HLA-Mismatched GPBSCs infusion therapy in refractory Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis: A observational study from a single center

Yue Song  
Capital Medical University Affiliated Beijing Friendship Hospital  
https://orcid.org/0000-0003-0898-5281

Jingshi Wang  
Capital Medical University Affiliated Beijing Friendship Hospital

Yini Wang  
Capital Medical University Affiliated Beijing Friendship Hospital

Zhao Wang ( wangzhao@ccmu.edu.cn )

Research

Keywords: hemophagocytic lymphohistiocytosis, Epstein-Barr virus, GPBSCs infusion, salvage therapy

Posted Date: June 2nd, 2020

DOI: https://doi.org/10.21203/rs.2.22776/v3

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Version of Record: A version of this preprint was published on July 1st, 2020. See the published version at https://doi.org/10.1186/s13287-020-01779-4.
Abstract

Background: Hemophagocytic lymphohistiocytosis (HLH) is a severe or even fatal inflammatory state. Epstein-Barr virus (EBV) infection associated HLH (EBV-HLH) is one of the most common secondary HLH and suffers a very poor prognosis. Allo-HSCT is often required for refractory EBV-HLH, but some patients still cannot proceed to the next allo-HSCT due to various factors. This study aimed to observe the efficacy of HLA-mismatched granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells (GPBSCs) infusion for refractory EBV-HLH.

Methods: A retrospective case-control study of refractory EBV-HLH patients with GPBSCs infusion from HLA-mismatched donors after chemotherapy (as GPBSCs group) and sole chemotherapy (as control group) was performed. Efficacy was evaluated 2 and 4 weeks and all patients were followed up until 1 March 2018.

Results: There were 18 cases who accepted infusion between March 2016 and Sep 2017 and 19 were randomly selected from refractory EBV-HLH patients who underwent salvage therapy during the same period for the control group. In GPBSCs group, WBC (p=0.017), Fbg (p=0.040), ferritin (p=0.039) improved significantly after treatment. The overall response rate was 66.7% (CR 22.2%, PR 44.4%). However, there is no significant differences in changes of WBC, HGB, PLT, TG, Fbg, Ferritin, AST, ALT, T-bil between two groups. Only the Fbg level was recovered better in the GPBSCs infusion group (p=0.003). In the GPBSCs group, EBV-DNA decreased significantly after 2 weeks (p=0.001) and 4 weeks (p=0.012) after treatment, and the effect of the decrease was significantly better than that of the chemotherapy alone group in 2 weeks but not 4 weeks (p2w=0.011, p4w=0.145). The median survival time in the infusion group was 20.4 weeks [95%CI 10.9, 29.9], and the median survival time in the control group was 10.8 weeks [95%CI 0-24.34]. In the short-term, the infusion group’s survival rate was better (2-month 88.89% vs. 52.63%, p=0.008; 3-month 83.33% vs. 47.09%, p=0.012), but there was no difference in OS (p=0.287).

Conclusions: Infusing GPBSCs combined with chemotherapy is effective, especially in decreasing EBV-DNA, performs better than chemotherapy alone, and improve short term survival rate. GPBSCs infusion is suggested as a bridging treatment method to allo-HSCT.

