

## HIV-associated lymphoma successfully treated with peripheral blood stem cell transplantation

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**Objective.** To evaluate feasibility, safety, and efficacy of peripheral blood stem cell collection (PBSCC) and autologous stem cell transplantation (ASCT), to treat patients diagnosed of high-risk or relapsed HIV-associated lymphoma (HIV<sup>+</sup> Ly), responding to highly active antiretroviral therapy (HAART).

**Methods.** Prospective and multicentric study in patients with high-risk or relapsed chemosensitive HIV<sup>+</sup> Ly, candidate for consolidation with ASCT. Eligibility criteria were similar to those of HIV<sup>-</sup> lymphoma. HAART was aimed to be maintained during the procedure.

**Results.** Fourteen patients were admitted. Adequate PBSCC was obtained from all patients (median CD34<sup>+</sup> cells was  $4.7 \times 10^6/\text{kg}$ ). Three patients died before ASCT; two had disease progression and one died from VHC-liver failure. Eleven transplanted patients showed neutrophil engraftment after a median time of 16 days (range, 9–33 days), and nine patients showed platelet engraftment after a median time of 20 days (range, 11–36 days). CD4<sup>+</sup> cell counts and HIV viral load (VL) were appropriately preserved along the procedure. No patients died from treatment-related complications. One patient died from lymphoma progression (day +19), and another died in complete remission (CR) with undetectable VL, 15 months after transplant, due to infection. One patient relapsed at 32 months after ASCT. The remaining eight patients are alive in CR with an event-free survival of 65% and a median follow-up of 30 months after ASCT (range, 7–36 months).

**Conclusions.** These results show that feasibility, safety, and efficacy of PBSCC and ASCT in HIV<sup>+</sup> Ly patients responding to HAART are similar to those observed in the HIV<sup>-</sup> lymphoma setting. © 2005 International Society for Experimental Hematology. Published by Elsevier Inc.

### Introduction

Patients with human immunodeficiency virus (HIV) infection have higher probability of developing diffuse aggressive non-Hodgkin lymphoma (NHL) and Hodgkin's disease (HD) compared with the general population [1–3]. HIV-associated lymphomas (HIV<sup>+</sup> Ly) frequently show high-risk criteria for relapse, aggressive histology, advanced stage, B symptoms, extra nodal involvement, and high International Prognostic Index (IPI  $\geq 2$ ) [4,5].

Highly active antiretroviral therapy (HAART) has proved to be effective in suppressing HIV replication, changing the natural history of HIV disease (improving immune function, reducing the incidence of opportunistic infections and HIV<sup>+</sup>-related tumors), and prolonging survival. However, the frequency of NHL and HD remains apparently increased in these patients, despite the use of HAART [6–9].

Before the advent of HAART, treatment of HIV<sup>+</sup> Ly with conventional chemotherapy resulted in important hematological toxicity and further impairment of the immune function. The better performance and immune status of patients on HAART allowed the use of more aggressive chemotherapy, achieving higher rates of complete remission (CR) [10–12]. Nevertheless, relapsed or refractory HIV<sup>+</sup> Ly patients

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showed poor survival results, even those that reached a second CR [13,14].

High-dose chemotherapy followed by autologous peripheral blood stem cell transplantation (ASCT) is the therapy of choice in relapsed or partially responding HIV<sup>-</sup> lymphoma patients [15]. Moreover this treatment has been employed in first-remission patients (CR-1) with poor risk features at diagnosis [16], particularly when IPI is greater than or equal to 2 or when more than one line of chemotherapy is needed to achieve CR-1.

Since the introduction of HAART, HIV infection could be considered similar to other chronic diseases. Thus ASCT could be an option to treat patients with relapsed HIV<sup>+</sup> Ly and even to consolidate patients with high-risk features at presentation [17,18]. In fact, high-dose chemotherapy and ASCT has been recently used in aggressive HIV<sup>+</sup> Ly [19–23].

We report on the long-term follow-up of patients with aggressive HIV<sup>+</sup> Ly treated with HAART and high-dose chemotherapy followed by ASCT performed in three Spanish institutions.

## Patients and methods

### Study design

Between April 2000 and May 2003, 14 consecutive male patients with aggressive HIV<sup>+</sup> Ly were admitted in a prospective, multicentric study of myeloablative chemotherapy followed by ASCT in three Spanish hospitals. The aim was to evaluate feasibility, safety, and efficacy of peripheral blood stem cell collection (PBSC) and ASCT in these patients. Survival analysis was performed in transplanted patients as well as in the entire group of candidate patients, on an intention-to-treat basis.

