OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION IN ADULT PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA IN THE ERA OF TYROSINE KINASE INHIBITORS: A RETROSPECTIVE ANALYSIS OF ITALIAN BLOOD AND MARROW TRANSPLANTATION SOCIETY (GITMO)

Anna Candoni[1], Alessandro Rambaldi[2], Renato Fanin[1], Andrea Velardi[3], William Arcese[4], Fabio Ciceri[5], Davide Lazzarotto[1], Federico Lussana[6], Jacopo Olivieri[1], Giovanni Grillo[7], Matteo Parma[8], Benedetto Bruno[9,10,] Federica Sora[11], Paolo Bernasconi[12], Riccardo Saccardi[13], Roberto Foà[14], Mariarosa Sessa[15], Paola Bresciani[16], Fabio Giglio[5], Alessandra Picardi[4], Alessandro Busca[10], Simona Sica[11], Katia Perruccio[3], Elisa Zucchetti[7], Elisa Diral[8], Anna Amelia Colombo[12], Stefano Tringali[17], Stella Santarone[18], Giuseppe Irrera[19], Stefano Mancini[20], Francesco Zallio[21], Michele Malagola[22], Francesco Albano[23], Angelo Michele Carella[24], Attilio Olivieri[25], Cristina Tecchio[26], Alida Dominietto[27], Adriana Vacca[28], Roberto Sorasio[29], Enrico Orciuolo[30], Antonio Maria Risitano[31], Agostino Cortelezzi[32], Sonia Mammoliti[33], Elena Oldani[6], Francesca Bonifazi[15], on behalf of GITMO.

[1]Università di Udine, Azienda Sanitaria-Universitaria Integrata Santa Maria Misericordia, Hematology and SCT Unit, UDINE, Italy, [2]Universita' degli studi di Milano e Azienda Socio Sanitaria Territoriale Papa Giovanni XXIII, Bergamo, Department of Oncology-Hematology, Bergamo, Italy, [3]Università di Perugia, Hematology and Clinical Immunology, Perugia, Italy, [4]Università 'Tor Vergata', Rome Transplant Network, Roma, Italy, [5]University Vita-Salute San Raffaele, Hematology and BMT Unit San Raffaele Scientific Institute, Milano, Italy, [6]Azienda Socio Sanitaria Territoriale Papa Giovanni XXIII, Bergamo, Department of Oncology-Hematology, Bergamo, Italy, [7]Ospedale Niguarda Ca' Granda, Department of Hematology Oncology, Milano, Italy, [8]Ospedale San Gerardo, Clinica Ematologica e Centro Trapianto di Midollo, Monza, Italy, [9]Università di Torino, Dip. di Biotecnologie Molecolari e Scienze per la Salute, Torino, Italy, [10]Presidio Molinette-AOU Città della Salute e della Scienza, Trapianto allogenico di cellule staminali-Dip. Oncologia, Torino, Italy, [11]Fondazione Policlinico Universitario "A.Gemelli", Istituto di Ematologia, Roma, Italy, [12]Fondazione IRCCS Policlinico San Matteo, Centro Trapianti SC Ematologia, Pavia, Italy, [13]Careggi University Hospital, Centro Trapianti di Midollo Osseo, Firenze, Italy, [14]Sapienza University, Policlinico 'Umberto I', Department of Cellular Biotechnologies and Hematology, Roma, Italy, [15]University Hospital S. Orsola-Malpighi, Institute of Hematology "L. and A. Seragnoli", Bologna, Italy, [16]University of Modena and Reggio Emilia, Section of Hematology, Modena, Italy, [17]AOR Villa Sofia-Cervello, UOS Trapianto di Midollo, Palermo, Italy, [18]Ospedale Civile, Dipartimento di Ematologia, Medicina Trasfusionale e

Biotecnologie, Pescara, Italy, [19]Grande Ospedale Metropolitano "Bianchi-Melacrino-Morelli", Centro Unico Regionale Trapianto Cellule Staminali e Terapie Cellulari "A. Neri", Reggio Calabria, Italy, [20]Azienda Ospedaliera San Camillo Forlanini, Centro Trapianti di Cellule Staminali, Roma, Italy, [21]Azienda Ospedaliera S. Antonio e Biagio, Unità Trapianti Midollo, Alessandria, Italy, [22]University of Brescia, ASST-Spedali Civili, Unit of Blood Diseases and Stem Cell Transplantation, Brescia, Italy, [23]University of Bari, Hematology and bone Marrow Transplantation, Bari, Italy, [24]Fondazione IRCCS Casa Sollievo della Sofferenza, UO Ematologia e Centro Trapianti TMO, San Giovanni Rotondo (FG), Italy, [25]University of Marche-Ospedali Riuniti, Hematology and Bone Marrow Transplantation, Ancona, Italy, [26]University of Verona, Haematology-Bone Marrow Transplant Unit, Verona, Italy, [27]IRCCS Ospedale Policlinico San Martino, U.O. Ematologia e Centro Trapianti, Genova, Italy, [28]P.O. "Roberto Binaghi", U.O. Ematologia-CTMO, Cagliari, Italy, [29]Azienda Ospedaliera S Croce e Carle, SC Ematologia, Cuneo, Italy, [30]Azienda Ospedaliero Universitaria Pisana, UO Ematologia, Pisa, Italy, [31]Università di Napoli, Department of Clinical Medicine and Surgery-BMT Unit, Napoli, Italy, [32]Università degli Studi e Ospedale Maggiore Policlinico di Milano, Milano, Italy, [33]GITMO Clinical Trials Office, Genova, Italy

ABSTRACT

Purpose: We conducted a retrospective, nationwide analysis to describe the clinical outcome of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) undergoing allogeneic hematopoietic stem cell transplantation (HSCT) after being treated with a TKI based therapy.

Patients and results: A total of 441 patients were included in the study. The median age at HSCT was 44 (range: 18-70). All 441 patients (100%) received TKI before HSCT (performed between 2005-2016). Of these patients, 404 (92%) were in cytologic complete remission (CR) while 37 (8%) had an active disease at the time of HSCT. Molecularly Measurable Residual Disease (MRD) was negative in 147 patients (36%) at the time of HSCT. The donor was unrelated in 46% of cases. The prevalent source of stem cells was peripheral blood (70%). The conditioning regimen was myeloablative in 82% of cases (TBI-based in 50%) and included ATG in 51% of cases. With a median follow-up after HSCT of 39.4 months (range: 1-145), the probability of Overall Survival (OS) at 1, 2 and 5 years was 69.6%, 61.1%, and 50.3%, respectively, with a median OS of 62 months. Progression Free Survival (PFS) at 1, 2 and 5 years was 60.2%, 52.1% and 43.7%, respectively. OS and PFS were significantly better in patients with CR and MRD-negativity at the time of transplant compared to patients with CR but MRD-positive (50% OS not reached vs. 36 months, P=0,015; 50% PFS not reached vs. 26 months, P=0.003). The cumulative incidence of relapse (CIR) at 5 years was significantly lower in patients with CR and MRD-negativity (19.5% vs. 35.4%, P=0.001). The non relapse mortality (NRM) after 1, 2, and 5 years was 19.1% (95%CI: 15.5-22.9), 20.7% (95%CI: 17-24.7), and 24.1% (95%CI: 20-28.5), respectively. The subgroup of patients with MRD-negative both at HSCT and at 3rd month after HSCT had a better outcome (5 year OS 70%). Conversely, the 37 patients who underwent HSCT with active Ph+ALL had a median OS and PFS of 7 and 5 months, respectively.

Conclusions: The median OS of all patients with Ph+ALL, treated with TKI based therapy and allografted in recent years at the GITMO Centers, is 62 months. The outcome of Ph+ALL patients undergoing HSCT after TKI therapy has improved (with a 2 yrs NRM of 20,7%), particularly for younger patients and those achieving a molecular remission before transplant (50% OS and PFS not reached). HSCT remains a standard of care consolidation treatment for Ph+ALL and only prospective randomized trials can suggest a survival benefit of non transplant based treatment strategies.

INTRODUCTION

Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia (Ph+ALL) accounts for approximately 25% of ALL cases in adults. Historically, it is characterized by a very unfavorable prognosis, with an overall survival (OS) rate of less than 20% after 5 years according to the major epidemiological studies available.[1,2] For many years, the treatment of Ph+ALL was based on intensive chemotherapy regimens followed by allogeneic stem cell transplantation (HSCT), which is still the only established procedure with curative intent for this disease.[2-4] Over the last decade, Tyrosine Kinase Inhibitors (TKI), mainly imatinib and dasatinib and more recently ponatinib, were gradually incorporated into both chemotherapy-based and chemotherapy-free induction regimens.[2, 5-15] The outcome improvement achieved by TKI based treatments has challenged the concept that a transplant based consolidation of remission is always mandatory.[16-18] In light of these recent treatment changes, an extensive activity of data reviewing has been done by several groups in order to update the results of HSCT in Ph+ALL in the era of TKIs paying attention to both pre and post-transplant phases.[2,12,14,19] To address this need, the European Society for Blood and Marrow Transplantation (EBMT) and the Japan Society for Hematopoietic Cell Transplantation (JSHCT) recently reviewed the outcome of Ph+ALL undergoing HSCT in their centers in the last decades (from 2000 to 2010 for EBMT and from 1990 to 2000 for the JSHCT).[14,19] The study reported herein is the most recent registry study that analyze the experience of the Italian Blood and Marrow Transplantation Society (GITMO) regarding the outcome of HSCT (performed between 2005 and 2016) for Ph+ALL patients in the era of TKI based therapy.