Background

Haemophagocytic lymphohistiocytosis (HLH) is a severe or even fatal inflammatory state caused by a hereditary or acquired immunoregulatory abnormality, non-malignant proliferation of lymphocytes and tissue cells, and secretion of a large number of inflammatory cytokines [1]. HLH is divided into two categories: primary and acquired. Acquired HLH is often associated with and caused by infections, malignancies, and autoimmune diseases [2]. Among the infections-associated HLH, Epstein–Barr virus (EBV) infection-associated HLH (EBV-HLH) is one of the most common. In previous studies, EBV-HLH patients suffered a much worse prognosis than patients with other types of infection-associated HLH, especially in adult HLH patients [3-5]. In 2015, a study of 61 cases with EBV-HLH reported a 1-year overall
survival (OS) of only 25.0% [6]. The current first-line treatment regimen, HLH-94 followed by allo-HSCT fails to trigger a response under ideal conditions in 30% of children with all HLH triggers [7]. In a study of 133 adults and adolescents with EBV-HLH, the non-response rate of the HLH-94 regimen was 52% [5]. The DEP (doxorubicin-etoposide-methylprednisolone) regimen and L-DEP (PEG-asparaginase and DEP regimen combination) regimen, which is used as a salvage therapy for refractory EBV-HLH, achieves a much better overall response rate and increase the chance to receive allo-HSCT, which improves survival [8-10]. However, some patients still cannot proceed to the next allo-HSCT due to various factors, such as financial limitations, disease activity, and a lack of time to find a suitable donor. It has been reported that granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cell (GPBSCs) infusion can mediate graft-versus-leukaemia (GVL) effects and hasten haematologic recovery without amplifying graft-vs-host disease (GVHD) [11]. Additionally, many studies have shown that infusion of HLA-mismatched donor GPBSCs combined with chemotherapy increased CR rates, improved survival, and avoided GVHD in AML patients [12-15]. In this study, patients with refractory EBV-HLH and the inability to undergo allo-HSCT were treated with GPBSCs infusion after chemotherapy. What is the effect of this treatment method on HLH? Can it contribute to haematologic recovery, EBV-DNA reduction, and prognosis improvement? Is it possible that GPBSCs infusion can be a bridge to allo-HSCT for these patients? These issues are discussed in this retrospective study.

Methods

Patients and donors

The study was approved by the Ethics Committee at Beijing Friendship Hospital. Written informed consent was obtained from each patient and/or their family or guardian before the treatment began.

A retrospective case-control study of refractory EBV-HLH patients with GPBSCs infusion from HLA-mismatched donors after chemotherapy (GPBSCs group) and sole chemotherapy (control group) was conducted. The patients in the control group were randomly selected from refractory EBV-HLH patients who underwent salvage therapy during the same period. Patients who were enrolled in this study fulfilled the following criteria: (1) patients satisfied HLH-2004 criteria at the time of initial diagnosis (the NK cell activity was tested by flow cytometry, ZL 201610013454.1) [1]; (2) high values for EBV-DNA copies in the peripheral blood (≥5.0*10^2 copies/ml) (tested with PCR assay); (3) primary HLH was excluded by HLH-related defective gene proteins PRF, GrB, XIAP, SAP, Munc13-4, Munc18-2, STX11, Rab27a, ITK, CD27, AP3B1 and whole genome sequencing if necessary, and lymphoma was excluded by repeated pathological biopsy of the focal area if there was an abnormal increase in FDG activity tested by positron emission tomography-computed tomography (PET-CT) and bone marrow biopsy; (4) treated with HLH-94 regimen (etoposide 150 mg/m2 twice weekly for 2 weeks and then weekly) and dexamethasone (initially 10 mg/m2 for 2 weeks followed by 5 mg/m2 for 2 weeks, 2.5 mg/m2 for 2 weeks, 1.25 mg/m2 for one week, and one week of tapering) [7] no less than 2 weeks before enrolment and did not achieve at least PR; and (5) patients are unable to perform allo-HSCT at that time point due to lack of donors, financial, patient and/or families’ refusal, and hesitating or other reasons, such as economic limitation.
EBV infection was confirmed by identifying a significantly increased number of EBV-DNA copies in the peripheral blood. In the absence of the accepted diagnostic criteria, refractory HLH was defined according to previous research findings [16] and our clinical experience as failure to achieve at least PR according to an evaluation 2 weeks after receiving HLH-94 induction therapy.

Before GPBSCs infusion, the HLA matching of donors and recipients was tested. The HLA matching of the GPBSCs infusion group was HLA-mismatched with relative donors. All the donors were haplo-identical and at least 5/10.

