Case Report

Iranian Journal of Immunology

December 2023; 20(4):466-472.

Rescue of HLH with T and B Lymphocyte Involvement Due to Epstein-Barr Virus by PD-1 Inhibitor/Ruxolitinib and Rituximab Combination Regimens: A Case Report

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ABSTRACT

Hemophagocytic lymphohistiocytosis (HLH) is a fatal clinical syndrome. The most common cause of secondary HLH is Epstein-Barr virus (EBV) infection. EBV-HLH is a common clinical disease with high mortality, easy relapse, and poor prognosis. Therefore, treating EBV-HLH with T and B lymphocyte involvement is challenging, and selecting an appropriate treatment regimen is critical. Moreover, research on how to evaluate the recurrence index after remission is scarce. In this study, we reported a case of EBV-HLH successfully treated with programmed cell death protein-1 (PD-1) inhibitor in combination with rituximab. The regimen had a good curative effect, and we successfully detected the trend of early recurrence. Our findings indicated that PD-1 inhibitor in combination with rituximab may help to treat EBV-HLH and maintain EBV-infected T and B whole-line lymphocytes.

Keywords: CD20 Monoclonal Antibody, Epstein-Barr Virus, Hemophagocytic Lymphohistiocytosis, Programmed Cell Death Protein-1, Salvage Therapy

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Cite this article as:
Zhu M, Zhang J, Shi Q, Sun X, Wang H, Sun M, Liu Y. Rescue of HLH with T and B Lymphocyte Involvement Due to Epstein-Barr Virus by PD-1 Inhibitor/Ruxolitinib and Rituximab Combination Regimens: A Case Report. *Iran J Immunol.* 2023; 20(4):466-472, doi: 10.22034/iji.2023.99254.2629.

Received: 2023-06-27
Revised: 2023-08-24
Accepted: 2023-08-27

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a group of lethal clinical syndromes. It can be divided into primary and secondary HLH according to the presence or absence of genetic defects. The most common cause of secondary HLH is Epstein-Barr virus (EBV) infection (1-3). Irrespective of the cause, an anti-hyperproliferative inflammatory factor regimen remains the current standard of care for HLH. The classic HLH-94 regimen is based on the combination of etoposide (VP-16) and dexamethasone (DXMS). However, EBV infects B lymphocytes by binding envelope protein gp350 to the CD21 on the surface of B cells (4). Primary EBV infection induces a strong virus-specific T cell response targeting lytic EBV-derived epitopes. EBV impairs the activation of $CD8^+$ and $CD4^+$T cells by interfering with different stages of the human leukocyte antigen (HLA) class I and class II antigen presentation pathway (5). HLH patients with T and B lymphocyte involvement are prone to refractory relapse, and CD20 monoclonal antibody alone cannot destroy EBV-infected T and natural killer (NK) cells. Therefore, given that patients do

not respond well to the traditional regimen, an effective alternative treatment is urgently required.

Here, we present the case of a patient diagnosed with EBV-HLH. After administering various treatments, she finally achieved initial remission and further exhibited a good response to maintenance therapy. This study explored the role of rituximab and programmed cell death protein-1 (PD-1) inhibitors in the treatment of EBV-HLH with T and B lymphocyte involvement.

CASE REPORT

A 17-year-old girl with no past medical history was admitted to our Hematology Department because of a fever (>38.5 °C) and cough that persisted for 10 days. Laboratory examination revealed the following: hemoglobin 105 g/L (normal: 115–150 g/L); white blood cells 0.72×10^9/L (normal: 3.5–9.5×10^9/L); neutrophils 0.42×10^9/L (normal: 1.8–6.3×10^9/L); platelet 32×10^9/L (normal: 125–350×10^9/L); biochemical: albumin 25.30 g/L (normal: 40–55 g/L); alanine aminotransferase 111.00 U/L (normal: 7–40 U/L); aspartate aminotransferase 176.00 U/L (normal: 13–35 U /L); alkaline phosphatase 143.00 U/L (normal: 35–100 U/L); triglycerides 4.09 mmol/L (normal: 0–1.7 mmol/L); coagulation routine: plasma fibrinogen 1.10 g/L (normal: 2–4 g/L); D-dimer assay 6.99 μg/mL (normal: 0–0.5 μg/mL); soluble CD25 (sCD25) 118610 pg/mL (normal: 0–6400 pg/mL); and ferritin >2000 ng/mL (normal: 13–150 ng/mL). In addition, active reticulocyte proliferation of the bone marrow was observed, demonstrating platelet phagocytosis. No significant immunophenotypic abnormalities were observed, and chromosome analysis revealed a normal female karyotype. Positron emission tomography-computed tomography showed increased 18F-deoxyglucose metabolism in the bone marrow and enlarged spleen. Head magnetic resonance imaging (MRI) was negative and demonstrated no neurological symptoms; therefore, we did not perform a lumbar puncture. The patient was diagnosed with HLH, with an H-Score of 294.

