

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

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## Keywords

***hemophagocytic lymphohistiocytosis, Epstein-Barr virus, GPBSCs infusion, salvage therapy***

## 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 ( $p_{2w}=0.011$ ,  $p_{4w}=0.145$ ). 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

Hemophagocytic 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 infection associated HLH, Epstein-Barr virus (EBV) infection associated HLH (EBV-HLH) is one of the most common. In previous studies, EBV-HLH patients suffer a much worse prognosis than other type of infection associated HLH, especially in adult HLH patients [3-5]. A previous study of 61 cases of EBV-HLH in 2015, reported a 1-year overall survival (OS) of only 25.0% [6]. Although the current first-line treatment regimen HLH-94 followed by allo-HSCT is effective, even under ideal conditions in children with all HLH triggers, 30% will not respond to HLH-94 [7]. In a study of adults and adolescents EBV-HLH 133 patients, the non-response rate of HLH-94 regimen is 52% [5]. Our center's DEP (doxorubicin-etoposide-methylprednisolone) regimen and L-DEP (PEG-asparaginase and DEP regimen combination) as the salvage therapy for refractory EBV-HLH achieve 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, time to find suitable donor, and so on. It has been reported that granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells (GPBSCs) infusion can mediate graft-versus-leukemia (GVL) effects and hasten hematologic recovery without amplifying graft-vs-host disease (GVHD) [11]. And then 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 inability to undergo allo-HSCT were treated with GPBSCs

infusion after chemotherapy. What is the effect of this treatment method in HLH? Whether it can help with hematologic recovery, EBV-DNA reduction, and prognosis improvement? Is it possible that GPBSCs infusion can be a bridge to allo-HSCT for these? These are discussed in this retrospective study.

## Methods

### Patients and donors

The study was approved by the Ethics Committee at Beijing Friendship Hospital approved by the ethical committee of the 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 (as GPBSCs group) and sole chemotherapy (as control group) was performed. The patients of control groups were randomly selected from refractory EBV-HLH patients who underwent salvage therapy during the same period. Patients who enrolled in this study fulfilled the following criteria: (1) patients satisfied HLH-2004 criteria at 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 ( $>5.0 \times 10^2$  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 is 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/m<sup>2</sup> twice weekly for 2 weeks and then weekly) and dexamethasone (initially 10 mg/m<sup>2</sup> for 2 weeks followed by 5 mg/m<sup>2</sup> for 2 weeks, 2.5 mg/m<sup>2</sup> for 2 weeks, 1.25 mg/m<sup>2</sup> for one week, and one week of tapering) [7] no less than 2 weeks before enrollment and did not achieve at least PR; (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 significantly increased EBV-DNA copies in the peripheral blood. In the absence of 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 the 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, the infusion of the cells was performed at 36 hours after chemotherapy regimen on day 0; (2) control group: only with chemotherapy. Chemotherapy regimen includes the salvage therapy: DEP regimen (liposomal doxorubicin 25 mg/m<sup>2</sup> day 1; etoposide 100 mg/m<sup>2</sup> 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/m<sup>2</sup> on day 5; liposomal doxorubicin (doxorubicin hydrochloride liposome injection) 25 mg/m<sup>2</sup>/day, day 1; etoposide 100 mg/m<sup>2</sup>/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 5ug/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 levels of soluble CD25, ferritin, and triglyceride; hemoglobin 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  $>0.5 \times 10^9/L$  in patients with an initial neutrophil count of  $<0.5 \times 10^9/L$ ; an increase by at least 100% to  $>2.0 \times 10^9/L$  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 which were indicted in 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  $\pm$  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 Wilcoxon rank sum test was used for others. Kaplan-Meier survival curves were used to analyze the patients' survival and the log-rank test to evaluate survival time.  $P < 0.05$  was considered to denote a significant difference. Sample size calculation using PASS 15 Power Analysis and Sample Size Software (2017, NCSS, LLC. Kaysville, Utah, USA) with the  $\beta = 0.1$  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 of these patients did not perform allo-HSCT at that time point were: lack of donors ( $n=3$ ), financial ( $n=3$ ), patient and/or families' refusal ( $n=5$ ). The other 7 patients were hesitating about HSCT at that time point. 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 GPBSCs group, parts of the patients' laboratory indicators improved significantly, including WBC ( $p=0.017$ ), Fbg ( $p=0.040$ ), 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 is no significant differences in 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 ( $p=0.003$ ), indicating that the GPBSCs group is similar to the chemotherapy alone group in terms of hematological recovery. The details are presented in Table 2.