Treatment methods

Two kinds of treatment methods for patients: (1) GPBSCs group: GPBSCs infusion from HLA-mismatched donors after chemotherapy, in which the infusion of the cells was performed at 36 hours after the chemotherapy regimen on day 0; (2) control group: treated only with chemotherapy. The chemotherapy regimen included salvage therapy: DEP regimen (liposomal doxorubicin 25 mg/m2 day 1; etoposide 100 mg/m2 was administered once on the first day of every week; methylprednisolone 15 mg/kg days 1 to 3, 2 mg/kg days 4 to 6, 1 mg/kg days 7 to 10, 0.75 mg/kg days 11 to 14, 0.5 mg/kg days 15 to 21, and 0.4 mg/kg days 22 to 28) and L-DEP regimen (PEG-asparaginase 2000 U/m2 on day 5; liposomal doxorubicin (doxorubicin hydrochloride liposome injection) 25 mg/m2/day, day 1; etoposide 100 mg/m2/day on the first day of every week; and methylprednisolone 15 mg/kg/day for days 1 to 3, 0.75 mg/kg/day for days 4 to 7, and 0.25 mg/kg/day for days 8 to 10).

Mobilization and apheresis of donor peripheral mononuclear cells

Apheresis of HLA-mismatched donor peripheral mononuclear cells was carried out after the donor was subcutaneously injected with 5 µg/kg G-CSF twice a day for 5 days. Donor cells were divided into aliquots and were cryopreserved in liquid nitrogen, but freshly collected cells were used in the first course.

Detection of donor chimerism and donor microchimerism

All patients in the GPBSCs infusion group were assessed for donor chimerism after infusion. Eight patients were evaluated for donor microchimerism at least 1 week and 2 weeks after infusion.

Evaluation criteria and observed indicators

Efficacy was evaluated 2 and 4 weeks after initiating therapy according to the criteria proposed by Marsh et al[16]. Complete response (CR) was defined as the normalization of all quantifiable symptoms and laboratory markers of HLH, including the levels of soluble CD25, ferritin, and triglyceride; haemoglobin levels; neutrophil and platelet counts; and alanine aminotransferase (ALT) levels. Partial response (PR) was defined as improvement in two or more of the following quantifiable symptoms and laboratory markers by 2 weeks: 1.5-fold decrease in soluble CD25 response; ferritin and triglyceride decreases of at least 25%; an increase of at least 100% to >0.5*10^9/L in patients with an initial neutrophil count of <0.5 *10^9/L; an increase by at least 100% to >2.0*10^9/L in patients with an initial neutrophil count of 0.5 to
2.0 \times 10^9/L; and a decrease of at least 50% in patients with initial ALT levels >400 U/L. Additionally, the subject's body temperature had to have reverted to normal ranges for either CR or PR to be diagnosed. Failure to achieve PR was defined as no response.

The observational indicators included symptoms and laboratory findings as indicated in the evaluation criteria. EBV-DNA copies in peripheral blood were also observed.

**Survival time**

Survival times were calculated from the date of diagnosis of refractory EBV-HLH. All patients were followed up until death or 1 March 2018, whichever occurred first.

**Statistical analysis**

SPSS 22.0 (IBM, USA) statistical software was adopted. Data that fit a normal distribution are presented as average ± standard deviation, and those that did not are presented as median and range. T-test (two-sided) was used for data that fit a normal distribution and homogeneity of variance, and the Wilcoxon rank-sum test was used for others. Kaplan–Meier survival curves were used to analyse the patients’ survival, and the log-rank test was used to evaluate survival time. P<0.05 was considered to denote a significant difference. Sample size calculation was performed using PASS 15 Power Analysis and Sample Size Software (2017, NCSS, LLC. Kaysville, Utah, USA) with \( \beta = 0.1 \) and \( \alpha = 0.05 \) (two independent proportions).

**Results**

**General conditions**

There were 18 cases of refractory EBV-HLH who accepted GPBSCs infusion between March 2016 and Sep 2017 as the infusion group. The reasons these patients did not receive allo-HSCT at that time point were: lack of donors (n=3), financial considerations (n=3), and patient and/or families’ refusal (n=5). The other 7 patients were hesitant about HSCT at that time point. In total, 19 patients were randomly selected from refractory EBV-HLH patients who underwent salvage therapy during the same period as the control group. The characteristics of the patients in the 2 groups are summarized in Table 1.