Inclusion criteria were: (1) patients under 65 years, diagnosed of chemotherapy-sensitive high-risk HIV<sup>+</sup> Ly, either in second CR (CR-2), in partial remission (PR), or in CR-1 with poor clinical or histological features (defined as age-adjusted IPI  $\geq 2$ , CR-1 after two or more lines of chemotherapy, or undertreated Burkitt lymphoma due to hematologic toxicity); (2) effective HAART, defined as conspicuously decreasing or undetectable HIV viral load (VL); (3) good performance status (Eastern Cooperative Oncology Group [ECOG] scale  $< 2$ ); (4) left ventricular ejection fraction higher than 50%; (5) creatinine level lower than 2 mg/dL and bilirubin level lower than 2 mg/dL; (6) absence of active opportunistic infections (OI); (7) approval of the therapeutic program by the hospital clinical ethics committee and signed informed consent obtained from all patients, according to the Declaration of Helsinki.

### Patients

The present study included 14 male patients (Table 1), with a median age of 39.5 years (range, 31–61 years), who were candidates to ASCT in three hospitals. Eleven patients had NHL (6 diffuse large B cell lymphoma, 2 Burkitt lymphoma, 1 Burkitt-like lymphoma, 2 anaplastic large-cell lymphoma, according to the Revised European American Lymphoma (REAL) classification for NHL [24]), and three patients were diagnosed of HD (2 mixed

cellularity, 1 lymphocytic depletion). Twelve showed advanced stages (9 IV-B, 1 IV-A, 1 III-B, 1 III-A) and two had bulky disease (stage I-A). Ten patients had one or more extra nodal involvement sites: bone marrow (5 patients), central nervous system (3 patients), lung (2 patients), gastrointestinal tract (2 patients), and liver (1 patient). Age-adjusted IPI was 3 in eight patients, 2 in five and 1 in one undertreated Burkitt lymphoma (median = 3). Four patients had simultaneous diagnosis of AIDS and HIV<sup>+</sup> Ly. According to the inclusion criteria, the indications to enter the current study for HIV<sup>+</sup> Ly patients were (Table 1): CR-2 in 4 patients; CR-1 after more than one line of chemotherapy in 2 patients; undertreated Burkitt lymphoma in CR-1 in 2 patients; CR-1 with IPI  $\geq 2$  in 2 patients; and PR chemotherapy-sensitive lymphoma in 4 patients.

### Methods

*Peripheral blood stem cell mobilization.* Patients were mobilized after achieving the best response to standard chemotherapy. Four different schedules were used: (1) standard dose of granulocyte colony-stimulating factor (G-CSF) (10  $\mu\text{g}/\text{kg}/\text{day} \times 5$  days) in patient no. 7; (2) high dose of G-CSF (20  $\mu\text{g}/\text{kg}/\text{day} \times 5$  days) in 6 patients (Patients no. 2, 4, 5, 6, 13, and 14); (3) cyclophosphamide (1.5  $\text{g}/\text{m}^2 \times 1$  day) plus high dose of G-CSF (20  $\mu\text{g}/\text{kg}/\text{day} \times 7$ –9 days) in 4 patients (Patients nos. 1, 3, 8, and 12); and (4) Ara-C (1  $\text{g}/\text{m}^2/12$  hours  $\times 2$  days) plus low dose of G-CSF (5  $\mu\text{g}/\text{kg}/\text{day} \times 7$  days) in 3 patients (Patients nos. 9, 10, and 11). PBSC was planned to be started once CD34<sup>+</sup> cells reached more than 10/ $\mu\text{L}$  in peripheral blood. A minimum of  $2 \times 10^6$  CD34<sup>+</sup> cells/kg was planned to be collected.

After programmed cell cryopreservation, hematopoietic progenitor cells were stored in an isolated chamber at  $-80^\circ\text{C}$ .

Conditioning regimen used included BEAM (BCNU 300  $\text{mg}/\text{m}^2/\text{day}$  on day  $-7$ ; etoposide 200  $\text{mg}/\text{m}^2/\text{day}$  and cytarabine 200  $\text{mg}/\text{m}^2/12$  hours on days  $-6$  to  $-3$ ; melphalan 140  $\text{mg}/\text{m}^2/\text{day}$  on day  $-2$ ) or BEAC (where melphalan was substituted by cytoxan 7.5  $\text{g}/\text{m}^2$  on day  $-2$ ). G-CSF (5  $\mu\text{g}/\text{kg}/\text{day}$ ) was started after ASCT and continued until hematopoietic engraftment. Engraftment was documented by increasing neutrophil and platelet peripheral blood counts unsupported by transfusions. Neutrophil engraftment was considered when more than  $0.5 \times 10^9/\text{L}$  was reached and platelet engraftment when more than  $20 \times 10^9/\text{L}$ , during 3 consecutive days, was achieved.