In terms of EBV, there was no difference in EBV-DNA levels at diagnosis ( $5.0 \times 10^5$  [ $1.5 \times 10^3$ ,  $7.7 \times 10^7$ ] vs.  $1.0 \times 10^6$  [ $2.0 \times 10^3$ ,  $5.2 \times 10^7$ ],  $p=0.090$ ) or before treatment ( $1.1 \times 10^5$  [ $<5.0 \times 10^2$ ,  $3.0 \times 10^6$ ] vs.  $5.7 \times 10^5$  [ $<5.0 \times 10^2$ ,  $5.2 \times 10^7$ ],  $p=0.951$ ) between two groups. In the GPBSCs group, EBV-DNA decreased significantly after 2 weeks ( $5.0 \times 10^3$  [ $<5.0 \times 10^2$ ,  $5.7 \times 10^5$ ],  $p=0.001$ ) and 4 weeks ( $4.1 \times 10^3$  [ $0$ ,  $4.4 \times 10^4$ ],  $p=0.012$ ) 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]. 13 of 18 in GPBSCs infusion group died (72.2%) as 7 of them were died of HLH progression, 1 died of aGVHD after GPBSCs infusion, 4 died of allo-HSCT related and 1 died of complications. The median survival time of infusion group was 20.4 weeks [95%CI 10.9, 29.9]. 15 of 19 in control group died (78.9%), 8 of them were died of HLH progression, 3 died of allo-HSCT related and 4 died of complications. The median survival time of 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 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 two groups. Also, there was no significant difference in overall survival (OS) between two groups ( $p=0.287$ ) (Figure 2).

### Graft-Versus-Host Disease (GVHD)

The median number of mononuclear (MNC) infused was  $7.60 \times 10^8$ /kg (range,  $5.1-12.2 \times 10^8$ /kg). The median CD34+ cell number infused was  $2.355 \times 10^6$ /kg (range,  $1.17-4.924 \times 10^6$ /kg). The number of infused cells was mainly to ensure the infusion was effective. 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-pointed treatment. Only 1 patient 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. all detected. There were 3 patients whose microchimerism was also detected again at 3 weeks and the microchimerism was also detected (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 first few weeks [7]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is often required for EBV-HLH, especially for adults and refractory/relapsed patients. It is thought that allo-HSCT is possible to 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 center, 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 perform allo-HSCT due to various factors. It has been reported that granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem

cells (GPBSCs) infusion can mediate GVL effects and hasten hematologic 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 can more effectively reduce EBV-DNA levels after 2 weeks and 4 weeks, but has no significant effect on the recovery of blood picture. The 3-month survival was improved, but no significant effect on overall survival.

GPBSCs infusion therapy in the treatment of leukemia reported in the 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 number of lymphocytes, CD34 cells, natural killer (NK) cells, as well as cytokines, contained in G-PBSCs may contribute roles in promoting hematopoietic recovery.[13,22]. However, in this study, there was no significant difference in the recovery of blood picture 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 leukemia. There may be possibility that the disease state of HLH is different from leukemia. The decline of the 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 treatment of leukemia. HLH needs to be controlled by controlling inflammatory cells and then the controlling of inflammatory factor storm [23]. However, the mechanism of hematopoietic recovery of GPBSCs is still unclear, especially when it's in HLH, and the specific internal mechanism still needs further study.