**Laboratory findings**

There was no difference in HLH features before treatment in the GPBSCs infusion group and the control group (including WBC, HGB, PLT, TG, Fbg, Ferritin, AST, ALT, T-bil) (P>0.05).

After 2 weeks of treatment in the GPBSCs group, some of the patients' laboratory indicators improved significantly, including WBC (p=0.017), Fbg (p=0.040), and ferritin (p=0.039). The overall response rate was 66.7% (12/18), with a CR rate of 22.2% (4/18) and a PR rate of 44.4% (8/18). However, there were no significant differences in WBC, HGB, PLT, TG, Fbg, Ferritin, AST, ALT, and T-bil between the two groups.
Only the Fbg level was recovered better in the GPBSCs infusion group (p=0.003), indicating that the GPBSCs group was similar to the chemotherapy alone group in terms of haematological recovery. The details are presented in Table 2.

In terms of EBV, there was no difference in EBV-DNA levels at diagnosis \((5.0\times10^5 [1.5\times10^3, 7.7\times10^7])\) vs. \(1.0\times10^6 [2.0\times10^3, 5.2\times10^7]\), \(p=0.090\) or before treatment \((1.1\times10^5 [<5.0\times10^2, 3.0\times10^6])\) vs. \(5.7\times10^5 [<5.0\times10^2, 5.2\times10^7]\), \(p=0.951\) between the two groups. In the GPBSCs group, EBV-DNA decreased significantly after 2 weeks \((5.0\times10^3 [<5.0\times10^2, 5.7\times10^5], p=0.001)\) and 4 weeks \((4.1\times10^3 [0, 4.4\times10^4], p=0.012)\) after treatment, and the effect of the decrease was significantly better than that of the chemotherapy alone group in 2 weeks but not 4 weeks \((p_{2w}=0.011, p_{4w}=0.145)\) (Figure 1).

**Survival analysis**

The statistical analysis of the survival times ended on March 1, 2018. The overall mortality of the 34 patients was 75.7% \((28/37)\), and the median survival time was 14.14 weeks \([0.6-116.4]\). A total of 13 of 18 in the GPBSCs infusion group died (72.2%); 7 of them died of HLH progression, 1 died of aGVHD after GPBSCs infusion, 4 died of allo-HSCT-related causes and 1 died of complications. The median survival time of the infusion group was 20.4 weeks \([95\% CI 10.9, 29.9]\). A total of 15 of 19 in the control group died (78.9%); 8 of them died of HLH progression, 3 died of allo-HSCT-related causes and 4 died of complications. The median survival time of the control group was 10.8 weeks \([95\% CI 0-24.34-116.4]\). In the first 3 months after treatment, the GPBSCs infusion group's survival was better than that of the control group \((2-month survival 88.89\% vs. 52.63\%, p=0.008; 3-month survival 83.33\% vs. 47.09\%, p=0.012)\). However, after 3 months, there was no difference between the two groups. In addition, there was no significant difference in overall survival (OS) between the two groups \((p=0.287)\) (Figure 2).

**Graft-versus-host disease (GVHD)**

The median number of mononuclear cells (MNCs) infused was \(7.60\times10^8/kg\) \((range, 5.1-12.2\times10^8/kg)\). The median infused CD34+ cell number was \(2.355\times10^6/kg\) \((range, 1.17-4.924\times10^6/kg)\). The number of infused cells was mainly to ensure that the infusion was effective. At present, there is no standard recommendations on the doses of infused cells for GPBSCs infusion therapy. The doses of CD34+ cells infused in this study were with reference to the doses of cells \((CD34+ 1.7\times10^6/kg, range, 1.1-4.6) *10^6/kg)\) infused in the study of Mei Guo et al\(^\text{[13]}\). Among the 18 patients who underwent GPBSCs infusion, 7 \((38.9\%)\) had acute GVHD (aGVHD) signs, 5 of which had liver dysfunction and gastrointestinal symptoms, and the other 2 cases had liver dysfunction or gastrointestinal symptoms. However, 6 of them improved after symptom-specific treatment. Only 1 patient experienced sustained and eventually died of aGVHD. The details of graft and GVHD are presented in Table 3.