Results of engraftment from the 7 HIV<sup>+</sup> Ly patients transplanted in Hospital Gregorio Marañón were compared with those of a historical group of 14 ASCT HIV<sup>-</sup> lymphoma patients transplanted at the same institution. These patients were matched for histological diagnosis, clinical staging, age, and age-adjusted IPI (Table 2). HIV<sup>-</sup> patients did not receive G-CSF after ASCT.

Patients received antibacterial, antifungal, and antiviral prophylaxis from the first day of conditioning regimen with ciprofloxacin 500 mg two times daily (BID) and fluconazol 200 mg BID, until engraftment, and acyclovir 800 mg BID until 30 days after ASCT. Additionally, trimetoprim-sulfametoxazol was given during conditioning treatment and after hematopoietic recovery. HAART (combination of at least three drugs including protease and transcriptase inhibitors) was aimed to be maintained during PBSC and ASCT. Toxicity was graded according to Bearman criteria [25].

CD34<sup>+</sup> and CD4<sup>+</sup> cell counts were performed with multiparametric analysis by flow cytometry (EPICS-XL, Coulter, Hialeah, FL, USA) according to the recommendations of the supplier.

**Table 1.** Patient characteristics, results of PBSCC, engraftment, and follow-up

| Patient no./<br>Hospital | Age  | Diagnosis/<br>Staging | Treatment pre-ASCT  | HAART           | Status pre-ASCT   | CD34 <sup>+</sup><br>cells collected<br>( $\times 10^6$ /kg) | Day of engraftment |       | Status at last follow-up            |
|--------------------------|------|-----------------------|---|-----------------|-------------------|--|--------------------|-------|-------------------------------------|
|                          |      |                       |   |                 |                   |  | Neutr.             | Plat. |                                     |
| 1/GM                     | 35   | HD/IV-B               | CMOPP-ABV $\times$ 6/CMOPP-ABV $\times$ 5                   | IDV/D4T/3TC     | CR-2              | 6.9  | 14                 | 17    | Alive in CR-3* (44 m)               |
| 2/GM                     | 38   | DLBCL/IV-B            | EPOCH $\times$ 4/ESHAP $\times$ 2                           | EFV/D4T/3TC     | CR-1; >1 L. Treat | 1.8  | 18                 | 36    | Alive in CR-1 (36 m)                |
| 3/GM                     | 58   | BL/I-A                | PETHEMA/EPOCH $\times$ 2                                    | EFV/ABC/D4T/3TC | CR-1              | 4.1  | 21                 | 35    | Alive in CR-1 (33 m)                |
| 4/GM                     | 55   | DLBCL/IV-B            | VACOPB/ESHAP $\times$ 1                                     | EFV/D4T/3TC     | CR-1; >1 L. Treat | 3.4  | 33                 | 455   | Alive in CR-1 (33 m)                |
| 5/GM                     | 31   | BL/IV-B               | EPOCH $\times$ 6  | EFV/ddI/3TC     | CR-1              | 5.2  | 21                 | 20    | Alive in CR-1 (32 m)                |
| 6/GM                     | 54   | HD/IV-B               | ABVD $\times$ 6/ESHAP $\times$ 7/BEACOPP                    | EFV/ddI/D4T     | CR-2              | 4.3  | 13                 | 18    | Alive in CR-1 (17 m)                |
| 7/GM                     | 39   | DLBCL/IV-A            | CHOP $\times$ 6+Rituximab $\times$ 4                        | RTC/LPV/3TC     | CR-1              | 2.6  | 16                 | 26    | Alive in CR-1 (7m)                  |
| 8/TP                     | 34   | HD/III-B              | ABVD/COPP-ABV+RT/ABVD                                       | NFV/D4T/3TC     | PR                | 3.2  | 17                 | -     | Dead from progression (0.6 m)       |
| 9/MV                     | 34   | DLBCL/I-A             | CHOP $\times$ 4+RT/ESHAP $\times$ 2                         | SQV/RTV/D4T/3TC | CR-2              | 18.1   | 9                  | 11    | Alive in CR-2 (28 m)                |
| 10/MV                    | 50   | ALCL/IV-B             | CHOP $\times$ 2/ESHAP $\times$ 3                            | EFV/D4T/3TC     | PR                | 16.7   | 11                 | 13    | Alive in CR-1 (16 m)                |
| 11/MV                    | 61   | DLBCL/IV-B            | CHOP $\times$ 6   | EFV/ddI/TDF     | CR-1              | 21.2   | 9                  | 20    | Dead from OI in CR-1 (15 m)         |
| 12/GM                    | 44   | DLBCL/IV-B            | CHOP $\times$ 6/ESHAP $\times$ 1/mini-BEAM/EPOCH $\times$ 3 | NFV/D4T/3TC     | CR-2              | 5.5  |                    |       | Dead from progression before ASCT   |
| 13/GM                    | 31   | BLL/IV-B              | EPOCH $\times$ 3/hyper-CVAD $\times$ 2/Rituximab $\times$ 4 | EFV/D4T/3TC     | PR                | 16   |                    |       | Dead from liver failure before ASCT |
| 14/TP                    | 40   | ALCL/III-A            | CHOP $\times$ 8/IFO VP-16+DHAP/mini-BEAM                    | NFV/D4T/3TC     | PR                | 4.18   |                    |       | Dead from progression before ASCT   |
| Median                   | 39.5 |                       |   |                 |                   | 4.7  | 16                 | 20    |                                     |