Interestingly, the GPBSCs infusion after chemotherapy was more effective at reducing EBV-DNA viral load than the regular chemotherapy. This may be similar to the GVL effect of GPBSCs in the treatment of leukemia: 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 leukemia is NK cells, and other cells, such as T cells, mainly function 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 happens in the early phase of allo-PBSCT, even if the total number of T cells decreased, the proportion of CD8+T 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 is not different from 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. This suggests that the effect of the reduction of EBV-DNA is achieved by the GVL-like effect of lymphocytes in the infused cells, just like in leukemia. With the patients who tested chimerism in this study, all of them were microchimerism except the patient mentioned above. Almost all patients with microchimerism did not develop mixed chimerism suggesting that GPBSCs is difficult to achieve donor chimerism or mixed chimerism. 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 for GPBSCs to effectively reduce EBV-DNA level. Due to the small sample size of this study, the correlation between the micro-chimerism rate and the EBV-DNA levels decline was not found. However, in the previous micro-transplant study, the micro-chimerism rate reached a peak at 7d-14d after infusion [11]. This is consistent with the efficacy of EBV-DNA's reduction was maximally in 2 weeks, but more data was needed to validate the relationship. However, the efficacy of EBV-DNA reduction cannot persist as in 4 weeks after treatment, there's no differences of EBV-DNA level between 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 who didn't have 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 amount of CD3+ cells infused was high. None of the 15 patients in the GPBSCs infusion group underwent GVHD prevention treatment. Whether it is DLI or haploid transplantation, although 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 did not use a pre-treatment 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 there is indeed one patient who died of severe GVHD in this study, although more considerations are of special circumstances, 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 effects in short terms. Allo-HSCT is still needed to achieve long-term remission [28]. Therefore, we suggest that use GPBSCs as a bridging treatment method to allo-HSCT, for those refractory EBV-HLH patients who do not have a chance to the allo-HSCT before.

## 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 short term survival rate. This effect is quite like GVL effect in leukemia but not exactly the same, which is probably achieved by the GVL-like effect of lymphocytes in the infused cells, and microchimerism may be important. However, the effects last in short terms. 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 do not have a previous chance to.

## 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-asparaginase 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.

## Declarations

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## 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.

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## Tables

Table 1 Clinical features of patients in two groups before treatment

Clinical features	No. of Patients	
	Infusion group (n=18)	Control group (n=19)
Median Age, years	21 [11-60]	29 [17-55]
Gender, Female: male	5:13	6:13
Fever	18 (100%)	19 (100%)
Splenomegaly/Hepatomegaly	16 (88.9%)	16 (84.2%)
Hemophagocytosis	14 (77.8%)	14 (73.7%)
NK cell activity <15.11%	6 (33.3%)	4 (21.1%)
soluble CD25 >6400pg/ml	14 (77.8%)	19 (100%)
EBV-DNA (copies/ml) (at diagnosis)	$5.0 \times 10^5$ [ $1.5 \times 10^3$ , $7.7 \times 10^7$ ]	$1.0 \times 10^6$ [ $2.0 \times 10^5$ , $1.0 \times 10^7$ ]