MATERIALS and METHODS

a) Study design.

This was a retrospective nationwide analysis based on registry data collected by GITMO. Inclusion criteria were: 1) diagnosis of Ph+ALL; age $\geq$ 18 years at transplant; 2) patients receiving first allogeneic HSCT from any donor (HLA-identical sibling donor (MSD), unrelated donor (UD) or alternative donor (haploidentical or cord blood) between 2005 and 2016 in a GITMO center; 3) TKI-based treatment prior to HSCT; 4) patients with available pre transplant MRD status, as well as complete clinical data and outcome. Data were extracted from the GITMO Registry (PROMISE Registry).

The endpoints of the study were: Overall Survival (OS), Progression-free Survival (PFS), cumulative incidence of Relapse (CIR), Non-relapse Mortality (NRM), cumulative incidence of extensive chronic GVHD (cGVHD), rate of MRD negativity and the rate of CR before and after transplant.

Data were centrally reviewed following the initial collection and queries were sent to the relevant parties for any conflicting and/or missing information.

All patients included in the registry signed an informed consent form. The study was conducted in compliance with current national and European legislation on clinical trials, in accordance with the Helsinki Declaration and the principles of good clinical practice (GCP). This study was approved by the GITMO board and by the institutional review board of the coordinating center (Hematology– University of Udine), and from institutional review board of all participating centers.

A list of Institutions reporting the data included in the current study is available in a Supplementary file.

b) Definitions.

Complete cytologic remission (CR) was defined as the absence of circulating blasts, less than 5% bone marrow (BM) blasts and a platelet count of $100x10^9$/L or higher. Relapse was defined as the reappearance of >5% leukemic cells in bone marrow aspirates or extramedullary leukemia in patients with previously documented CR.

Cytogenetic analysis was performed using the G-banding technique. Complete Cytogenetic Remission (Cytogenetic CR) was defined as having obtained a normal cytogenetic result in at least 20 metaphases.

BCR-ABL transcripts were detected by real-time quantitative polymerase chain reaction (PCR) according to validated methods.[21,22] Minimal Residual Disease (MRD) negativity was defined as undetectable BCR-ABL mRNA transcripts by real-time qPCR. Investigators were asked to provide MRD data at the time of transplant (within 30 days prior to procedure) and after HSCT.

Acute graft versus host disease (aGVHD) was computed when graded > 1 based on standard criteria and requiring therapy. Chronic GVHD (cGVHD) was classified as none, limited or extensive (EcGVHD) according to Seattle criteria.[23] The aGVHD persisting or progressing after day 100 was scored as cGVHD.

The timing of HSCT, the conditioning regimen, GVHD prophylaxis and post HSCT timing of BCR-ABL monitoring were defined by each Institution according to their active protocols.

c) Statistical analysis.

To compare baseline characteristics or outcome measures among subgroups, we used the Chi-square or Fisher's exact test for categorical variables, the Student's t-test for normally distributed variables and the Mann-Whitney or Kruskal-Wallis test for non-normally distributed variables. Median follow-up time was calculated among survivors.

Overall survival (OS) was defined as the time from HSCT to death, regardless of the cause. PFS was considered to be survival following HSCT with no evidence of relapse or progression. Death from any cause was considered as an event for OS, whereas relapse/progression and death from any cause were considered as events for PFS. OS and PFS were computed using the Kaplan-Meier method. Univariate and multivariate analyses were performed using the Cox proportional hazard method.

The cumulative incidence method was used for computing CIR, NRM and extensive cGvHD in a competing risks setting. CIR was estimated by considering relapse as the event of interest and death without relapse as a competing event. NRM was defined as death without evidence of relapse or progression, with relapse as a competing event.

Extensive cGVHD was estimated by considering the occurrence of extensive cGVHD as an event of interest and death without cGVHD as a competing risk, with leukemia relapse treated as a competing risk if it occurred without prior GVHD.

Predictive analyses for extensive cGVHD, CIR and NRM were based on the proportional hazard model for subdistribution of competing risk. Univariate and multivariate analyses were then performed using Gray's test and the proportional subdistribution hazard regression model developed by Fine and Gray. In general, a stepwise backwards procedure was used to construct a set of independent predictors for each endpoint. All predictors with a P-value less than 0.20 were

considered, and then sequentially removed if the P-value in the multiple model was above 0.05. All tests were 2-sided. The type I error rate was fixed at 0.05. However, the whole procedure and the final model accounted for manual adjustments, i.e. exclusion of variables with partial overlapping information (collinearity) or categorization of continuous or non-dichotomous categorical variables according to clinical relevance. Factors considered were: patient sex, disease characteristics at diagnosis (presence of hyperleukocytosis, defined as $>30x10^9$ WBC/L; additional cytogenetic abnormalities beyond t(9:22)), pre-transplant therapeutic strategy (use of TKI + steroids vs. TKI + chemotherapy), the patient's age at transplantation, donor sex, disease status at time of transplantation (1^{st} CR vs. 2^{nd} and subsequent CR vs. advanced disease), donor type (matched sibling vs. unrelated donor vs. alternative donor-i.e. haploidentical or cord blood), source of stem cells (peripheral blood vs. bone marrow vs. cord blood), year of transplantation, time from diagnosis to transplant, CMV serostatus of recipient and donor, hematopoietic cell transplantation specific comorbidity index (HCT-CI) at transplant, Karnofsky performance status (KPS) at transplant ($\geq$ 90 vs. <90), molecular remission status at transplant, type of conditioning (reduced-intensity conditioning-RIC vs. myeloablative-MAC) and use of antithymocyte globulin (ATG) and total body irradiation (TBI) in the conditioning regimen. Patient age and year of transplantation were analyzed as continuous variables. In order to evaluate the impact of the molecular response measured in the first 3 months after transplant on survival endpoints, a landmark analysis was applied for OS and PFS using a landmark day 90 after HSCT. Analyses were performed using Stata 12.0 (Statacorp, College Station, Texas).

RESULTS

a) Patients and Ph+ALL status at HSCT.

A total of 441 patients were included in the study. The main clinical findings are reported in **TABLE 1**. The median age at transplantation was 44 (range: 18-70). Additional karyotype abnormalities were reported in 30% of evaluable cases (124/416). All 441 patients (100%) received TKI before transplant: 80 (18%) had received TKI + steroids while 361 (82%) had received TKI + chemotherapy. The patients in this second group, compared to patients treated with TKI + steroids alone, had a significantly higher leukocyte count at ALL diagnosis (P=0.001). The median interval between the diagnosis of Ph+ALL and HSCT was 7.67 months (range: 2.3-78.8) without significant differences between the 2 pre HSCT treatment groups (P=0.83). Patients who received HSCT in 1st CR had a shorter interval between diagnosis to HSCT (median: 6.83 months) than patients who received transplants in >1st CR (median: 11.43 months) and patients with active disease at HSCT (median: 10.03 months, p-value for any difference among groups <0,001).

Of these 441 patients, 404 (92%) were in complete cytologic remission at the time of HSCT, while 37 (8%) had an active disease. In detail, of the 404 patients in CR at the time of HSCT, 337 patients (83%) were in 1st CR, while 67 patients (17%) were in 2nd or subsequent CR. A significantly higher percentage of patients transplanted in >1st CR had been treated with CHT+TKI before HSCT (20% vs. 4%, P<0,001).

There were 147/404 patients in CR and MRD-negative (36%) at the time of HSCT. A significantly higher proportion of patients treated with TKI + steroids were MRD-negative at the time of transplantation (40/76-53%) compared to patients treated with TKI + CHT (107/328-33%) (P=0.001).

b) Transplantation characteristics and outcome.

Patients received a variety of HSCT preparative regimens based on existing available protocols at time of treatment. Only 27% (118/441) of HSCT procedures were performed from 2005 to 2010, while 323/441 (73%) were performed from 2011 to 2016. The main characteristics of the HSCT are reported in **TABLE 2**. The donor was unrelated in 46% of cases and the prevalent source of stem cells was peripheral blood-PB (70%). The conditioning regimen was myeloablative (MAC) in 82% of cases, and it was TBI-based in 50% of cases. ATG was used in 51% of cases. HCT-CI (available in 402 cases) ranged from 0-2 in 95% of patients. The median follow-up from the transplant was 39.4 months (range of 1-145).

