Successful autologous stem cell transplantation for relapsed lymphoma with peripheral blood stem cells cryopreserved for ten years.

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Introduction

Autologous stem cell transplantation (SCT) has been shown to be an effective therapy for patients with recurrent diffuse large B-cell lymphoma (DLBCL). In recurrent follicular lymphoma, several Phase II studies suggest that salvage treatment followed by consolidation with autologous SCT can result in prolonged disease-free survival (DFS) whereas conventional dose chemotherapy is likely to produce consecutive remissions of shorter duration each time. These studies demonstrate that autologous SCT provides an important survival benefit in patients with chemosensitive recurrences of not only DLBCL but follicular lymphoma and should currently be considered a treatment of choice. However, it is often challenging to harvest a sufficient number of stem cells at the time of relapse after the patients have received multiple chemotherapies that may reduce the stem cell pool. One of the potential solutions for this is to cryopreserve peripheral blood stem cells (PBSC) at the early time point before significant stem cell damage due to chemotherapy and radiotherapy and stored for several years. (Journal of Hematopoietic Cell Transplantation Vol. 1 No. 1; 29–32, 2012)
Case report

A 32-year-old female was diagnosed with t(14; 18)-positive follicular lymphoma grade 2 in 1998 on a gastric biopsy. Bone marrow involvement was also documented at that time, thus it was concluded that she had a stage IV disease before the start of the therapy. Subsequently, she was treated with 6 cycles of CHOP and obtained a CR. She had a potential risk of relapse with/without transformation, and was expected that she would be eligible for autologous SCT at the time of relapse. SCH at the time of first CR would likely have a higher stem cell yield than that at the time of second CR. In addition, because her marrow was involved with disease at the time of presentation but was cleared after induction chemotherapy, it was felt that this might be the best opportunity to harvest high quality PBSC. PBSC were harvested for 2 days (referred as to D1 and D2) following mobilization by subcutaneous filgrastim injection for 3 consecutive days (300 µg, 600 µg, and 300 µg). The harvested cells were pelleted by centrifugation and resuspended in autologous plasma harvested concurrently, then mixed with an equal volume of CP+1 medium (Kyokuto Seiyaku Kogyo, Tokyo, Japan). The final cell suspension contained 6% HES, 5% dimethylsulfoxide (DMSO), and 4% human albumin. The cell concentration was adjusted not to exceed 1×10⁹ cells/ml. The cell suspension was then injected into a plastic bag (Cryocyte®, Baxter Healthcare), frozen in a program freezer (Cryo Med), and stored in liquid nitrogen. At each harvest, ANC, CD34⁺ cells and colony forming unit of granulocytes and/or macrophages (CFU-GM) using MethCult H4434 (STEMCELL Technologies) were assayed. The obtained ANC, CD34⁺ and CFU-GM were as follows: 7.86×10⁹, 39.2×10⁶ and 492.4×10⁴ for D1 and 14.6×10⁹, 406.2×10⁶ and 2993.5×10⁴ for D2, respectively (Table 1). Unexpectedly, she remained in CR for 10 years without further therapeutic intervention.

Her lymphoma recurred in 2008 with symptoms of stomach discomfort. Upper gastrointestinal endoscopy revealed multiple erosions and stenosis in her duodenal bulb. After admission to our hospital, she developed jaundice (maximum total bilirubin of 3.5 mg/dl) due to lymphoma invasion from duodenal bulb to hepatic portal region. She was treated with 4 cycles of R-DeVIC, and reached second CR which was confirmed by computed tomography and upper gastrointestinal endoscopy. Because of her good response to salvage chemotherapy, we considered that her lymphoma would be well controlled by consolidation with either rituximab maintenance or possibly autologous PBSCT and that allogeneic SCT would not be necessary at that point although she had a