Furthermore, she demonstrated no symptoms of herpes or edema, important clinical manifestations of chronic EBV infection. To clarify the primary disease, we tested the patient for HLH-related genes (Supplement 1), which showed negative for *adaptor-related protein complex 3 subunit beta 1 (AP3B1), biogenesis of lysosomal organelles complex 1 subunit 6 (BLOC1S6), CD27, interleukin-2-inducible tyrosine kinase (ITK), lysosomal trafficking regulator (LYST), magnesium transporter 1 (MAGT1), Nod-like receptor family CARD-containing 4 protein (NLRC4), perforin 1, src homology 2 domain–containing protein 1A (SH2D1A), unc-13 homolog D (UNC13D), X-linked inhibitor of apoptosis (XLAP),* and other common hemophagocytic genes. Furthermore, the T cell rearrangement gene test showed negative for the *T cell receptor beta chain (TCRB)*, *T cell receptor delta chain (TCRD)*, and *T cell receptor gamma locus (TCRG)* genes. Tumor markers and immune disease antibody spectrum were also negative. The whole blood EBV DNA quantification was 1 510 000 copies/mL (normal: <400 copies/mL), with anti-viral capsid antigen (VCA)-immunoglobulin (Ig) M (-), anti-VCA-IgG (+), and anti-EBV nuclear antigen-IgG (-). According to the HLH-94 regimen (DXMS+VP16) (Supplement 2 for Treatment Option details), induction therapy, anti-infection treatment, blood count increase, and liver and stomach protection were administered as adjuvant therapy. The patient's condition was assessed throughout the treatment period. Compared with the previous results, her ferritin, triglyceride, EBV DNA, interleukin (IL)-6, IL-10, and other inflammatory factors levels did not decrease significantly (Fig. 1). Lymphocytes were further sorted using flow cytometry, and the EBV DNA copies in each line of lymphocytes were calculated using real-time polymerase chain reaction.