The probability of survival (OS) at 1, 2, 3 and 5 years from transplant was 69.6% (95%CI: 65-73.8), 61.1% (95%CI: 56.2-65.7), 52.4% (95%CI: 46.8-57.1) and 50.3% (95%CI: 44.9.-55.4), respectively. The probability of Progression Free Survival (PFS) at 1, 2, 3 and 5 years was 60.2% (95%CI: 55.7-64.7), 52.1% (95%CI: 47.4-56.8), 45.1% (95%CI: 40.2-50) and 43.7% (95%CI: 38.7-48.7), respectively.

Among patient in CR, OS and PFS from transplant were significantly better in MRD-negative patients in comparison to those MRD-positive at the time of HSCT (P =0.015 for OS and P=0.003 for PFS) (**FIGURES 1A-D**). The cumulative incidence of Relapse (CIR) at 2 and 5 years was 27.9% (95% CI: 23.6-32.3) and 31.8% (95%CI: 27.1-36.5), respectively (**FIGURE 2A**). As expected, CIR was significantly lower in patients who were MRD-negative at the time of transplant compared to MRD-positive ones (Gray's test: SHR=0.47 [0.31–0.73], P=0.001) (**FIGURE 2B).** For the whole population of patients, the cumulative incidence of Non Relapse Mortality (NRM) at 1, 2, 3 and 5 years was 19.1% (95%CI: 15.5-22.9), 20.7% (95%CI: 17-24.7), 24.1% (95%CI: 20-28.5) and 24.1% (95%CI: 20-28.5), respectively (**FIGURE 2C**).

Acute GVHD requiring therapy was documented in 41% of cases and chronic GVHD requiring therapy was documented in 29%. The cumulative incidence of extensive cGVHD at 2 and 5 years from HSCT was 18.5% (95%CI: 14.9-22.4) and 19.8% (95%CI: 16-23.9), respectively (**FIGURE 2D**). A total of 96/441 patients (22%) died of NRM. In detail, the causes of NRM were: GVHD in 49/96 (51%), Infections in 33/96 (35%), PTT in 6/96 (6%), VOD in 1/96 (1%) and other causes in 7/96 (7%).

Of note, the 37 patients transplanted with active Ph+ALL had a very unfavorable post transplant outcome, with a median OS and PFS, from HSCT, of 7 and 5 months, respectively (**FIGURES 1C and 1D**).

By univariate analysis (**TABLE 3**), many factors were associated with a favorable OS including: younger age, shorter interval between diagnosis and 1st CR, early disease phase (1st CR) at the time of transplantation, CR with MRD negativity at the time of transplantation, KPS >90%, a matched sibling or unrelated donor. The inclusion of TBI in the conditioning regime proved to be an additional favorable factor for PFS **(TABLE 3).**

However, by multivariate analysis, as shown in **TABLE 4**, favorable predictive factors for OS and PFS remained: the use of a matched sibling or unrelated donor (P=0.001), being in 1st CR at the time of HSCT (P <0.001), a CR with MRD negativity at the time of transplantation, a younger age

(P=0.013 for OS), a transplant performed in the most recent years (P=0.008) and the use of TKI and steroids prior to HSCT (P=0.008).

Early disease phase, MRD negativity (P=0,008), lower age and more recent year of transplant were found to significantly predict also the incidence of Relapse. Of note no significant effect of donor type was seen while the use of TBI in the conditioning regimen (P=0.001) confirms its protective effect on relapse.

The incidence of extensive chronic GVHD was significantly associated, in multivariate analysis, to HLA mismatching (alternative vs. UD and sibling; p=0.019), a female donor (P=0.01) and to the use of ATG (P=0.011) which was not associated instead to relapse risk.

c) Impact of Disease Status at the 3rd month post HSCT on Outcome.

421 patients were re-evaluated for MRD within the 3rd month from HSCT: 302/421 (72%) were MRD-negative. In particular, 177 (60%) of the 294 patients being MRD-positive or with active disease), were converted to MRD-negative within the 3rd month after HSCT (**Supplemental Table 1**).

A landmark analysis for OS and PFS showed that post transplant MRD negativity (at 3th month) had a significant favorable effect on OS and PFS post HSCT (landmark analysis for OS and PFS-**Supplemental Figure 1**). However, roughly one third (100/302) of the 302 patients being MRD-negative at the 3rd month after HSCT, molecularly relapsed thereafter, with a median time of 8 months from transplant (range; 5-108). Unfortunately, in these cases of molecular relapse, a systematic evaluation of BCR-ABL mutations was not available, and therefore, these data are not evaluable in this study.

d) TKIs post HSCT.

TKI inhibitors were used in 40% of patients (178/441) in the post-transplant phase. In 74% of patients, TKIs were used as preemptive (53%-94/178) or prophylactic (21%-38/178) therapy, before cytological relapse. The strategy of TKIs use post HSCT (prophylaxis, preemptive, cytological recurrence) was very variable and not shared across the various centers. As expected, time for TKI start was earlier in the prophylaxis (median 102 days, range: 17-259) and pre-emptive (median 128 days, range: 26-3233) groups, than in the relapse group (median 192 days, range 35-964).

Of the 38 patients starting TKI post HSCT prophylactically, 13 (34%) had a molecular relapse after a median of 5.1 months (range: 0.9-47.6 months); of these, 5 had a concurrent and 3 developed later cytological relapse, while 5 never relapsed cytologically. Ninety-four patients started TKI after HSCT because of molecular relapse (pre-emptive treatment): 38 (40%) had a cytological relapse

after a median of 4.8 months (range 0.4-76.6 months) after start of TKI. In the group of patients treated pre-emptively (complete data available for 88 patients), the probability of survival free of cytological relapse or death (from start of TKI) was 64.7% (95%CI: 53.2%-74%), 57% (95%CI: 45.1%-67.3%) and 55.1% (95%CI: 43%-65.6%) at 1, 2 and 5 year, respectively.

Dasatinib was used after HSCT in 45.5% of cases (81/178), followed by imatinib in 35.3% (n=63), ponatinib in 16.3% (n=29), and nilotinib in 2.2% (n=4). When analyzed according to treatment strategy, dasatinib was still the most used TKI in the prophylactic (45%, n=17) and relapse groups (59%), whereas in the pre-emptive group imatinib was chosen in most cases (46%, n=43). As expected, ponatinib was mostly employed in the relapse group (n=14, 48% of 29 patients using ponatinib after HSCT).

DISCUSSION

For many years, the curative options for Ph+ALL have been extremely limited, and chemotherapy followed by allogeneic HSCT has remained the primary standard of care of this disease. This approach allowed for a 5-year OS of 30-35%, whereas in patients not treated with HSCT, the expected 5-year OS was not greater than 10-15%.[11,17,24] In recent years, the therapeutic scenario for adults with Ph+ALL has fortunately improved with the introduction of new drugs including the second and third generation inhibitors of tyrosine kinases (TKI), followed by anti-CD19 and anti-CD22 monoclonal antibodies (Blinatumomab and Inotuzumab), and, more recently, the cell therapies with chimeric antigen receptor T cells (CAR-T cells).[25-31] Some of these approaches are currently used in second line, but a shift to the frontline setting is foreseeable, while use as maintenance therapy or MRD treatment represent other forthcoming options.[32] The efficacy of TKIs in Ph+ALL treatment is without question considering their ability to obtain the CR with MRD negativity. Nevertheless, few data and studies are available today regarding the outcome of therapeutic programs including both TKI and HSCT and, to date, HSCT is still considered a standard of care to consolidate remission in Ph+ALL, even though recent results suggest that long lasting remission can be achieved and maintained even without transplantation.[10,25,33-35] The retrospective nationwide analysis we present in this paper was undertaken to evaluate the clinical outcome of adult Ph+ALL patients who underwent HSCT in the TKIs-based therapy era. In keeping with previous reports, our results confirm the potential curative effect of HSCT in patients with Ph+ALL (**TABLE 5**).[14,19] The long-term follow-up of this large analysis of patients receiving TKIs treatment prior to HSCT indicates that survival can be achieved close to 50%, a significant improvement compared to the pre-TKI era. A point of strength of this study is the inclusion of a large number of patients undergoing HSCT in a relatively short period of time (73% of HSCT included were performed from 2011 to 2016), as well as the long follow-up duration. In addition, all included patients received TKI before HSCT and had MRD evaluation before transplantatation consistently determined by quantitative polymerase chain reaction (RQ-PCR).