**Donor chimerism and donor microchimerism**

Only one patient in the GPBSCs group developed mixed chimerism, and the highest peripheral chimerism rate was 93.45% \((3w after treatment)\). This patient eventually died of aGVHD. In the other 10 patients who
underwent microchimerism detection, micro-chimerism was detected (0.008%-3.26%). In 2 weeks after treatment, microchimerism was detected in 8/8. There were 3 patients whose microchimerism was also detected again at 3 weeks (Table 3).

**Discussion**

EBV-HLH suffers a poorer prognosis than other subtypes of secondary HLH, especially relapsed and refractory EBV-HLH [17,18]. Without effective treatment, short-term mortality is high, and most patients with relapsed EBV-HLH die within the first few weeks [7]. Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is often required for EBV-HLH, especially for adults and refractory/relapsed patients. It is thought that allo-HSCT can induce immune reconstitution, thus enabling patients to effectively eliminate EB virus[3,19]. In one Japanese cohort, allo-HSCT resulted in an 85.7% 10-year OS for patients with EBV-HLH [20]. In a retrospective analysis from our centre, 27.1% of patients with EBV-HLH received allo-HSCT, and the final survival rate was 52.78% [21]. However, some patients in the actual situation are unable to receive allo-HSCT due to various factors. It has been reported that granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cell (GPBSCs) infusion can mediate GVL effects and hasten haematologic recovery without amplifying GVHD in AML [11]. Considering the important position of allo-HSCT, we tried to use GPBSCs infusion as a salvage treatment in patients with refractory EBV-HLH. This study found that for patients with refractory EBV-HLH, GPBSCs infusion therapy could more effectively reduce EBV-DNA levels after 2 and 4 weeks but had no significant effect on the recovery of blood parameters. The 3-month survival was improved, but there was no significant effect on overall survival.

The GPBSCs infusion therapy in the treatment of leukaemia reported in previous literature can significantly shorten the recovery time of white blood cells and platelets [11]. GPBSCs is a stimulated cell infusion. The current speculation is that, in contrast to unstimulated DLI, the larger numbers of lymphocytes, CD34 cells, natural killer (NK) cells, as well as cytokines, contained in G-PBSCs may contribute roles in promoting haematopoietic recovery.[13,22]. However, in this study, there was no significant difference in the recovery of blood parameters in the GPBSCs infusion group compared with the uninfused group, although similar levels of donor chimerism were observed in this study, which was consistent with the previous study of leukaemia. It is possible that the disease state of HLH is different from that of leukaemia. The decline in blood cells in HLH is mainly related to the production and action of a large number of inflammatory mediators [19]. This is different from the bone marrow suppression caused by chemotherapy drugs in the treatment of leukaemia. HLH needs to be controlled by controlling inflammatory cells and then controlling the inflammatory factor storm [23]. However, the mechanism of haematopoietic recovery of GPBSCs is still unclear, especially in the context HLH, and the specific internal mechanism still needs further study.