### Table 1. Summary of cell processing

<table>
<thead>
<tr>
<th></th>
<th>ANC</th>
<th>CD34⁺ cells</th>
<th>CFU-GM</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBSCH Day 1</td>
<td>7.86×10⁹ ANC</td>
<td>39.2×10⁶</td>
<td>63.0 /10⁵ ANC</td>
<td>N.A.</td>
</tr>
<tr>
<td>(2 bags)</td>
<td></td>
<td></td>
<td>Total 492.4×10⁴ (7.63×10⁴/kg)</td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>14.6×10⁹ ANC</td>
<td>406.2×10⁶</td>
<td>205.0 /10⁵ ANC</td>
<td>N.A.</td>
</tr>
<tr>
<td>(4 bags)</td>
<td></td>
<td></td>
<td>Total 2993.5×10⁴ (46.4×10⁵/kg)</td>
<td></td>
</tr>
<tr>
<td>Quality check</td>
<td>Test tube from Day 1</td>
<td>N.A.</td>
<td>N.D.</td>
<td>6.0/10⁵ ANC</td>
</tr>
<tr>
<td>harvest</td>
<td></td>
<td></td>
<td>Total 47.2×10⁴ (0.73×10⁵/kg)</td>
<td></td>
</tr>
<tr>
<td>Test tube from Day 2</td>
<td>N.A.</td>
<td>N.D.</td>
<td>67.0/10⁵ANC</td>
<td>93.2</td>
</tr>
<tr>
<td>harvest</td>
<td></td>
<td></td>
<td>Total 978.2×10⁴ (15.2×10⁵/kg)</td>
<td></td>
</tr>
<tr>
<td>Bag from Day 1</td>
<td>18.5×10⁹ ANC</td>
<td>425.8×10⁶</td>
<td>82.7/10⁵ANC</td>
<td>95.5</td>
</tr>
<tr>
<td>harvest (1 of 2 bags)</td>
<td></td>
<td></td>
<td>Total 649.7×10⁴ (10.1×10⁵/kg)</td>
<td></td>
</tr>
<tr>
<td>PBSCT.remaining 5 bags</td>
<td>18.5×10⁹ ANC</td>
<td>425.8×10⁶</td>
<td>Total 3239.7×10⁴</td>
<td>61.4</td>
</tr>
</tbody>
</table>

1 values were calculated based on those assayed at cryopreservation.
2 lower viability was noted after the contents of all 5 bags were combined.
N.A., not applicable; N.D., not done
sister and a brother. We then questioned if the PBSC harvests which had been stored for 10 years could be a source of stem cells for autologous PBSCT. We performed quality checks on small scale test vials which had been frozen in liquid nitrogen from 2 consecutive harvests allocated for quality checks. The viability of thawed cells was 92.6% for D1 and 93.2% for D2, respectively (Table 1). The number of progenitor cells in the vials was assayed following density-gradient separation on Ficoll-Hypaque (GE Health Science) due to cell clumps. The obtained CFU-GM per $1 \times 10^7$ mononuclear cells was 6 for D1 and 67 for D2, respectively. We subsequently thawed only one of the six frozen PBSC bags, due to uncertainty of the quality of the cells as a result of the observation of significant cell clump formation in the test vials. We thawed one of the two bags harvested on the first day whose number of CD34$^+$ cells was lower than those harvested on the second day. The viability of cells in the bag was 95.5% and it contained $489 \times 10^4$ CFU-GM and $1464 \times 10^4$ BFU-E corresponding to $7.6 \times 10^{-3}/kg$ CFU-GM and $22.7 \times 10^{-3}/kg$ BFU-E, respectively (Table 1). It has been shown that PBSC containing $>20 \times 10^4$ CFU-GM/kg or $>2-2.5 \times 10^4$ CD34$^+$ cells/kg is sufficient for rapid and sustained engraftment. In addition, the sample turned to be negative for t(14;18) translocation when determined by polymerase chain reaction. Based on these encouraging data, we thought the remaining 5 bags should contain a sufficient number of stem cells for hematological reconstitution and decided to proceed with autologous PBSCT. The preconditioning regimen consisted of three consecutive days of melphalan (65 mg/m$^2$) and thiotepa (200 mg/m$^2$) on days −5, −4 and −3. On day 0, the patient received PBSCT consisting of $2.87 \times 10^7$ of ANC, $4.43 \times 10^7$ of CD34$^+$ and $32.3 \times 10^8$ of CFU-GM, respectively (see Table 1), followed by subcutaneous G-CSF at a dose of 300 $\mu$g for 9 consecutive days followed by 75 $\mu$g for 1 day. The lower viability of cells was observed after the contents of all 5 bags were combined (Table 1). The patient developed stomatitis (grade 3) and febrile neutropenia (grade 3) following PBSCT and had 4 day-long diarrhea (grade 3) from preconditioning, but she recovered by day 13. She achieved a granulocyte count $>500/mm^3$ on day 10 and self-supporting platelet count $>20 \times 10^3/mm^3$ on day 12. She was discharged from our hospital on day 21. Currently, she remains free from lymphoma and other complications for more than 3.5 years.