As expected, for patients in CR, the main parameter that negatively affected outcomes was undergoing HSCT with measurable levels of MRD. Patients with negative MRD before HSCT had a 5-year OS and PFS significantly better compared with those with measurable levels of MRD. In keeping with other previous studies and registry data the improved clinical outcome was mainly due to a reduction in the cumulative incidence of relapse (CIR) in MRD negative patients.[14,19,36-38] This observation emphasize the importance of achieving a robust remission before HSCT and with this aim innovative treatments, such as second and third generation TKIs and anti-CD19 and anti-CD22 monoclonal antibodies, should be strongly considered before transplantation. Not surprisingly, the

importance of achieving a complete molecular remission holds true also for patients who are excluded from HSCT as part of the front-line treatment and the long-term PFS that has been reported in some patients achieving MRD negative status without HSCT highlights the need of future prospective clinical trials comparing transplantation with the available innovative treatments based on TKIs and/or immunotherapy.[33,34,39] The interesting results coming from some recent but not randomized clinical trials, confirm the clinical relevance of achieving a complete molecular remission and also pose a challenge to the absolute indication to perform an allogeneic transplant in MRD negative patients, as indispensable post-remission therapy.[25,33]

Despite the evident advantage of being molecularly negative before transplant, our data confirm that HSCT is able to convert a significant number of patient (60% of cases) who are MRD positive at time of conditioning. Moreover, the subgroup of patients with MRD-negative both at HSCT and at 3rd month after HSCT had a better outcome with a 5 year OS of 70%. Conversely, the presence, at conditioning, of a documented active disease (relapsed or refractory) was associated with a very dismal outcome with a median OS and PFS of 7 and 5 months, respectively. This observation clearly points out that Ph+ALL patients with refractory disease should be offered new experimental treatments able to induce at least a hematologic response before being considered eligible to transplant.[25-31]

Despite some progress, the NRM, even if reduced, remains a major problem (20% in this recent cohort) affecting the decision to advise an allogeneic HSCT to these patients.[14,19] A transplant from a mismatched donor remains significantly associated to a higher risk of NRM most likely as a consequence of more GvHD. In this study a large proportion of patients (49%) did not receive an in vivo T cell depletion and we observed a relatively high incidence of extensive chronic GvHD (5 yrs-cumulative incidence of 19%) and this should be carefully considered when planning future clinical trials with specific GvHD prophylaxis.[40] Moreover, the deep molecular remission achievable in a proportion of patients before transplant strongly suggest to consider reduced intensity conditioning regimens in Ph+ALL, particularly when MRD negative and older than 50 years. In this setting, preliminary uncontrolled studies suggest that RIC regimens may represent a good alternative to myeloablative conditioning (MAC) regimen.[35,41]

Another controversial issue is the use of TKIs in the post-transplant period to prevent the risk of relapse. In this study, treatment with TKIs after HSCT was performed in 40% of patients, in most cases as a pre-emptive treatment. As expected, outside the setting of a prospective clinical trial, there was a significant lack of uniformity as to the criteria of when and how to start treatment with TKIs after transplant. It is extremely important that, quite recently, the EBMT Acute Leukemia Working Party published a position paper about the use of post-transplant TKIs.[42] These

recommendations clearly underline the need that the MRD assessments have to become a standard part of disease monitoring post HSCT, in order to offer a timely initiation of an effective treatment with TKIs to minimize the risk of leukemia relapse.[39,42-44]

Additional limitations of the current study need to be considered, including the heterogeneity of TKIs-based programs (with or without chemotherapy) used to achieve remission before transplant, the type of conditioning regimens and GvHD prophylaxis. All of these limitations preclude any definitive conclusions regarding the optimal standard of care in this context. In addition, our registry data base did not contain specific information about BCR/ABL mutations, such as the T315I and others, and TKIs related complications.

In conclusion, our data confirm that alloHSCT is a potentially curative treatment for Ph+ALL patients with an excellent outcome for those obtaining a molecular remission before transplant. Innovative treatment approaches with targeted molecules will be crucial to further improve this scenario, reducing the risk of relapse after transplantation and NRM. In addition, prophylactic or preemptive post-transplant therapy with TKIs should be adopted to minimize the risk of leukemia relapse. Future prospective clinical trials comparing post-remission strategy with or without HSCT are urgently need to define the best evidence-based consolidation among Ph+ALL patients achieving molecular remission through the incorporation of innovative drugs into frontline treatment. For the present, waiting for new and consolidated data, we suggest to offer HSCT for all suitable patients with Ph+ALL in first CR, taking into consideration also patients' preference.

REFERENCES

1. Fielding A. Current treatment of Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia. Hematology Am Soc Hematol Educ Program 2011;2011:231-7.
2. Chiaretti S, Foa' R. Management of Adult Ph-positive acute Lymphoblastic Leukemia. Hematology 2015;406-411.
3. Fielding A, Goldstone AH. Allogeneic hematopoietic stem cell transplant in Philadelphia-positive acute lymphoblastic leukemia. Bone Marrow Transplant 2008;41(5):447-453.
4. Fielding AK, Rowe JM, Richards SM, et al. Prospective outcome data on 267 unselected adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia confirms superiority of allogeneic transplantation over chemotherapy in the pre-imatinib era: results from the International ALL Trial MRC UKALLXII/ECOG2993. Blood 2009;113(19):4489-4496.
5. Bassan R, Rossi G, Pogliani EM, et al. Chemotherapy-phased imatinib pulses improve long-term outcome of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Northern Italy Leukemia Group protocol 09/00. J Clin Oncol. 2010;28(22):3644-52.
6. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med 2013;369(19):1783-1796.
7. Carpenter PA, Johnston L, Fernandez HF, et al. Post-transplant feasibility study of nilotinib prophylaxis for high-risk Philadelphia chromosome positive leukemia. Blood 2017;130(9):1170-1172.
8. Vignetti M, Fazi P, Cimino G, et al. Imatinib plus steroids induces complete remission and prolongs survival in elderly Philadelphia chromosome–positive patients with acute lymphoblastic leukemia without additional chemotherapy: results of the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) LAL 0201-B protocol. Blood 2007;109(9):3676-3678.
9. Foà R, Vitale A, Vignetti M, et al. Dasatinib as a first-line treatment for adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL). Blood 2011;118(25):6521-6528.
10. Leoni V, Biondi A. Tyrosine kinase inhibitors in BCR-ABL positive acute lymphoblastic leukemia. Haematologica 2015;100(3):295-299.

11. Yilmaz M, Kantarjian H, Ravandi-Kashani F, et al. Philadelphia chromosome-positive acute lymphoblastic leukemia in adults: current treatments and future perspectives. Clin Adv Hematol Oncol 2018;16(3):216-223.

12. Kebriaei P, Saliba R, Rondon G, et al. Long-term follow-up of allogeneic hematopoietic stem cell transplantation for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: impact of tyrosine kinase inhibitors on treatment outcomes. Biol Blood Marrow Transplant 2012;18(4):584-92.

13. Ravandi F, Jorgensen JL, Thomas DA, et al. Detection of MRD may predict the outcome of patients with Philadelphia chromosome-positive ALL treated with tyrosine kinase inhibitors plus chemotherapy. Blood 2013; 122(7):1214-1221.

14. Brissot E, Labopin M, Beckers MM, et al. Tyrosine kinase inhibitors improve long-term outcome of allogeneic hematopoietic stem cell transplantation for adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia. Haematologica 2015;100(3):392-399.

15. Ma L, Hao S, Diong C, et al. Pre-transplant achievement of negativity in minimal residual disease and FAB L1 morphology predict superior out come after allogeneic transplant for Philadelphia chromosome positive ALL: an analysis of Southeast Asian patients. Leuk Lymphoma 2015;56(5):1362-9.

16. Litzow MR. Should anyone with Philadelphia chromosome-positive ALL who is negative for minimal residual disease receive a hematopoietic stem cell transplant in first remission ? Best Pract Res Clin Haematol 2016;29(4):345-350.

17. Ravandi F. Current management of Philadelphia chromosome positive ALL and the role of stem cell transplantation. Hematology Am Soc Hematol Educ Program 2017;2017(1):22-27.

18. El Fakih R, Jabbour E, Ravandi F, et al. Current paradigms in the management of Philadelphia chromosome positive acute lymphoblastic leukemia in adults. Am J Hematol 2018;93(2):286-295.

19. Nishiwaki S, Imai K, Mizuta S, et al. Impact of MRD and TKI on allogeneic Hematopietic cell transplantation for Ph+ALL: a study from the adult ALL WG of the JSHCT. Bone Marrow Transplantation 2016;51:43-50.

20. Chen H, Liu KY, Xu LP, et al. Administration of imatinib after allogeneic hematopoietic stem cell transplantation may improve disease-free survival for patients with Philadelphia chromosome positive acute lymphoblastic leukemia. J Hematol Oncol 2012;5:29.

21. Gabert J, Beillard E, van der Velden VH, et al. Standardization and quality control studies of "real-time" quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia—a Europe Against Cancer program. Leukemia 2003;17(12):2318-5.

22. van der Velden VH, Panzer-Grumayer ER, Cazzaniga G, et al. Optimization of PCR-based minimal residual disease diagnostics for childhood acute lymphoblastic leukemia in a multi-center setting. Leukemia 2007;21(4):706-13.

23. Shulman HM, Sullivan KM , Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med, 69 (1980): 204-217

24. Litzow MR, Fielding AK, Luger S, et al. The evolving role of chemotherapy and hematopoietic cell transplants in Ph-positive acute lymphoblastic leukemia in adults. Bone Marrow Transplant. 2017;52(12):1592-1598.