GM: Hospital Gregorio Marañón (Madrid, Spain); TP: Hospital Trias i Pujol (Barcelona, Spain); MV: Hospital Marqués de Valdecilla (Santander, Spain); HD: Hodgkin's disease; DLBCL: diffuse large B cell lymphoma; BL: Burkitt lymphoma; ALCL: anaplastic large-cell lymphoma; BLL: Burkitt-like lymphoma; PETHEMA: vincristine, daunorubicine, cyclophosphamide, L-asparaginase, prednisone, intrathecal (methotrexate, cytarabine, hydrocortisone); RT: radiotherapy; IFO: ifosfamide; IDV: indinavir; D4T: stavudine; 3TC: lamivudine; EFV: efavirenz; ABC: abacavir; ddI: didanosine; RTV: ritonavir; LPV: lopinavir; NFV: nelfinavir; SQV: saquinavir; TDF: tenofovir; CR-1: first complete remission; CR-2: second complete remission; CR-3: third complete remission; >1 L.Treat: more than one line of treatment; PR: partial remission; OI: opportunistic infection; Neutr.: neutrophils; Plat: platelets; m: months.

\*This patient relapsed 32 months after ASCT.

**Table 2.** Comparison of clinical features, number of CD34<sup>+</sup> cells infused, and engraftment in HIV<sup>+</sup> Ly and HIV<sup>-</sup> lymphoma (control group) patients transplanted in Hospital G.U. Gregorio Marañón

|   | HIV <sup>+</sup> Ly<br>n = 7 | Control group<br>n = 14 | p value* |
|---|------------------------------|-------------------------|----------|
| Diagnosis   |                              |                         |          |
| NHL   | 5                            | 10                      | 1.0      |
| HD  | 2                            | 4                       |          |
| IPI at diagnosis  |                              |                         |          |
| 0–1   | 1                            | 5                       | 0.6      |
| 2–3   | 6                            | 9                       |          |
| Stage at diagnosis  |                              |                         |          |
| I–II  | 1                            | 4                       | 0.6      |
| III–IV  | 6                            | 10                      |          |
| Age <sup>§</sup>  | 39 (31–58)                   | 49 (33–61)              | 0.7      |
| CD34 <sup>+</sup> infused (×10 <sup>6</sup> /kg) <sup>§</sup> | 3.9 (1.6–6.5)                | 3.7 (2.5–14.5)          | 0.45     |
| Engraftment (days) <sup>§</sup>                               |                              |                         |          |
| Neutrophils   | 18 (13–33)**                 | 13 (9–16)***            | 0.004    |
| Platelets   | 26 (17–455)                  | 12.5 (8–330)            | 0.004    |

\*p value according to *t*-test/Fisher's exact test or Mann-Whitney test.

§median (range).

\*\*HIV<sup>+</sup> Ly received G-CSF post-ASCT (see text).

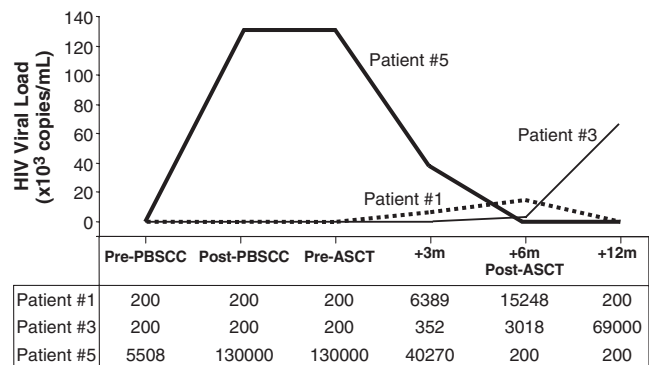
\*\*\*HIV<sup>-</sup> lymphoma patients did not receive G-CSF post-ASCT.