Interestingly, GPBSCs infusion after chemotherapy was more effective at reducing EBV-DNA viral load than regular chemotherapy. This may be similar to the GVL effect of GPBSCs in the treatment of leukaemia: the lymphocytes of the infused donor cells act synergistically with the recipient's immune
system to delete EBV-infected cells, thereby reducing Epstein-Barr virus load. However, prior studies suggested that the main mediator of GVL in leukaemia is NK cells, and other cells, such as T cells, mainly function in GVL by interacting with the recipient's immune system \([13,24]\). However, in HLH, EB virus clearance mainly relies on EB virus-specific CD8+ T cells \([25]\). Previous studies have found that EBV-specific CD8+ T recombination occurs in the early phase of allo-PBSCT; even if the total number of T cells decreased, the proportion of CD8+ T is still elevated, but this phenomenon was not observed in cord blood transplantation \([26]\). The GVL effect of DLI is mainly through donor chimerism or mixed chimerism \([24]\). In this study, only one patient achieved mixed chimerism after infusion of cells, although the number of cells infused was not different from those of other patients. This patient eventually died of GVHD, but the EBV viral load of this patient decreased significantly, and EBV-DNA turned negative after 4 weeks, suggesting that the effect of the reduction of EBV-DNA is achieved by the GVL-like effect of lymphocytes in the infused cells, similar to that observed in leukemia. With the patients who were tested for chimerism in this study, all of them had microchimerism except the patient mentioned above. Almost all patients with microchimerism did not develop mixed chimerism, suggesting that donor or mixed chimerism is difficult to achieve with GPBSC infusion. However, with the persistence of micro-chimerism, a small number of donor cells persist in the patient; this micro-chimerism may be the main reason by which GPBSCs effectively reduce the EBV-DNA level. Due to the small sample size of this study, no correlation between the micro-chimerism rate and the decline in EBV-DNA levels was found. However, in the previous micro-transplant study, the micro-chimerism rate reached a peak at 7-14 days after infusion \([11]\). This is consistent with the efficacy of EBV-DNA's reduction, which was maximal in 2 weeks, but more data are needed to validate the relationship. However, the efficacy of EBV-DNA reduction cannot persist, as in 4 weeks after treatment, there is no difference in EBV-DNA level between the 2 groups. We speculate that the loss of differences in EBV viral load are related to possible depletion of GPBSCs. Considering the transient efficacy of GPBSCs infusion, allo-HSCT should be taken into consideration as soon as HLH is controlled. Therefore, GPBSCs infusion may play a role in bridging the allo-HSCT, especially for those patients who have not had a previous transplant opportunity.

Except for the one patient mentioned above, who achieved mixed chimerism and suffered severe GVHD causing death, only 6 of the remaining 14 patients developed aGVHD signs, and all of them improved rapidly after symptomatic treatment, even if the number of CD3+ cells infused was high. None of the 15 patients in the GPBSCs infusion group underwent GVHD prevention treatment. Regardless of DLI or haploid transplantation, although the GVL effect is very impressive, the GVHD is also very severe \([27]\). Although GPBSCs infusion is a kind of transplantation with infused donor cells and chemotherapy, it was not used as a pretreatment regimen with strong immunosuppressive effects similar to haploid transplantation \([11]\). No severe immunosuppression significantly reduces the incidence and severity of GVHD after infusion. However, considering that one patient died of severe GVHD in this study, more considerations are required for special circumstances, and close monitoring of GVHD after infusion is still necessary.

In terms of survival, the survival rate of the GPBSCs infusion group was better than that of the control group within 3 months, but there was no significant difference in the long-term survival. This may
suggest that GPBSCs infusion can help with refractory EBV-HLH, but only provides effects in the short term. Allo-HSCT is still needed to achieve long-term remission [28]. Therefore, we suggest using GPBSCs as a bridging treatment method to allo-HSCT for those refractory EBV-HLH patients who did not have a previous opportunity for allo-HSCT.

Conclusion

Infusing HLA-mismatched donor GPBSCs combined with chemotherapy is effective in refractory EBV-HLH. It can decrease EBV-DNA levels in 2 and 4 weeks, and it performs better than chemotherapy alone in 2 weeks. Infusing HLA-mismatched donor GPBSCs combined with chemotherapy and can also improve the short-term survival rate. This effect is similar to the effect of GVL in leukaemia, but it is not exactly the same, which is probably due to the GVL-like effect of lymphocytes in the infused cells, and microchimerism may be important. However, the effects are short-term. After the exhaustion of donor cells, allo-HSCT is still needed. GPBSCs infusion is suggested as a bridging treatment method to allo-HSCT for those refractory EBV-HLH patients who have not had a previous opportunity for transplantation.

Declarations

Acknowledgements

We thank the patients and their families for participating in our study.