25. Jabbour E, Pui CH, Kantarjian H. Progress and Innovations in the Management of Adult Acute Lymphoblastic Leukemia. JAMA Oncol. 2018. [Epub ahead of print]

26. Martinelli G, Boissel N, Chevallier P, et al. Complete hematologic and molecular response in adult patients with relapsed/refractory Philadelphia chromosome positive B-precursor acute lymphoblastic leukemia following treatment with blinatumomab: results from a phase II, single-arm, multicenter study. J Clin Oncol 2017;35(16):1795-1802.

27. Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. N Engl J Med 2016;375(8):740–753.

28. Kantarjian H, Stein A, Gökbuget N, Fielding AK, et al.. Blinatumomab versus Chemotherapy for Advanced Acute Lymphoblastic Leukemia. N Engl J Med. 2017 Mar 2;376(9):836-847.

29. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remission in Leukemia. N Engl J Med 2014; 371:1507-1517.

30. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018 Feb 1;378(5):439-448.

31. Park JH, Rivière I, Gonen M, et al. Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia. N Engl J Med. 2018 Feb 1;378(5):449-459.

32. Gökbuget N, Dombret H, Bonifacio M, et al. Blinatumomab for minimal residual disease in adults with B-cell precursor acute lymphoblastic leukemia. Blood 2018;131(14):1522-1531.

33. Jabbour E, Kantarjian H, Ravandi F, et al. Combination of hyper-CVAD with ponatinib as first-line therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia: a single-centre, phase 2 study. Lancet Oncol 2015;16(15):1547-1555.

34. Short NJ, Jabbour E, Sasaki K, et al. Impact of complete molecular response on survival in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood 2016;128(4):504-7.

35. Bachanova V, Marks D.I., Zhang M.J.,et al.Ph+ ALL patients in first complete remission have similar survival after reduced intensity and myeloablative allogeneic transplantation: impact of tyrosine kinase inhibitor and minimal residual disease Leukemia 2014;28:658-665.

36. Lussana F, Intermesoli T, Gianni F, et al. Achieving Molecular Remission before Allogeneic Stem Cell Transplantation in Adult Patients with Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia: Impact on Relapse and Long-Term Outcome. Biol Blood Marrow Transplant 2016;22(11):1983-1987.

37. Balduzzi A, Di Maio L, Silvestri D, Songia S, Bonanomi S, Rovelli A, Conter V, Biondi A, Cazzaniga G, Valsecchi MG. Minimal residual disease before and after transplantation for childhood acute lymphoblastic leukaemia: is there any room for intervention? Br J Haematol. 2014;164(3):396-408.

38. Bar M, Wood BL, Radich JP, Doney KC, Woolfrey AE, Delaney C, Appelbaum FR, Gooley TA. Impact of minimal residual disease, detected by flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute lymphoblastic leukemia. Leuk Res Treatment. 2014;2014:421723.

39. Wieduwilt MJ. In the Era of BCR-ABL1 Inhibitors, Are We Closing the Survival Gap Between Allogeneic Hematopoietic Cell Transplantation and Chemotherapy for Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia in First Complete Remission ? Biol Blood Marrow Transplant 2018;24(4):637-638.

40. Kröger N, Solano C, Wolschke C, Bandini G, et al. Antilymphocyte Globulin for Prevention of Chronic Graft-versus-Host Disease. N Engl J Med. 2016 Jan 7;374(1):43-53.

41. Mohty M, Labopin M, Volin L, et al. Acute Leukemia Working Party of EBMT. Reduced-intensity versus conventional myeloablative conditioning allogeneic stem cell transplantation for patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation. Blood. 2010 25;116(22):4439-43.

42. Giebel S, Czyz A, Ottmann O, et al. Use of tyrosine kinase inhibitors to prevent relapse after allogeneic hematopoietic stem cell transplantation for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: A position statement of the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. Cancer 2016;122(19):2941-51

43. Cazzaniga G, De Lorenzo P, Alten J, et al. Predictive value of minimal residual disease in Philadelphia-chromosome-positive acute lymphoblastic leukemia treated with imatinib in the European intergroup study of post-induction treatment of Philadelphia-chromosome-positive acute lymphoblastic leukemia, based on immunoglobulin/T-cell receptor and BCR/ABL1 methodologies. Haematologica 2018;103(1):107-115.

44. Soverini S, Vitale A, Poerio A, et al. Philadelphia-positive acute lymphoblastic leukemia patients already harbor BCR-ABL kinase domain mutations at low levels at the time of diagnosis. Haematologica 2011;96(4):552-557.

TABLE 1. Patients and Ph+ALL characteristics (n 441).

Characteristics	**All evaluable cases**
Age at diagnosis	43 (16-68)
Gender (M/F)	230/211
Ph+ALL Mature phenotype	39/397 (9.8%)
Additional Cytogenetic Abnormalities	124/416 (30%)
BCR-ABL Transcript type	
p190	289/437 (66%)
p210	104/437 (24%)
p190+p210	30/437 (7%)
WBC at diagnosis (average) [x10^9/L]	59,1 (0,2-451)
WBC at diagnosis (median) [x10^9/L]	23,9
WBC > 30 [x10^9/L]	160/374 (43%)
WBC > 100 [x10^9/L]	62/374 (17%)
Number of TKI lines (average)	1,3 (1-3)
Number of TKI lines (median)	1,0
Time from diagnosis to SCT (median)	7,67 (2,3–78,8)
Ph+ALL STATUS at SCT	
CR	404/441 (92%)
1° CR	337/404 (83%)
CR >1°	67/404 (17%)
REL/REF	37/441 (8%)
MRD STATUS at SCT	
CR, MRD-positive	257/404 (64%)
CR, MRD-negative	147/404 (36%)

TABLE 2. HSCT characteristics.

	All (441 pts)
Median age (range) at transplant	44 (18-70)
Donor type:	
Sibling HLA id	159/441 (36%)
MUD	201/441 (46%)
Haplo	68/441 (15%)
Cord Blood	13/441 (3%)
Source of Stem Cells:	
BM	117/441 (27%)
PB	311/441 (70%)
CB	13/441 (3%)
Conditioning regimen:	
MAC	362/441 (82%)
RIC	79/441 (18%)
TBI based	221/441 (50%)
GVHD prophylaxis	
ATG	226/441 (51%)
CNI+ MTX/MMF	352/441 (80%)
Use of PT-CY	23/441 (5%)
Other	66/441 (15%)
HCT–CI (available in 402 pt)	
0-2	382/402 (95%)
≥3	20/402 (5%)
GVHD	
aGVHD requiring therapy (grade > 1)	181/441 (41%)
cGVHD requiring therapy (limited or extensive)	127/441 (29%)

CNI: calcineurin inhibitors; MTX: methotrexate, MMF:mofetil mycophenolate; PT-CY: post-transplant cyclophosphamide

TABLE 3. Univariate analysis of predictors for Overall Survival, Progression-Free Survival, Incidence of Relapse, Non-Relapse Mortality and Chronic Extensive GVHD.