HIV viral load was analyzed in plasma by direct quantification of HIV-1-RNA (Quantiplex HIV-1 RNA 3.0-bDNA, Bayer, Tarrytown, NY, USA). Undetectable VL was considered when less than 200 copies/mL was found.

**Statistical analysis.** For continuous variables, mean ± SD or median values and ranges (minimum and maximum) were calculated and compared with the *t*-test or Mann-Whitney test. Proportions were compared using the Chi-square test or Fisher's exact test. Time to event was calculated in months and the method of Kaplan-Meier was used for survival estimations (close-out date for this analysis was March 1, 2004). All reported *p* values are two-sided and *p* values less than 0.05 were considered significant. Data were analyzed using SPSS version 9.0 software (SPSS, Inc, Chicago, IL, USA).

## Results

All 14 patients had HIV infection stage C3. At the time of PBSCC and ASCT, they showed a good performance status (ECOG < 2) without OI. Prior to mobilization, patients had received a median of 2 different lines of conventional-dose chemotherapy (Table 1). They all were on HAART at the time of stem cell collection, and none of them received AZT within HAART (Table 1). HAART was discontinued in patient #1 during the conditioning treatment. This patient showed an increase in VL after day +20, which was reverted by resuming HAART (Fig. 1). Patient #3 showed increasing VL values, 12 months post-ASCT, needing a new HAART combination (Fig. 1). Patient #5, who had poor adherence

**Figure 1.** Viral load evolution in three patients (#1, #3, and #5) with positive determinations. ASCT = autologous stem cell transplantation; PBSCC = peripheral blood stem cell collection.

to HAART before PBSCC and ASCT, showed increasing VL values, which subsequently decreased after adequate HAART intake (Fig. 1). The rest of the patients remained with undetectable VL.

An adequate number of CD34<sup>+</sup> cells (median of 4.7 × 10<sup>6</sup>/kg, range 1.8–21.2) was collected from all patients (Table 1). The PBSCC yield was higher in the three patients (Patients nos. 9, 10, 11; Table 1) mobilized with Ara-C in one of the hospitals, with a median CD34<sup>+</sup> cells/kg of 18.1 × 10<sup>6</sup> (range, 16.7–21.2), than in the rest of the patients, with a median CD34<sup>+</sup> cells/kg of 4.2 × 10<sup>6</sup> (range, 1.8–16). Two patients (Patients 8 and 12) needed 2 rounds of mobilization (schedules 2 and 3; see Patients and Methods) and one patient (Patient 2) required 4 identical rounds, in order to collect the targeted amount of CD34<sup>+</sup> cells. Hematopoietic stem cells were separately stored for a median of 36 days (range, 16–69 days) before infusion.

Three patients died after PBSCC, two (Patients 13 and 14) from lymphoma progression and one (Patient 12) from VHC-liver failure (Table 1). Therefore, ASCT was performed in 11 patients (78.5%), 9 of which were in CR (6 in CR-1, 3 in CR-2) and 2 in PR at the time of transplantation.

BEAM was used as conditioning regimen in 10 patients and BEAC in one. Seven patients (7/11, 63%) needed intravenous nutrition due to mucositis (Bearman's criteria grade II). Patient no. 1 developed liver toxicity grade II; a liver biopsy obtained on day +22 post-ASCT showed changes consistent with drug toxicity. Patient no. 4 had a subdural hematoma (day +20 post-ASCT) with favorable evolution without surgical treatment. No apparent interference between HAART and conditioning regimen was observed.