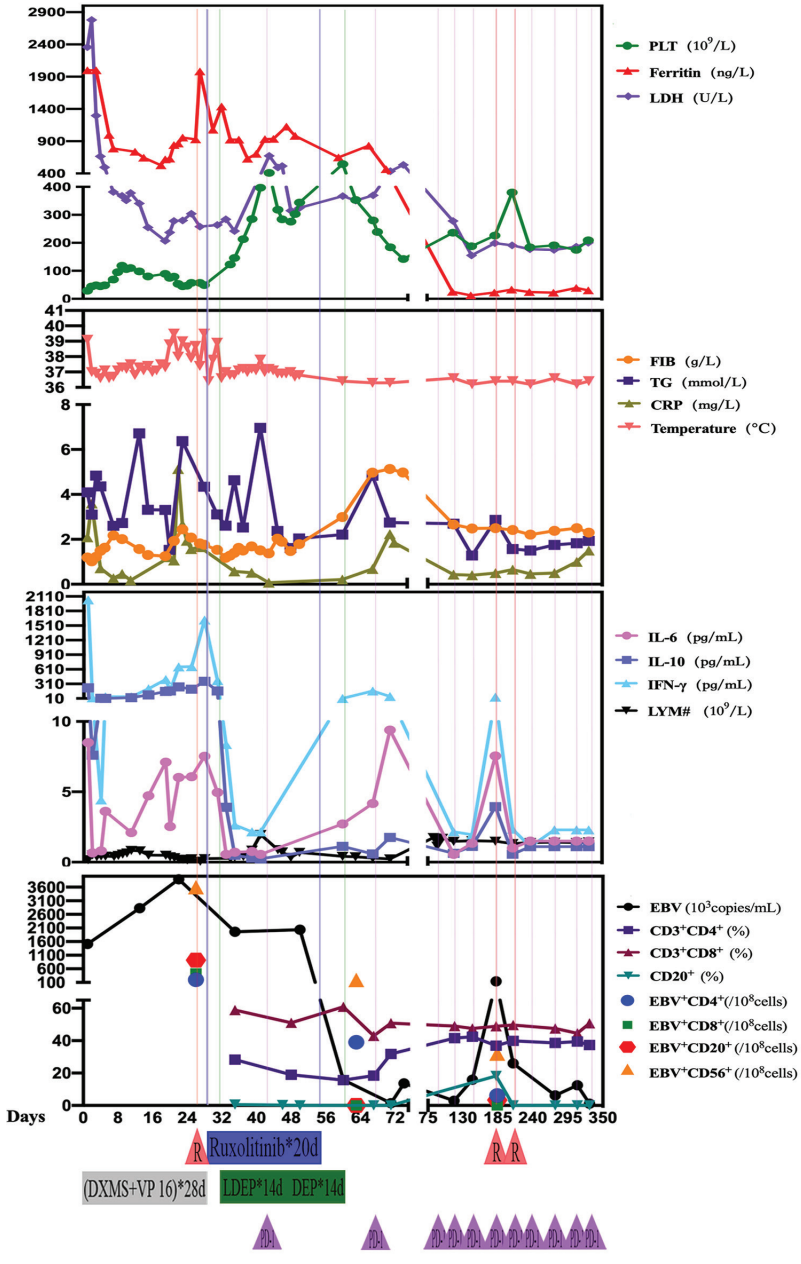

Fig. 1. The treatment status of our patients, as well as changes in this patient's body temperature and HLH-related laboratory parameters during treatment. DXMS: Dexamethasone; VP-16: Etoposide; DOX: Doxorubicin liposome; L: PEG-aspargase; Pred: Methylprednisolone

The DNA copy number was 1350/10^7 cells in $CD3^+CD4^+$ cells, 2150/10^7 cells in $CD3^+CD8^+$ cells, 3980 /10^7cells in $CD3^-CD20^+$ cells, and 25500/10^7cells in $CD56^+$ cells, suggesting that EBV had infected T, B, and NK cell populations. Rituximab was administered to eliminate the infected B cells, while Thyrosine Kinase Inhibitor (Ruxolitinib) was given to suppress the strong immune response. The patient received LDEP and DEP regimen as salvage therapy (doxorubicin liposome+VP-16 +PEG-aspargase+methylprednisolone) (Supplement 2 for Treatment Option details). During treatment, the levels of inflammatory factors such as IL-6 and IL-10 significantly decreased, whereas ferritin, triglycerides, and EBV remained high. Therefore, we administered a PD-1 inhibitor, camrelizumab, to intervene in T lymphocytes, following which the patient's clinical symptoms improved, and she was discharged from the hospital. Subsequently, monthly maintenance

therapy with PD-1 inhibitors was initiated. During re-examination, the relevant indicators decreased significantly, and the sCD25 level returned to the normal range. However, 5 months after discharge, the patient experienced fatigue and anorexia, her blood test showed EBV copies and $CD20^+$ cells, and interferon γ (IFN-γ) levels increased. Rituximab was immediately administered to inhibit the expression of B lymphocytes, following which she quickly recovered. Subsequently, PD-1 inhibitor treatment was continued as maintenance therapy. To date, no other complications have been reported for 40 months.

DISCUSSION

HLH is a clinical syndrome mainly characterized by pathological inflammatory responses caused by hereditary or acquired immune dysfunction. These reactions are induced by the abnormal activation and proliferation of lymphocytes, monocytes, and other phagocytic cells, which secrete many inflammatory cytokines (6). Early diagnosis and treatment are essential to reduce the mortality of HLH. Two key points should be considered in HLH treatment: reducing excessive inflammatory response and treating the cause of hemophagocytic disease.