		OS	OS	PFS	PFS	NRM	NRM	RI	RI	EcGVHD	EcGVHD
Variable	*Comparison*	HR (95%CI)	p-value	HR (95%CI)	p-value	SHR (95%CI)	p-value	SHR (95%CI)	p-value	SHR (95%CI)	p-value
Age	*per 10 y more*	1.13 (1.01 - 1.28)	**0.037**	1.09 (0.97 - 1.21)	0.143	1.36 (1.13 - 1.62)	**0.001**	0.88 (0.76 - 1.01)	0.074	1.1 (0.92 - 1.31)	0.291
Male recipient	*Yes vs no*	1.16 (0.88 - 1.54)	0.297	1.22 (0.94 - 1.58)	0.131	1.41 (0.95 – 2.1)	0.089	1.03 (0.73 - 1.45)	0.877	0.98 (0.63 - 1.51)	0.922
WBC at diagnosis [x10^9/L]	*> 30 vs < 30*	1.24 (0.91 - 1.69)	0.18	1.11 (0.83 - 1.49)	0.473	1.02 (0.66 - 1.58)	0.93	1.14 (0.77 - 1.67)	0.51	1 (0.63 - 1.59)	0.996
Additional karyotypic abnormalities	*Yes vs no*	1.05 (0.77 - 1.43)	0.77	1.05 (0.78 - 1.4)	0.754	0.94 (0.6 - 1.47)	0.771	1.09 (0.74 - 1.61)	0.657	1.06 (0.67 - 1.7)	0.795
Transplant year	*each year later*	0.96 (0.92 - 1.01)	0.119	0.97 (0.93 - 1.01)	0.178	0.98 (0.92 - 1.04)	0.525	0.96 (0.91 - 1.02)	0.177	0.95 (0.89 - 1.01)	0.129
Pre transplant therapy	*TKI + CHT vs TKI + steroide*	0.8 (0.58 - 1.11)	0.177	0.78 (0.58 - 1.05)	0.106	0.86 (0.54 - 1.35)	0.51	0.81 (0.55 - 1.19)	0.283	1.46 (0.82 - 2.61)	0.202
Interval diagnosis to CR1	*> median vs < median*	1.51 (1.14 - 2)	**0.005**	1.54 (1.18 - 2)	**0.001**	1.36 (0.92 - 2.03)	0.127	1.42 (1 - 2)	**0.047**	0.75 (0.49 - 1.17)	0.207
Disease status at HSCT	*CR2+ vs CR1*	2 (1.4 - 2.86)	**p < 0.001**	2.03 (1.45 - 2.84)	**p < 0.001**	1 (0.56 - 1.8)	0.988	2.42 (1.58 - 3.71)	**p < 0.001**	0.59 (0.28 - 1.23)	0.159
	Adv vs CR1	3.46 (2.28 - 5.27)	**p < 0.001**	3.67 (2.49 - 5.42)	**p < 0.001**	1.54 (0.79 - 3)	0.202	3.31 (1.94 - 5.63)	**p < 0.001**	0.8 (0.35 - 1.84)	0.601
MRD status at HSCT	*neg vs pos*	0.59 (0.43 - 0.82)	**0.002**	0.56 (0.42 - 0.76)	**p < 0.001**	0.96 (0.63 - 1.46)	0.849	0.43 (0.28 - 0.65)	**p < 0.001**	1.7 (1.1 - 2.62)	**0.018**
HCT-CI	*HCT-CI ≥ 3 vs < 3*	0.83 (0.53 - 1.3)	0.417	0.69 (0.45 - 1.05)	0.082	0.8 (0.43 - 1.5)	0.492	0.69 (0.39 - 1.2)	0.19	0.68 (0.33 - 1.41)	0.305
KPS at HSCT	*KPS ≥90%*	0.62 (0.45 - 0.85)	**0.003**	0.76 (0.56 - 1.04)	0.091	0.78 (0.49 - 1.24)	0.295	0.86 (0.56 - 1.32)	0.49	1.08 (0.61 - 1.91)	0.79
Donor type	*Sibling vs MUD*	0.88 (0.64 - 1.22)	0.444	0.91 (0.67 - 1.22)	0.517	0.59 (0.36 - 0.97)	**0.039**	1.25 (0.86 - 1.82)	0.25	1.39 (0.88 - 2.2)	0.157
	Alternative vs MUD	1.8 (1.26 - 2.57)	**0.001**	1.67 (1.2 - 2.33)	**0.003**	1.78 (1.11 - 2.84)	**0.017**	1.15 (0.71 - 1.87)	0.562	0.54 (0.25 - 1.17)	0.117
Female donor	*Yes vs no*	0.87 (0.65 - 1.17)	0.363	0.86 (0.66 - 1.12)	0.259	0.89 (0.59 - 1.34)	0.575	0.91 (0.64 - 1.3)	0.609	1.7 (1.1 - 2.63)	**0.016**
CMV serostatus	*D-/R- vs D+/R+*	0.76 (0.45 - 1.29)	0.313	0.88 (0.56 - 1.39)	0.584	0.74 (0.33 - 1.68)	0.478	0.98 (0.57 - 1.69)	0.948	1.1 (0.56 - 2.17)	0.787
	D-/R+ vs D+/R+	1.23 (0.89 - 1.71)	0.204	1.08 (0.79 - 1.47)	0.637	1.64 (1.06 - 2.53)	**0.027**	0.7 (0.45 - 1.09)	0.112	0.68 (0.38 - 1.21)	0.188
	D+/R- vs D+/R+	0.87 (0.49 - 1.54)	0.629	0.93 (0.55 - 1.57)	0.787	1.08 (0.49 - 2.36)	0.85	0.86 (0.42 - 1.74)	0.666	1.3 (0.62 - 2.72)	0.485
Conditioning regimen	*MAC vs RIC*	0.83 (0.59 - 1.17)	0.289	0.84 (0.61 - 1.17)	0.305	0.86 (0.53 - 1.4)	0.546	0.86 (0.56 - 1.33)	0.497	0.97 (0.55 - 1.69)	0.91
Source of HSC	*BM vs PB*	1.04 (0.75 - 1.43)	0.832	1.03 (0.76 - 1.38)	0.867	1.24 (0.8 - 1.94)	0.333	0.83 (0.55 - 1.25)	0.369	0.74 (0.44 - 1.26)	0.267
	CB vs PB	1.83 (0.9 - 3.73)	0.098	1.66 (0.85 - 3.24)	0.141	2.11 (0.81 - 5.5)	0.127	0.96 (0.34 - 2.71)	0.943	0.34 (0.05 - 2.33)	0.27
Use of ATG	*Yes vs no*	0.82 (0.62 - 1.09)	0.177	0.88 (0.68 - 1.15)	0.357	0.78 (0.53 - 1.17)	0.229	1.03 (0.73 - 1.45)	0.878	0.54 (0.34 - 0.85)	**0.007**
TBI-containing regimen	*Yes vs no*	0.86 (0.65 - 1.14)	0.305	0.72 (0.55 - 0.94)	**0.014**	1.16 (0.78 - 1.72)	0.471	0.56 (0.39 - 0.79)	**0.001**	1.45 (0.93 - 2.26)	0.099

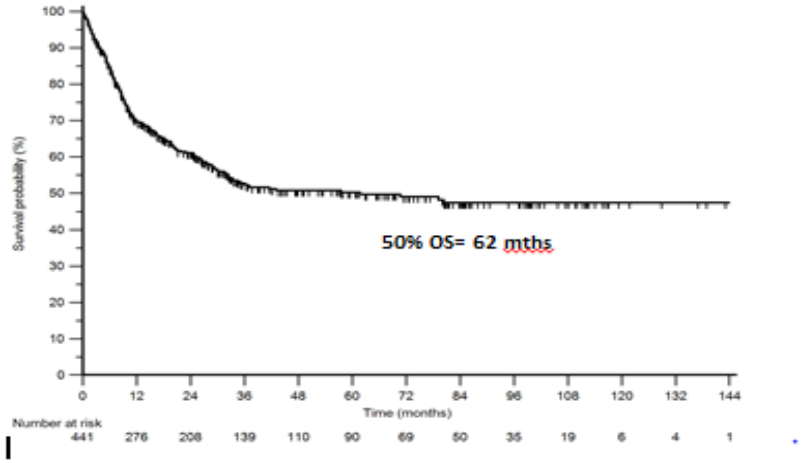
TABLE 4. Multivariate Analysis of significant predictors for Overall Survival, Progression-Free Survival, Incidence of Relapse, Non-Relapse Mortality and Chronic Extensive GVHD.

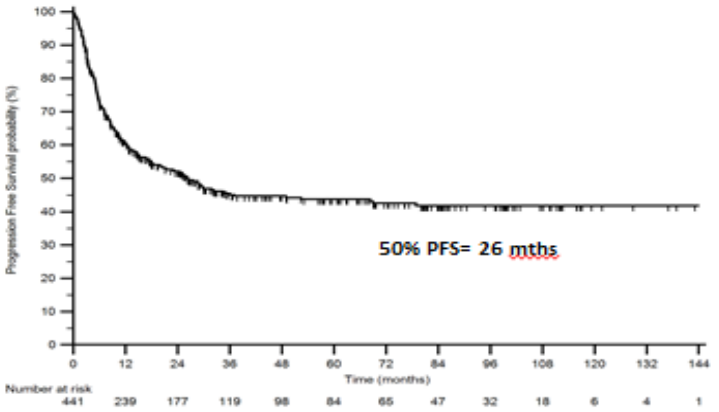
	Variable	*Comparison*	HR (95%CI)	p-value
Overall Survival (OS)	**Donor**	Alternative vs other (Sibling + UD)	1.77 (1.26 - 2.49)	0.001
	Disease status at Allo-SCT	CR2+ vs CR1	1.99 (1.39 - 2.87)	p < 0.001
		Adv vs CR1	2.75 (1.76 - 4.31)	p < 0.001
	Age	per 10y more	1.17 (1.03 - 1.32)	0.013
	MRD status at Allo-SCT	neg vs pos	0.65 (0.46 - 0.91)	0.011
	Pre Allo-SCT strategy	TKI + CHT vs TKI + steroids	0.7 (0.5 - 0.97)	0.035
	Transplant year	each year later	0.94 (0.9 - 0.98)	0.008
Progression-free Survival (PFS)	**Donor**	Alternative vs other (Sibling + UD)	1.61 (1.16 - 2.22)	0.004
	Disease status at Allo-SCT	CR2+ vs CR1	2.05 (1.46 - 2.89)	p < 0.001
		Adv vs CR1	2.71 (1.78 - 4.11)	p < 0.001
	MRD status at Allo-SCT	neg vs pos	0.6 (0.44 - 0.81)	0.001
	Pre Allo-SCT strategy	TKI + CHT vs TKI + steroids	0.63 (0.46 - 0.86)	0.003
	Transplant year	each year later	0.95 (0.91 - 1)	0.036
Non-relapse Mortality (NRM)	**Donor**	Sibling vs UD	0.58 (0.35 - 0.94)	0.027
		Alternative vs UD	1.74 (1.09 - 2.8)	0.021
	Age	per 10y more	1.4 (1.16 - 1.68)	p < 0.001
	Male recipient	Yes vs no	1.52 (1.01 - 2.28)	0.047
Relapse incidence (RI)	**Disease status at SCT**	CR2+ vs CR1	2.55 (1.66 - 3.91)	p < 0.001
		Adv vs CR1	2.38 (1.39 - 4.06)	0.002
	Age	per 10y more	0.8 (0.69 - 0.93)	0.003
	MRD status at SCT	neg vs pos	0.54 (0.35 - 0.85)	0.007
	TBI-containing regimen	Yes vs no	0.52 (0.36 - 0.75)	0.001
	Transplant year	each year later	0.94 (0.89 - 1)	0.047
Chronic Extensive GVHD (EcGVHD)	**Donor**	Alternative vs other (Sibling + UD)	0.41 (0.2 - 0.87)	0.019
	Use of ATG	Yes vs no	0.56 (0.35 - 0.88)	0.011
	Female donor	Yes vs no	1.77 (1.15 - 2.74)	0.01
	MRD status at Allo-SCT	neg vs pos	1.74 (1.13 - 2.68)	0.012