Table 2 Comparison between two treating groups

	Before		p value	2 weeks		p value
	GPBSCs	control		GPBSCs	control	
WBC	1.36 [0.2, 2.4]	1.8 [0.5, 7.4]	0.134	2.68 [0.17, 15.38]	3.95 [0.2, 12]	0.696
HGB	81.5±17.701	94.63±27.132	0.092	82.75±18.614	97.19±17.608	0.032
PLT	49.5 [12, 340]	45 [3, 499]	0.893	47 [3, 313]	88 [26, 451]	0.102
TG	2.59 [0.6, 12.78]	2.05 [1.39, 5.29]	0.929	1.89 [1.02, 8.86]	1.63 [0.74, 4.11]	0.093
Fbg	1.04 [0.84, 2.91]	1.045 [0.3, 2.12]	0.252	2.16 [0.77, 4.24]	1.065 [0.53, 2.74]	0.003
Ferritin	3843.1 [28, 24174]	3207 [118, 455000]	0.909	3352.5 [255, 40699]	1433 [16, 7549]	0.068
AST	52.5 [13.3, 229.9]	65 [26, 766]	0.150	35.7 [12.8, 181]	28 [8, 680.2]	0.261
ALT	58 [14, 252]	76 [10, 310]	0.425	59 [6, 502]	34.5 [4, 227]	0.123
T-bil	18.725 [8.01, 85.66]	50.925 [7.48, 289.66]	0.088	33.51 [9.36, 169.14]	17.34 [1.84, 192.69]	0.551
EBV-DNA	1.1*10 <sup>5</sup> [<5.0*10 <sup>2</sup> , 3.0*10 <sup>6</sup> ]	5.7*10 <sup>5</sup> [<5.0*10 <sup>2</sup> , 5.2*10 <sup>7</sup> ]	0.304	5.0*10 <sup>3</sup> [<5.0*10 <sup>2</sup> , 5.7*10 <sup>5</sup> ]	6.6*10 <sup>4</sup> [<5.0*10 <sup>2</sup> , 9.0*10 <sup>7</sup> ]	0.011
ECOG	2.78	2.63	0.606	2.56	2.42	0.645

Table 3 Graft details and donor microchimerism rate after GPBSCs infusion

Patient	Graft		Donor microchimerism rate (%)		
	mononuclear (cells/kg)	CD34+ (cells/kg)	1w	2w	3w
1	12.2	4.56	37.4	86.0	93.89
2	5.5	2.31	0.024	0.081	
3	9.7	2.115	0.023	0.014	0.005
4	5.5	2.59	-		
5	5.1	1.17	0.846	0.148	
6	5.2	2.08	0.17		
7	6.4	1.4	-		
8	11.6	2.43	0.15	0.25	
9	10.1	2.4	-		
10	9.7	4.924	11.21	0.15	0.01
11	16.4	3.19	-		
12	5.3	1.325	0.002	0.001	
13	9.6	3.11	0.008		
14	6.1	2.04	-		
15	8.8	2.65	-		
16	16.5	2.31	0.37	0.31	0.034
17	5.1	2.448	-		
18	5.7	1.824	3.26	0.76	