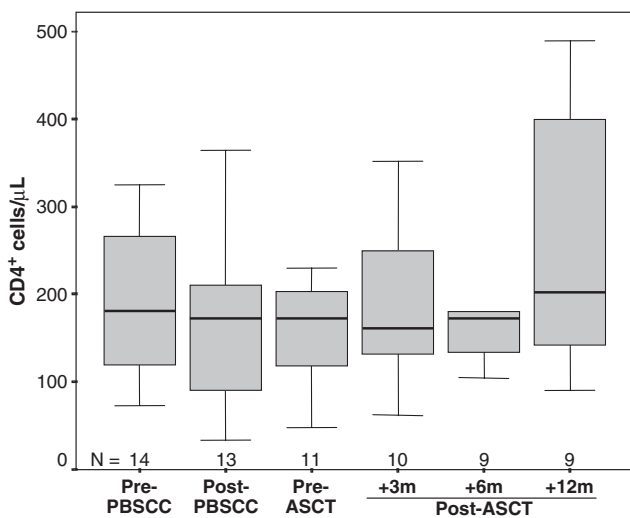
The median of CD34<sup>+</sup> cells infused was 4 × 10<sup>6</sup>/kg (range 1.6–21.2). G-CSF (5 µg/kg/day) was started at a median of 7 days (range, 4–21 days) after ASCT and maintained until neutrophil engraftment (median of 6 days; range, 2–32 days). Neutrophil engraftment was achieved in all 11 patients (Table 1), at a median of 16 days (range, 9–33 days). Platelet engraftment was achieved in nine patients, at a median of 20 days (range, 11–36 days). Patient no. 8 died of HIV<sup>+</sup> Ly

progression before platelet recovery (day +19) and Patient no. 4, although platelet transfusion independent from day +49, reached platelet counts above  $20 \times 10^9/L$  15 months after ASCT. Bone marrow studies in this patient (days +240 and +510) showed evidence of hemosiderosis and mild megaloblastoid changes with normal cytogenetic analysis.

All patients had fever during the neutropenic period, with characterized origin in eight of them. Positive blood cultures were obtained in 6 patients. Three patients had gram-positive bacteremia (*Streptococcus viridans* in two patients and *Staphylococcus hominis* in one) and the other three had gram-negative bacteremia (*Fusobacterium*, *Proteus*, and *E. coli*). During this period three patients showed clinical pneumonia (due to *Legionella pneumophila* in Patient no. 8, due to *Aspergillus* in Patient no. 10, and of unknown microbiological origin in Patient no. 2). Two patients developed *Clostridium difficile* enterocolitis. All infections were successfully treated.

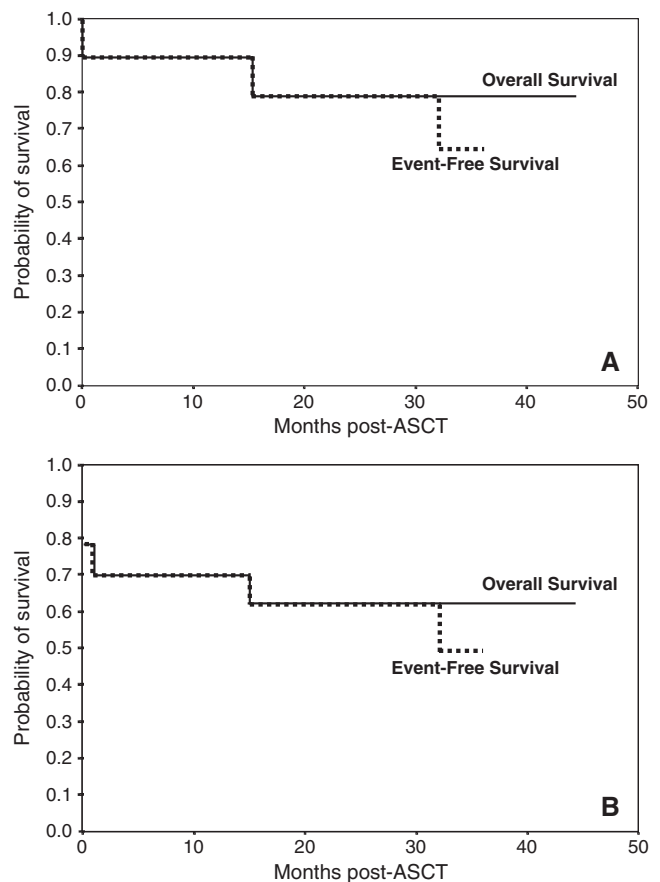
After engraftment, Patient no.1 developed cytomegalovirus infection (CMV-Ag<sup>+</sup>) on day +35, successfully treated with ganciclovir. Moreover, this patient developed uncomplicated disseminated *Herpes zoster* infection 12 months after transplant. Patient no. 4 was treated for pneumonia due to *Staphylococcus* at 30 months and Patient no. 11 showed a resolved *Varicella zoster* infection after 3 months post-ASCT and he died as a consequence of an obscure gastrointestinal infection, 15 months post-ASCT, remaining in CR with undetectable VL.