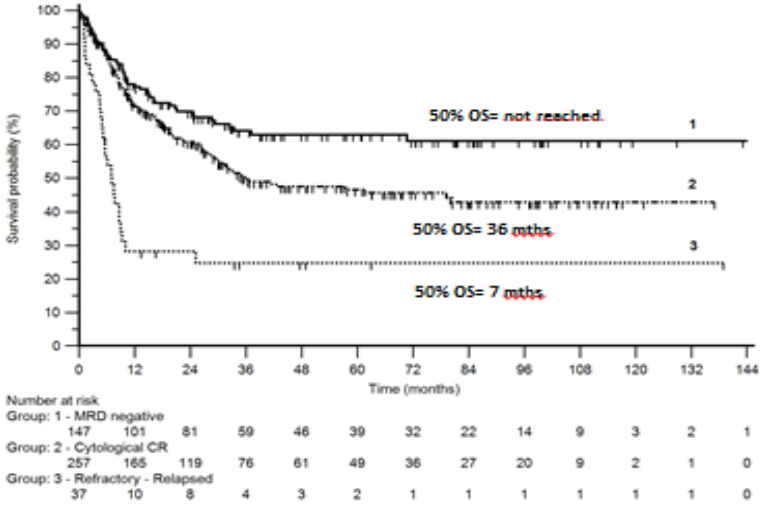
TABLE 5. Comparison between GITMO study and EBMT/JSHCT studies.

	GITMO study 2018	**EBMT study (BRISSOT 2015)**	**JSHCT study (NISHIWAKI 2016)**
patients number	**441**	**473**	**432**
Period of time	Retrospective (2005-2016)	Retrospective (2000-2010)	Retrospective (1990-2000)
Pre-transplant TKI -n°of pts (%)	441 (100%)	390 (82,5%)	432 (100%)
Age at HSCT-Median (range)	45 (19-70)	42 (18-70)	43 (16-68)
Status at HSCT	CR 92% (1° CR 83%) RIC/REL 8%	1° CR (100%)	1° CR (100%)
MRD-negative at HSCT	36%	65%	64%
myeloablative regimen	82%	79%	86%
Transplants from HLA-id sibling donor	36%	49%	32%
OS	5 yrs: 50,3% 63% MRD- 47% MRD+	5 yrs 46%	4 yrs 67% MRD- 55% MRD+
PFS	5 yrs: 44% 57% MRD- 40% MRD+	5 yrs 38%	4 yrs 60% MRD- 46% MRD+
CIR	5 yrs 19,5% MRD- 35,4% MRD+	5 yrs 36%	4 yrs 19% MRD- 29% MRD+
NRM	5 yrs 24%	5 yrs 26%	4 yrs 21% MRD- 25% MRD+
Post-transplant TKI - n° of pt (%)	178/441 (40%)	157/319 (49%)	103/425 (24%)
aGVHD	41%	40% (a 100 gg)	7-9%
cGVHD	29%	53% (a 5 aa)	32-33%
Impact of MRD negativity	Favorable on OS, PFS, CIR	No impact on OS, PFS, CIR	Favorable on OS, PFS, CIR

FIGURE 1. A: Overall Survival, from transplant, of the whole transplanted population (median OS= 62 months). B: Progression Free Survival, from transplant, of the whole transplanted population (median PFS= 26 months). C: OS according to status at Allo-SCT (1.MRD neg; 2. Cytologic CR; 3 Refractory or Relapsed)-MRD neg vs MRD pos, P= 0,0015. D: PFS, from transplant, according to status at Allo-SCT (1.MRD neg; 2. Cytologic CR; 3 Refractory or Relapsed)-MRD neg vs MRD pos, P=0,003.







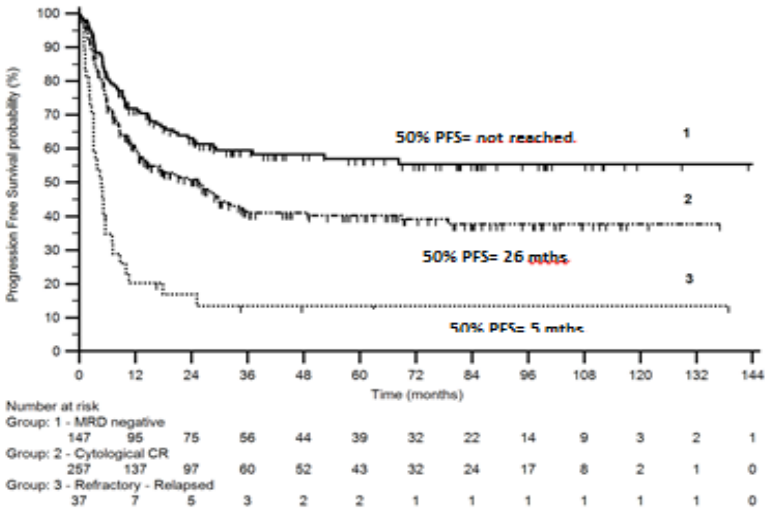
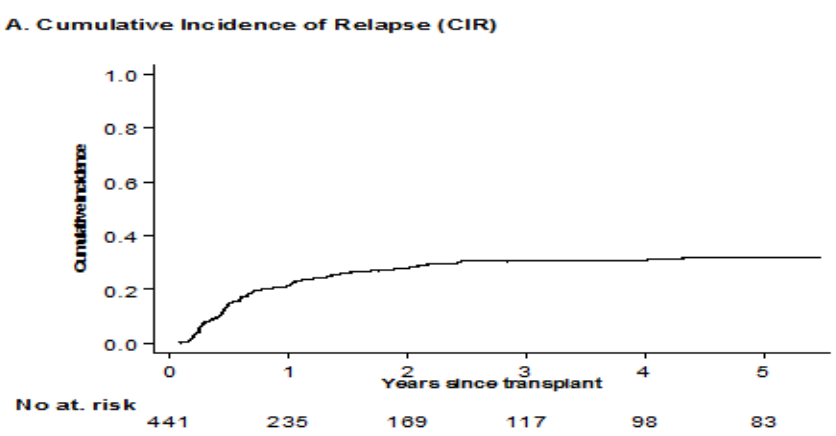
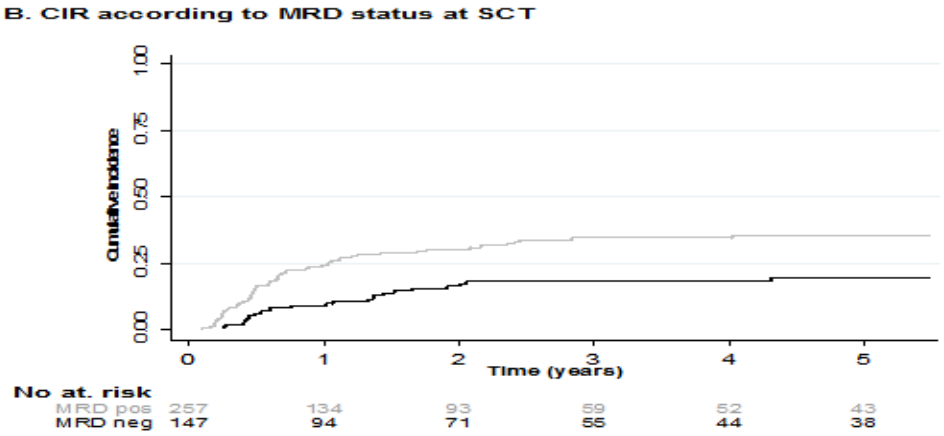
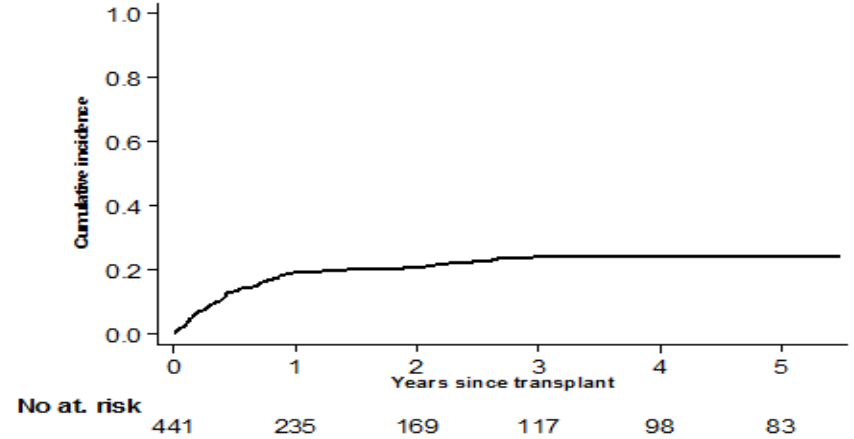
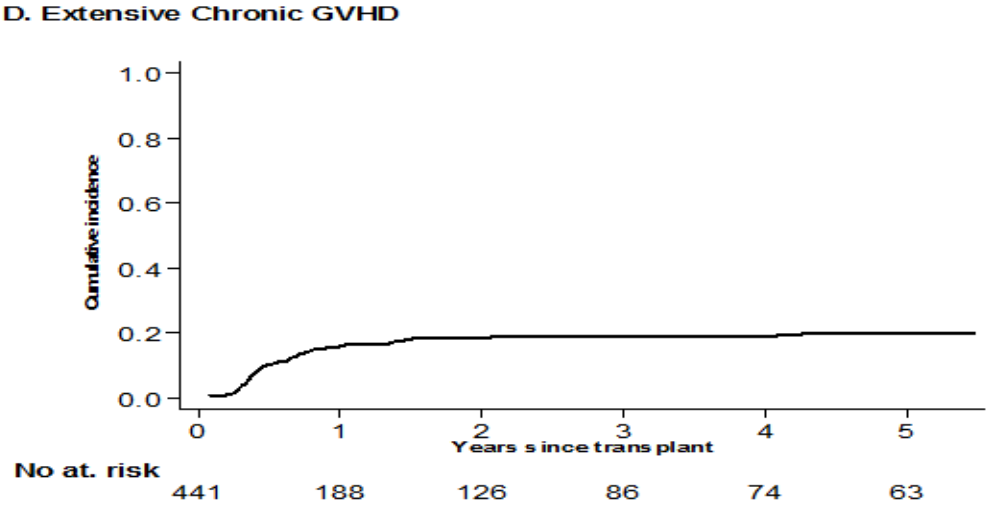