LFT, liver function test; GI, gastrointestinal

**Figures**

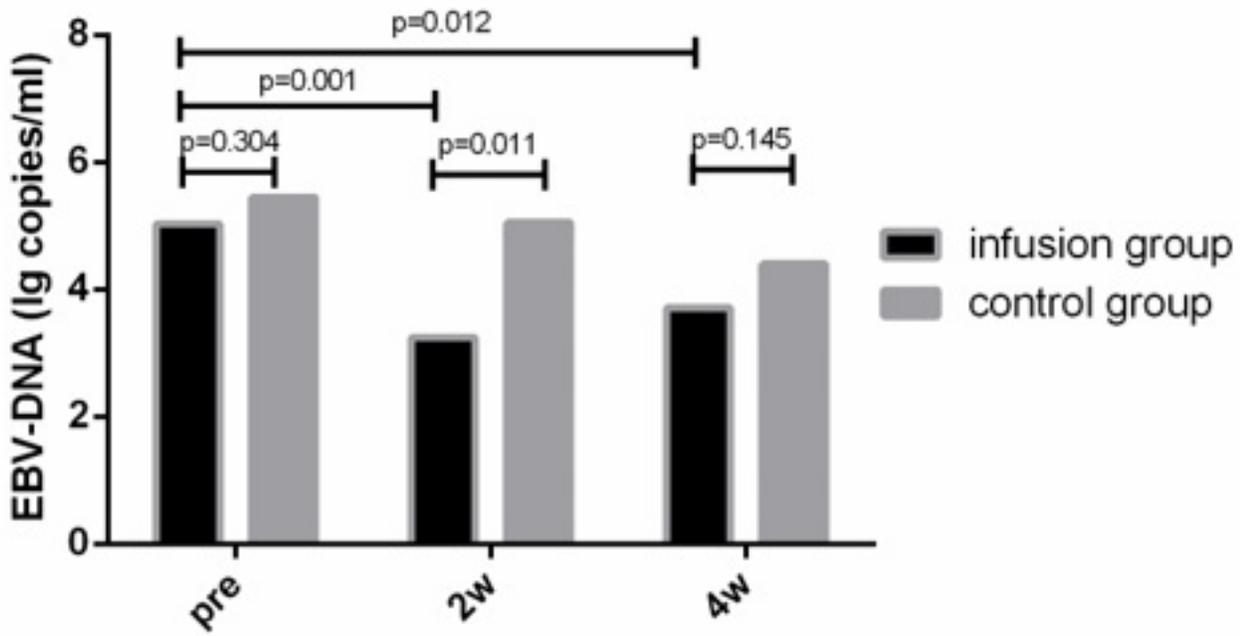


Figure 1

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 ( $p_{2w}=0.011$ ,  $p_{4w}=0.145$ ).

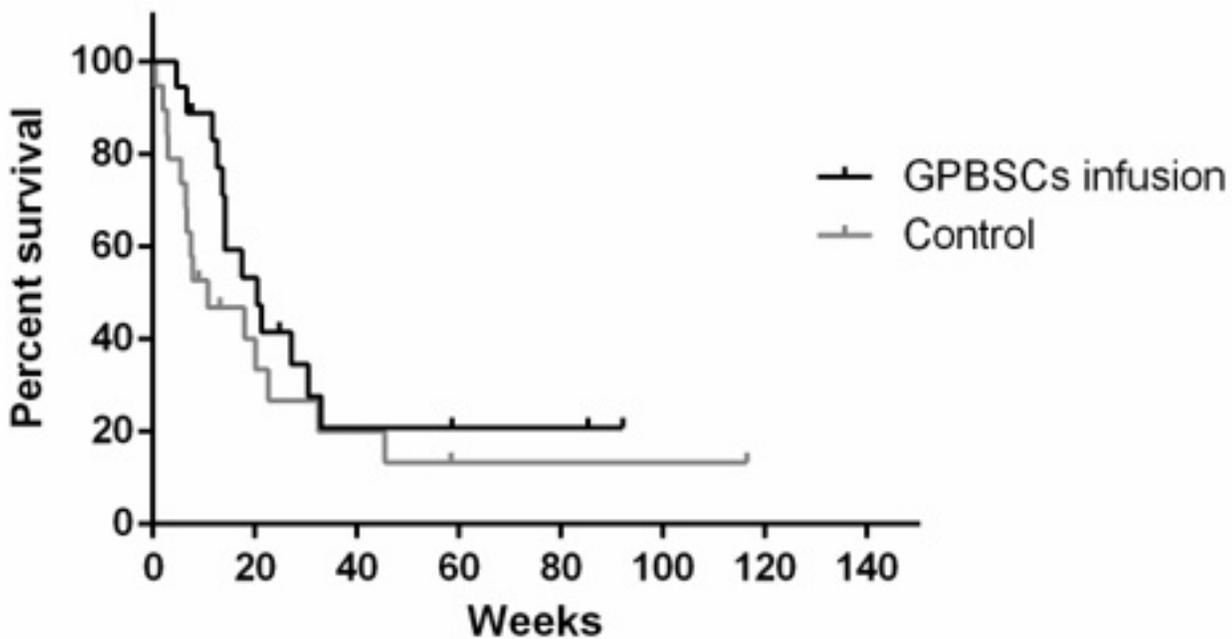


Figure 2

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