33	-2=Not evaluable -1=No data	I 3
Date of first complete remission	DOCR1_		D 8
Risk group	RG	1=Poor 2=Good	I 1

Treatment

	Code	Forma
<i>Induction IA</i>		
Date of start Induction	DOSI_	D 8
Date of end Induction (date of last administration)	DOENDI_	D 8
<i>Induction IB</i>		
...		

Transplantation

	Var Name	Code	Forma t
Considered eligible for transplantation	ELHSCT	1=No 2=Yes 9=Not known	I 1
Search for unrelated donor	DONOR	1=No 2=Yes 9=Not known	I 1
No. of siblings	SIBL	-1=No data	I 2
Hystocompatibility of siblings	HYSTO	1=No 2=Yes 9=Not known	I 1
Date of HLA typing	DOHLA_		D 8
Date of transplantation	DOHSCT_		D 8
Phase of transplantation	PHSCT	1=In 1 st CR 2=Resistant disease	I 1
Type of transplantation	THSCT	1=Autologous 2=Allogeneic (gen. HLA-id, related) 3=Allogeneic (fen. HLA-id, related) 4=Allogeneic (not HLA-id, related) 5=Allogeneic (not specified) 6=Allogeneic (HLA-id, unrelated) 7=Allogeneic (not HLA-id, unrelated) 8=Syngeneic (identical twins) 9=No data	I 1
Source of transplantation	SHSCT	1=BM 2=PBSC 3=Cord blood Give your own code for other types	I 1

Follow-up

	Var Name	Code	Forma t
Date of first relapse	DOREL_		D 8
Site of first relapse	TOREL	1=BM 2=CNS 3=Testis 4=BM+CNS 5=BM+Testis Give your own code for other sites	I 2
Date of death	DODEAD_		D 8
Cause of death	CODEAD	1=Progressive ALL 2=HSCT related 3=2 nd tumor 4=Sepsis 5=Pneumonia 6=Other infection 7=Haemorrhage 8=MOF 9=Other (specify in the note space) 99=Not known	I 2
Phase of death	PH_DEAD	1=Before start therapy 2=In induction, before CR 3=In first CR 4=After relapse 5=After 2 nd neoplasm	I 1
Date of last follow-up	DOLFU_	<i>Equals date of death, if patient died</i>	D 8
Date of 2° malignant neoplasm	DOSMN_		D 8
Type of 2° malignant neoplasm	T_SMN	Give your own codes	I 2
Note space	NOTE		C 40

Appendix V Serious Adverse Event Report

STI571 - GLIVEC® CSTI571AIT07	ID <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	
	Center No. Subject No.	
	Subject's initials <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	
	1. 2. fam.	
Randomization number <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>		