Funding

This work was supported by the National Natural Science Foundation of China (No.81871633); Beijing Natural Science Foundation (No.7181003); Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding (ZYLX201702); Beijing Municipal Administration of Hospitals' Ascent Plan (DFL20180101); Beijing Municipal Administration of Hospitals Incubating Program (PX2018003); Beijing Municipal Administration of Hospitals’ Youth Program (QML20160102); and Beijing Municipal Administration of Hospitals’ Ascent Plan (DFL20180101); Beijing Municipal Administration of Hospitals Clinical Technology Innovation Project (XMLX201803).

Availability of data and materials

The datasets used during the current study are available from the corresponding author on request.

Authors’ contributions

ZW contributed to the design of the study. YNW and JSW helped with the study design and data analyses. YS conducted the data analysis and wrote the manuscript. All authors approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

**Consent for publication**

We have obtained consent to publish from the participants and/or their families or guardian.

**Ethics approval and consent to participate**

The study was approved by the Ethics Committee at Beijing Friendship Hospital. Written, informed consent was obtained from each patient and their family or guardian before the treatment began.

**Abbreviations**

Allo-HSCT: Allogeneic hematopoietic stem cell transplantation; ALT: Alanine aminotransferase; CR: Complete response; DEP regimen: Doxorubicin hydrochloride liposome, etoposide, and methylprednisolone; EBER: EBV encoded small RNA; EBV: Epstein–Barr virus; GVHD: Graft versus host disease; HLH: Hemophagocytic lymphohistiocytosis; L-DEP regimen: PEG-asparagase plus DEP regimen; NK: Natural killer; OS: Overall survival; PR: Partial response; VP-16: Etoposide; GPBSCs: granulocyte colony-stimulating factor mobilized peripheral blood stem cells.

**References**


**Tables**

Table 1 Clinical features of patients in two groups before treatment

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>No. of Patients</th>
<th>Infusion group (n=18)</th>
<th>Control group (n=19)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age, years</td>
<td></td>
<td>21 [11-60]</td>
<td>29 [17-57]</td>
<td>0.368</td>
</tr>
<tr>
<td>Gender, Female: male</td>
<td></td>
<td>5:13</td>
<td>6:13</td>
<td>0.800</td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td>18 (100%)</td>
<td>19 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Splenomegaly/Hepatomegaly</td>
<td></td>
<td>16 (88.9%)</td>
<td>16 (84.2%)</td>
<td>0.677</td>
</tr>
<tr>
<td>Hemophagocytosis</td>
<td></td>
<td>14 (77.8%)</td>
<td>14 (73.7%)</td>
<td>0.772</td>
</tr>
<tr>
<td>NK cell activity&lt;15.11%</td>
<td></td>
<td>6 (33.3%)</td>
<td>4 (21.1%)</td>
<td>0.401</td>
</tr>
<tr>
<td>soluble CD25&gt;6400pg/ml</td>
<td></td>
<td>14 (77.8%)</td>
<td>19 (100%)</td>
<td>0.100</td>
</tr>
<tr>
<td>EBV-DNA (copies/ml) (at diagnosis)</td>
<td></td>
<td>5.0<em>10^5 [1.5</em>10^3, 7.7*10^7]</td>
<td>1.0<em>10^6 [2.0</em>10^3, 5.2*10^7]</td>
<td>0.090</td>
</tr>
</tbody>
</table>