**Discussion**

We report here a patient with recurrent follicular lymphoma (grade 3) who was successfully treated with high-dose chemotherapy and autologous PBSCT. However, this potentially unusual treatment course raises several issues to discuss. It is still controversial whether patients with recurrent follicular lymphoma in second CR should be treated with high-dose chemotherapy with SCT support. It has been shown that high-dose chemotherapy with autologous PBSCT prolongs progression-free survival and overall survival and should be considered especially in patients with short first CR after chemotherapy containing rituximab, but the role of PBSCT has to be redefined when rituximab can be used for maintenance therapy after second CR.

Furthermore, it has been shown that in selected younger patients with a high-risk profile, a potentially curative allogeneic SCT with dose-reduced conditioning may be considered in relapsed disease. From these points of view, our patient was young (42 years old) at the time of PBSCT, therefore allogeneic SCT as consolidation therapy may have been an option because she had two siblings. However, she also had PBSC that had been cryopreserved for 10 years and turned out to contain a sufficient number of stem cells without lymphoma cell contamination. Autologous PBSCT was considered to be almost free from transplant-related mortality whereas allogeneic SCH was not yet standard clinical practice back in 1998 before introduction of the reduced-intensity conditioning (RIC). FL has a high frequency of relapse, and can transform to aggressive lymphoma. The patient was young at FL diagnosis, so her treatment with high-dose chemotherapy supported by autologous PBSCT was a reasonable option. There have been several reports on long term cryopreservation of PBSC focusing on recovery of CD34$^+$ cells, progenitor cells and immunocompetent cells. Recently, Veeraputhiran et al. have reported based on data from 262 samples that viability of thawed PBSC and engraftment of white blood cells and platelets are not affected by either various cryostorage periods (<1 year, 1–9 years and >9 years) and DMSO concentrations (5% versus 10%). Spurr et al. reported based on data from 40 harvests that stem cell collections could remain adequate for safe transplantation after up to 14 years of cryostorage, but also pointed out the necessity of some precautions such as harvesting higher than normal numbers of stem cells in collections intended for long-term storage and repeating in vitro assays on harvests after long-term storage prior to transplan-
tation. The impact of long-term storage and DMSO concentration on recovery of viable CD34+ cells and lymphocytes and lymphocyte subsets has been analyzed by Liseth et al. They showed that recovery of viable cells was significantly better in 5% DMSO than in 10% whereas the frequencies of several T lymphocyte subsets but not NK cells showed DM-DO-dependent differences. Collectively, long-term cryopreservation for more than 10 years is possible and could be standard clinical practice. We suggest that a patient who is young and has a high risk of relapse should be harvested at first CR to prepare for autologous SCT at second CR. In conclusion, our experience confirms previous reports that PBSC cryopreserved for around 10 years are sufficient for stem cell rescue following high dose chemotherapy.

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Author’s contribution

Y. I., K. H., F. M., M. T., A. H., Y. Y., M. W., S. M. and M. L. performed transplantation and collected data; S. S. and F. M. conducted stem cell assays; Y. I., F. M., Y. A., M. O. and N. E. analyzed data and wrote the manuscript.

Conflict of Interest Disclosure

No author of this paper has a conflict of interest.

References