SERIOUS ADVERSE EVENT REPORT							Page 1 of 3
1. REPORT TYPE: ☐ Initial ☐ Follow-up		2. Country:			3. CASE ID:		
I. ADVERSE EVENT INFORMATION							
4. DATE OF BIRTH:	5. AGE yrs./mo.	6. RACE ☐ Caucasian ☐ Oriental ☐ Black ☐ Other	7. SEX ☐ Male ☐ Female	8. HEIGHT cm	9. WEIGHT kg	10. ONSET OF FIRST SIGN/SYMPTOM OF SAE day month year	
11. SERIOUS ADVERSE EVENT(S) IN MEDICAL TERMS (diagnosis if possible) Case description of the above SAE (include related sign/symptoms, treatment, course/outcome and suspected cause of the SAE) (continue on p.3 if more space is required): Is the event due to lack of efficacy? ☐ No ☐ Yes Is the event due to progression of underlying illness? ☐ No ☐ Yes		12. CHECK ALL APPROPRIATE TO EVENT ☐ Patient died day month year ☐ Involved or prolonged inpatient hospitalization ☐ Involved persistence of significant disability or incapacity ☐ Life-threatening Other Seriousness Criteria: ☐ Congenital anomaly/birth defect ☐ Other significant medical events					
II. TRIAL DRUG INFORMATION							
13. TRIAL DRUG(S) AT OR BEFORE ONSET OF SAE (If blinded, provide drug package no.) TRIAL DRUG PACKAGE NO.: Comments (Continue on P.3 if more space is required): Drug code broken ☐ No ☐ Yes				14. LAST VISIT/WEEK BEFORE ONSET OF SAE VISIT NO.: WEEK NO.:			
15. DOSES AT OR BEFORE ONSET OF SAE (total daily dose or specify if other- ADD additional pages)		16. ROUTE OF ADMINISTRATION		17. THERAPY DATES			
				FROM: day month year day month year TO: day month year			
18. TRIAL INDICATION		19. THERAPY DURATION UNTIL ONSET OF FIRST SIGNS/SYMPTOM OF SAE		20. TIME ELAPSED BETWEEN LAST DRUG ADMINISTRATION AND ONSET OF FIRST SIGNS/SYMPTOM OF SAE			
		hrs/days/months		mins/hrs/days/months			
III. HISTORY							
21. PATIENT'S PAST MEDICAL HISTORY (e.g. co-existing medical conditions such as disease, allergies, similar experiences)							
IV. MANUFACTURER INFORMATION (FOR INTERNAL USE ONLY)							
22. DATE MANUFACTURER NOTIFIED OF SAE				23. DATE OF THIS REPORT			
day month year				day month year			
24. NAME AND ADDRESS OF REPORTING MANUFACTURER							
Novartis Farma S.p.A. Largo Boccioni 1 21040 - Origgio (VA)							
PLEASE FAX FORM TO LOCAL CS&E FAX NO. 02-96703051							

SERIOUS ADVERSE EVENT REPORT

Page 2 of 3

25. CONCOMITANT DRUGS RELEVANT TO THE SAE (exclude therapy to treat SAE)

26. COMMENTS (if adverse event is considered to be caused by a comedication, please note it here)

27. ACTION TAKEN (mark all as appropriate)

☐ No Action Taken	☐ Trial drug permanently discontinued due to this adverse event	☐ Concomitant medication taken
☐ Trial drug dosage adjusted/ temporarily interrupted*	☐ Non-drug therapy given**	☐ Hospitalization/prolonged hospitalization

* If ticked, enter new dosage information in field 11

** If ticked, provide therapeutic measures in field 11

28. TEST/LABORATORY FINDINGS (enter only those findings necessary for SAE diagnosis or course description)

29. COMMENTS ON TEST/LABORATORY FINDINGS (Provide normal ranges on Pg. 3 if not already provided.)
(If the SAE is a laboratory abnormality, enter comments on clinical findings and/or treatment in field 11.)

30. OUTCOME OF THE PATIENT/SAE day month year

- ☐ Completely recovered Date of recovery: ☐ Condition still present and unchanged
☐ Recovered with sequelae ☐ Condition deteriorated
☐ Condition improving ☐ Death Autopsy: ☐ No ☐ Yes

31. ASSESSMENT OF CAUSALITY
Relationship to study drug: ☐ Not suspected ☐ Suspected

V.	INFORMATION SOURCE
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[illegible]

PLEASE FAX FORM TO LOCAL CS&E FAX NO. 02-96703051

STI571 - GLIVEC®
CSTI571AIT07

ID

Center No.

Subject No.

Subject's initials

1. 2. fam.

Randomization
number

SERIOUS ADVERSE EVENT REPORT

Page 3 of 3

1. REPORT TYPE: ☐ Initial ☐ Follow-up

3. CASE ID:

FOR ADDITIONAL INFORMATION:

V. INFORMATION SOURCE

32. NAME, ADDRESS AND TELEPHONE NUMBER OF INVESTIGATOR

33. REPORTING DATE BY INVESTIGATOR/PERSON
REPORTING EVENT

Signature

day month year

PLEASE FAX FORM TO LOCAL CS&E FAX NO. 02-96703051