CD4<sup>+</sup> cell counts did not show significant changes before and after PBSCC with median of 186/ $\mu$ L (range, 72–325) and 172/ $\mu$ L (range, 33–364) respectively ( $p = 0.349$ ). Similarly, although CD4<sup>+</sup> cells transiently decreased after ASCT (Fig. 2), the median CD4<sup>+</sup> cell counts observed 12 months after ASCT (200/ $\mu$ L) were not significantly different from those observed before ASCT (172/ $\mu$ L) ( $p = 0.138$ ) (Fig. 2).



**Figure 2.** Follow-up of CD4<sup>+</sup> cell counts. ASCT = autologous stem cell transplantation; PBSCC = peripheral blood stem cell collection.

The clinical follow-up of the 11 ASCT patients is shown in Table 1. From the nine patients in CR pre-ASCT, seven remain alive and in CR. Patient no. 1 relapsed 32 months after ASCT, but is alive in third CR and is receiving a new line of conventional-dose chemotherapy. Patient no. 11 died in CR-1 from a gastrointestinal infection 15 months after ASCT. From the two patients that were in PR pre-ASCT, one (Patient no. 8) died from HIV<sup>+</sup> Ly progression 19 days after ASCT and the other (Patient no. 10) achieved CR post-ASCT and persists in CR after 14 months. Therefore, overall survival for the 11 transplanted patients is 81% at 15 months, with a median follow-up of 28 months (range, 0.6–44 months). Furthermore, eight patients remain in CR, for an event-free survival of 65% at 32 months, with a median follow-up of 30 months (range, 7–36 months) (Fig. 3A). Analysis of the 14 candidate patients, on an intent-to-treat basis, rendered an overall survival of 63.5% at 15 months, with a median follow-up of 17 months (range, 0–44 months) and an event-free survival of 51% at 32 months, with a median follow-up of 30 months (range, 7–36 months) (Fig. 3B).



**Figure 3.** Survival analysis of autologous stem cell transplantation (ASCT) in HIV<sup>+</sup> Ly patients when the 11 transplanted patients are considered (A), or the whole series of patients ( $n = 14$ ) on an intention-to-treat analysis (B).

## Discussion

HIV<sup>+</sup> Ly patients have usually had a worse prognosis compared with HIV<sup>-</sup> lymphoma patients. The advent of HAART has changed the course of HIV infection, allowing a better treatment of HIV<sup>+</sup> Ly. Conventional chemotherapy schedules currently reach CR rates higher than 70% and median overall survival longer than two years [26,27]. Nevertheless, treatment options for patients with relapsed or refractory HIV<sup>+</sup> Ly are scanty and show poor results, achieving overall responses of less than 50%, with CR rates lower than 10%, and a median survival of less than 1 year [13,28–30].

Currently, high-dose chemotherapy followed by ASCT is the first option for HIV<sup>-</sup> lymphoma patients with poor-risk prognostic features. Therefore, after HAART was introduced, rescue and consolidation of high-risk HIV<sup>+</sup> Ly with high-dose chemotherapy followed by ASCT has been attempted [20,31–33]. Gabarre et al. [31] reported their experience in 14 relapsed or resistant HIV<sup>+</sup> Ly patients, of which 6 were alive in CR after a median follow-up of 10 months (range, 1–31 months), while the remaining patients died from lymphoma (7) or HIV progression (1). Re et al. [33] also treated 10 resistant or relapsed HIV<sup>+</sup> Ly (NHL and HD) patients. From nine evaluable patients, six remained in CR after a median follow-up of 8 months (range, 2–17 months) after ASCT. None died from transplant-related complications. Krishnan et al. [32] have similarly treated 19 high-risk HIV<sup>+</sup> Ly patients, 9 of them in an early stage of their disease, either CR-1 or first PR. Sixteen of them remained in CR post-ASCT, with a median follow-up of 27.5 months (range, 6–57.5 months). Three patients died, one from transplant-related toxicity and two from lymphoma progression.

Inclusion criteria in the present study were similar to those used to treat high-risk HIV<sup>-</sup> lymphoma patients. Interestingly, 6 of 11 (54.5%) patients were transplanted in CR-1 due to concurrent poor prognostic features. In particular, recent studies supporting a better outcome for HIV<sup>-</sup> patients with age-adjusted IPI greater than or equal to 2 transplanted in CR1 [34,35] induced us to adopt a similar criterion. Other concurrent poor prognostic features considered were requirement of more than one line of therapy to reach CR-1, or Burkitt histology unable to complete the appropriate chemotherapy schedule due to hematologic toxicity.