FIGURE 2. [A] Cumulative Incidence of Relapse at 2 and 5 yrs. 27,9% (95% CI:23,6-32,3) and 31,8% (95%CI:27,1-36,5). **[B] CIR** according to MRD status at SCT. Gray test: SHR= 0.47 (0.31–0.73), p=0,001; CI at 1,2,5 years for **MRD neg: 9.1%** (5.1%-14.5%), **16.3%** (10.6%-23.1%), **19.5%** (13%-27%); CI at 1,2,5 years for **MRD pos**: **24.3%** (19.2%-29.8%), **30.3%** (24.6%-36.2%), **35.4%** (29.1%-41.7%). **[C] NRM** at 1,2,3,5 years: 19.1% (15.5%-22.9%), 20.7% (17%-24.7%), 24.1% (20%-28.5%), 24.1% (20%-28.5%). **[D] Cumulative Incidence of extensive cGVHD** at 1,2,3,5 years: 16% (12.7%-19.6%), 18.5% (14.9%-22.4%), 18.8% (15.2%-22.7%), 19.8% (16%-23.9%)

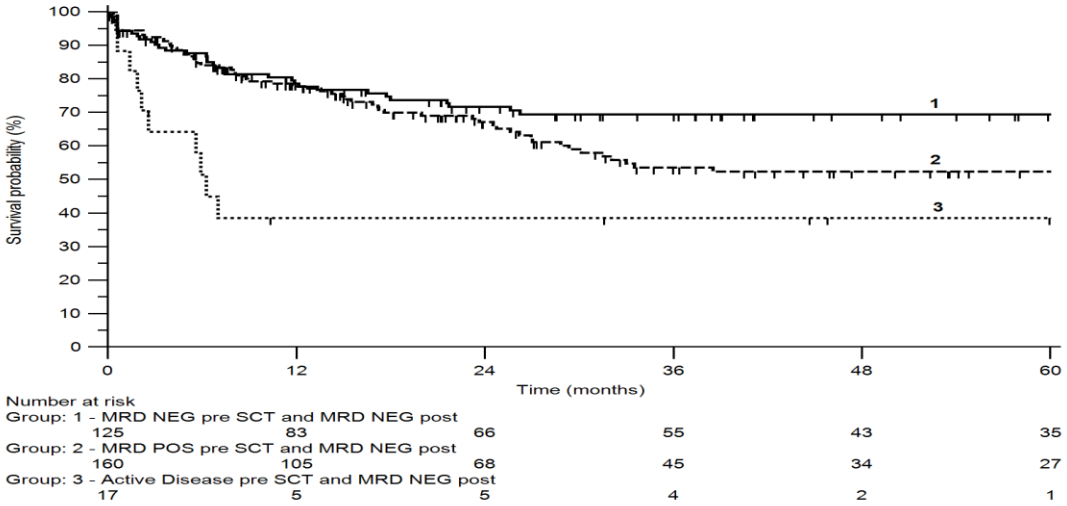


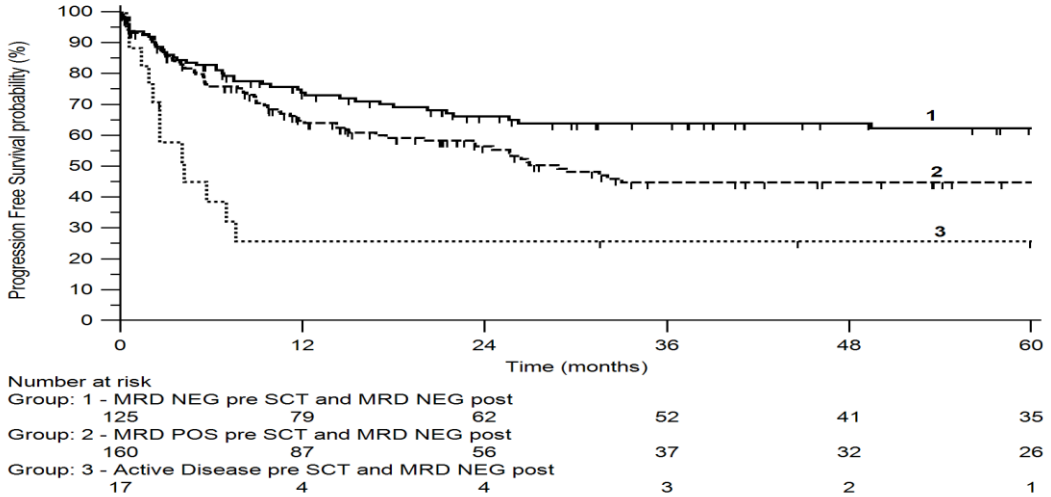






SUPPLEMENTAL FIGURE 1. OS and PFS in MRD NEG patients at 3rd month after Allo-SCT according to MRD Status pre Allo-SCT (group 1. MRD neg at Allo-SCT and MRD neg at 3rd month after Allo-SCT; group 2. MRD pos at Allo-SCT and MRD neg at 3rd month after Allo-SCT; group 3. Active disease at Allo-SCT and MRD neg at at 3rd month after Allo-SCT). OS at 5 years: group 1=70% , group 2=53%, group 3=39%. PFS at 5 years: group 1=62% , group 2=45%, group 3=26%.





SUPPLEMENTAL TABLE 1. MRD Negativity at 3rd month post Allo-SCT according to MRD status pre Allo-SCT.

Pre SCT MRD Status	N° of MRD-negative at 3rd month post SCT	OS at 2, 3, 5 yrs	PFS a 2, 3 e 5 yrs
MRD-negative = 147	125/140 (89%)	125 MRD-negative at SCT and at 3rd month: 72%, 70%, **70%**	125 MRD-negative at SCT and at 3rd month: 66%, 64%, **62%**
MRD-positive = 257	160/246 (65%)	160 MRD-negative at 3rd month: 67%, 54%, 53%	160 MRD-negative at 3rd month: 56%, 45%, 45%
Refractory/Relapse = 37	17/35 (49%)	17 MRD-negative at 3rd month: 39%, 39%, 39%	17 MRD-negative at 3rd month: 26%, 26%, 26%
Total cases = 441	**302/421 (72%)**	302 MRD-negative at 3rd month: 67%, 60%, 60%	302 MRD-negative at 3rd month: 59%, 52%, 52%