Table 2 Comparison between two treating groups
<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>p value</th>
<th>2 weeks</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>GPBSCs</td>
<td>control</td>
<td>GPBSCs</td>
<td>control</td>
</tr>
<tr>
<td></td>
<td>1.36 [0.2, 2.4]</td>
<td>1.8 [0.5, 7.4]</td>
<td>2.68 [0.17, 15.38]</td>
<td>3.95 [0.2, 12]</td>
</tr>
<tr>
<td>HGB</td>
<td>81.5±17.701</td>
<td>94.63±27.132</td>
<td>82.75±18.614</td>
<td>97.19±17.608</td>
</tr>
<tr>
<td>TG</td>
<td>2.59 [0.6, 12.78]</td>
<td>2.05 [1.39, 5.29]</td>
<td>1.89 [1.02, 8.86]</td>
<td>1.63 [0.74, 4.11]</td>
</tr>
<tr>
<td>Fbg</td>
<td>1.04 [0.84, 2.91]</td>
<td>1.045 [0.3, 2.12]</td>
<td>2.16 [0.77, 4.24]</td>
<td>1.065 [0.53, 2.74]</td>
</tr>
<tr>
<td>Ferritin</td>
<td>3843.1 [28, 24174]</td>
<td>3207 [118, 455000]</td>
<td>3352.5 [255, 1433]</td>
<td>3207 [118, 455000]</td>
</tr>
<tr>
<td>ALT</td>
<td>58 [14, 252]</td>
<td>76 [10, 310]</td>
<td>59 [6, 502]</td>
<td>34.5 [4, 227]</td>
</tr>
<tr>
<td>T-bil</td>
<td>18.725 [8.01, 85.66]</td>
<td>50.925 [7.48, 289.66]</td>
<td>33.51 [255, 1433]</td>
<td>17.34 [1.84, 192.69]</td>
</tr>
<tr>
<td>EBV-DNA</td>
<td>1.1<em>10^5 [&lt;5.0</em>10^2, 5.2*10^7]</td>
<td>5.7<em>10^5 [&lt;5.0</em>10^2, 5.2*10^7]</td>
<td>5.0<em>10^3 [&lt;5.0</em>10^2, 5.2*10^7]</td>
<td>6.6<em>10^4 [&lt;5.0</em>10^2, 9.0*10^7]</td>
</tr>
<tr>
<td>ECOG</td>
<td>2.78</td>
<td>2.63</td>
<td>0.606</td>
<td>2.56</td>
</tr>
</tbody>
</table>

Table 3 Graft details and donor microchimerism rate after GPBSCs infusion

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mononuclear (cells/kg)</th>
<th>CD34+ (cells/kg)</th>
<th>1w</th>
<th>2w</th>
<th>3w</th>
<th>4w</th>
<th>Organ system affected</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.2</td>
<td>4.56</td>
<td>37.4</td>
<td>86.0</td>
<td>93.89</td>
<td>elevated LFTs, rash, GI symptoms</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.5</td>
<td>2.31</td>
<td>0.024</td>
<td>0.081</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.7</td>
<td>2.115</td>
<td>0.023</td>
<td>0.014</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.5</td>
<td>2.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.1</td>
<td>1.17</td>
<td>0.846</td>
<td>0.148</td>
<td></td>
<td>elevated LFTs, GI symptoms</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.2</td>
<td>2.08</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6.4</td>
<td>1.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>11.6</td>
<td>2.43</td>
<td>0.15</td>
<td>0.25</td>
<td></td>
<td>GI symptoms</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>10.1</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9.7</td>
<td>4.924</td>
<td>11.21</td>
<td>0.15</td>
<td>0.01</td>
<td>elevated LFTs, GI symptoms</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>16.4</td>
<td>3.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5.3</td>
<td>1.325</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
<td>elevated LFTs</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>9.6</td>
<td>3.11</td>
<td>0.008</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>6.1</td>
<td>2.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>8.8</td>
<td>2.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>16.5</td>
<td>2.31</td>
<td>0.37</td>
<td>0.31</td>
<td>0.034</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>5.1</td>
<td>2.448</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>5.7</td>
<td>1.824</td>
<td>3.26</td>
<td>0.76</td>
<td></td>
<td>elevated LFTs, GI</td>
<td>II</td>
<td></td>
</tr>
</tbody>
</table>

LFT, liver function test; GI, gastrointestinal

Figures
Figure 1

EBV-DNA before, 2 weeks and 4 weeks after treatment between two groups. There was no difference in EBV-DNA levels before treatment (p=0.304) between two groups. In the GPBSCs group, EBV-DNA decreased significantly after 2 weeks (p=0.001) and 4 weeks (p=0.012) after treatment, and the effect of the decrease was significantly better than that of the chemotherapy alone group in 2 weeks but not 4 weeks (p2w=0.011, p4w=0.145).

Figure 2

Overall survival (OS) between two groups (p=0.259)