Overall survival of this group of transplanted patients was 81% at 15 months, with an event-free survival of 65% at 32 months (Fig. 3A). One patient in PR progressed and died from lymphoma early after transplantation, one in CR-2 relapsed after ASCT, and one more died in CR and with undetectable VL from an obscure gastrointestinal OI, 15 months post-ASCT. Analysis of the 14 candidate patients, on an intent-to-treat basis, rendered an overall survival of 63.5% at 15 months and an event-free survival of 51% at 32 months (Fig. 3B). In this study, as well as in those previously reported, transplant-related mortality has not been relevant. Moreover, the apparently low relapse rate observed

could be related to the use of high-dose therapy followed by ASCT earlier in the clinical history of lymphoma evolution, as also stated by Krishnan et al. [32].

All of our patients were responding to HAART combinations (excluding AZT), which were maintained along the PBSCC and transplantation procedure. Interestingly, the association of HAART with mobilization and high-dose chemotherapy schedules did not cause an increase in liver or gastrointestinal toxicity in our patients, unlike previously reported [36]. Furthermore, in agreement with other authors [31–33], we did not find particular organ toxicities or OI among the HIV<sup>+</sup> Ly transplanted patients. None of our patients died of HIV disease progression, suggesting that ASCT does not have an ominous effect on HIV disease, provided HAART is maintained.

Of note is the observation of a delayed neutrophil and platelet engraftment (median of 16 and 20 days, respectively), compared to those reported by other investigators [21,32,33]. This observation is consistent with the lower median number of CD34<sup>+</sup> cells collected from our patients ( $4.7 \times 10^6/\text{kg}$ ) compared to that reported by other groups [21,32,33], with the exception of those patients mobilized with Ara-C + G-CSF in our series, which had a better collection yield (median of  $18.1 \times 10^6/\text{kg}$ ) and a faster engraftment (Table 1). Although an appropriate explanation for these findings is not obvious, the different chemotherapy regimens used in the treatment of HIV<sup>+</sup> Ly, the different HAART combinations used, and the different mobilization schedules used could dramatically influence the number of CD34<sup>+</sup> cells collected.

In order to get deeper insight into these findings, PBSCC and engraftment after ASCT in HIV<sup>+</sup> Ly have been compared with those of a matched historical control group composed of HIV<sup>-</sup> lymphoma patients transplanted in one hospital (Hospital Gregorio Marañón, Madrid; Table 2). HIV<sup>+</sup> Ly patients showed a significant later engraftment of neutrophils and platelets, in spite of the routine use of G-CSF post-ASCT in these patients. Nevertheless, no differences were observed in the amount of CD34<sup>+</sup> cells collected and infused between both groups. Therefore, other factors related to HAART or HIV infection, which can damage the bone marrow environment, could be invoked to explain these findings. Therefore, in the HIV<sup>+</sup> Ly setting, it might be worth trying to use grafts containing the highest number of CD34<sup>+</sup> cells possible [21,32,33], in order to reduce the time to engraftment.

As other authors have reported [31–33], our study has shown a mild decrease in CD4<sup>+</sup> cell counts after ASCT (Fig. 2), recovering levels similar to those observed pre-ASCT 12 months after transplantation. HAART was aimed to be maintained during PBSCC and ASCT and VL remained undetectable in those patients who properly adhered to HAART. However, patient #1 discontinued HAART during conditioning and subsequently showed a significant VL increase in three weeks, which was controlled after resuming

HAART (Fig. 1). Patient #5, with poor adherence to HAART, gained VL control after proper intake of HAART during hospitalization. These observations suggest that HAART should be maintained all along the procedure to properly control HIV infection, mostly considering the excellent tolerance and the absence of remarkable interactions in the context of PBSCC and ASCT.

Despite the small number of patients included in this study, several conclusions may be raised: (1) PBSCC rendered an adequate number of CD34<sup>+</sup> cells to perform ASCT in patients with HIV<sup>+</sup> Ly; (2) incidence of toxicities or OI was not particularly increased in this setting; (3) engraftment was delayed compared to HIV<sup>-</sup> lymphoma patients; therefore, G-CSF could be useful following ASCT; and (4) provided HAART was maintained, CD4<sup>+</sup> counts and VL were appropriately controlled all along the procedure. Within this scenario, our results show the feasibility, safety, and efficacy of high-dose chemotherapy followed by ASCT, as consolidation or rescue therapy in high-risk HIV<sup>+</sup> Ly patients. Therefore, ASCT may be used for the treatment of HIV<sup>+</sup> Ly patients in a similar way as it is used for HIV<sup>-</sup> patients. Finally, ASCT might be of greater survival benefit when used in early phases of the disease, particularly when poor prognostic features are present.

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