HLH can be distinguished as primary and secondary HLH based on gene mutations. In most cases, primary HLH has a childhood onset, characterized by hereditary and familial-specific gene mutations. However, in secondary HLH, EBV infection is the most common cause, accounting for approximately 70% (7). Conversely, some studies have found that irrespective of the type of HLH, EBV is involved in the course of HLH (8). In this study, we sequenced only HLH-related genes. *Coronin 1A (CORO1A), interferon regulatory factor 8 (IRF8), ITK, MAGT1, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta (PIK3CD), lipopolysaccharide-responsive and beige-like anchor protein (LRBA),* and *minichromosome maintenance complex component 4 (MCM4)* are known as EBV-susceptible genes. *MAGT1*, a magnesium transporter subtype 1, is involved in the disorder in magnesium $(Mg)^{2+}$ homeostasis after EBV infection. Decreased level of intracellular free Mg^{2+} leads to defective expression of PD-1 in $CD8^+$T cells (9). IL2-inducible T cell kinase encodes an intracellular tyrosine kinase expressed in T cells. *ITK* can fine-tune the T cell receptor (TCR) cascade, transforming changes in TCR signal intensity into differential gene expression programs (10). Hence, in the absence of *ITK*, cell functions other than T cell activation are impaired, making the body more susceptible to EBV infection (11). *ITK* is also an important kinase in the PD-1 pathway, which mediates many phosphorylation events downstream of PD-1 ligation (12). No mutations in the above genes were detected in our patient, which explains the good PD-1 effect.

Our patient had a clear diagnosis of EBV-HLH and required immediate treatment. The current HLH treatment is based on the HLH-94 regimen (13). In a 2017 prospective study involving 369 patients, the early addition of cyclosporine (HLH-04 protocol) showed no improvement in patient outcomes (14, 15). The HLH-94 regimen is a combination of DXMS+VP-16, both of which can inhibit inflammatory factors secretion, and VP-16 can selectively and rapidly clear activated T lymphocytes. However, >30% of patients did not respond to this traditional regimen (16). Similarly, our patient exhibited a poor response to induction therapy. In such cases of response failure, choosing the next regimen can be challenging. Hence, we considered the two key points mentioned above: reducing the inflammatory response and treating EBV infection. We administered the patient Janus kinase (JAK) inhibitor ruxolitinib to inhibit inflammation. After ruxolitinib treatment is initiated in HLH patients, ferritin and fibrinogen levels usually return to normal within 7–30 days (17). Furthermore, several

studies have reported that ruxolitinib in combination with the HLH-94 protocol demonstrated a good response in relieving inflammatory storms in HLH (18, 19). In addition, we chose the LDEP/DEP regimen as salvage therapy. For lymphoma or EBV-related HLH, DEP is a feasible treatment method (20). The addition of pegaspargase to the DEP regimen for EBV-HLH helps prevent and control the disease and acts as a bridging therapy for transplantation (21).

At present, there is no specific effective targeted therapy for EBV infection. Considering the expression of EBV in the patient's lymphocyte subsets, we used rituximab 100 mg to suppress EBV-infected B lymphocytes. After 14 days of LDEP treatment, the triglyceride, ferritin, and EBV levels remained high. Despite the benefits, chemotherapy can lower immune system cells, such as white blood cells, and impair the immune system's ability to clear the underlying EBV infection. Although the patient was administered a strong immunosuppressive treatment, the inhibition rate of underlying EBV-infected lymphocytes was insufficient, which explained why the patient did not achieve disease stability. As the HLH-related inflammatory factor storm was under control, we administered one dose of camrelizumab. Studies have shown that the expression level of PD-1 on the surface of $CD8^+$T cells increases after EBV or other viral infection, and it was hypothesized that PD-1 blockade could reverse EBV-related immunosuppression, thereby restoring the immune response required to clear EBV infection (22, 23). Surprisingly, post-PD-1 inhibitor treatment, our patient's symptoms improved significantly, following which she was discharged.

In the HLH-94 regimen, although VP-16 is used to target activated T cells, long-term high-dose hormone treatment suppresses immune inflammation and also kills immune cells, which is not helpful for the eradication of potential EBV and may cause HLH relapse after a single chemotherapy. Ruxolitinib was administered to our patient after the first course of treatment. However, it mainly calmed the inflammatory storm and did not affect EBV infection, the real syndrome initiator. EBV initially infects naive B cells and can remain latent in the healthy memory B cell pools for a long time. Chemotherapy regimens containing rituximab are more effective against EBV-HLH with only and mainly B lymphocytes infected by EBV (24). Once EBV is activated in the body, NK cells and $CD8^+$T cells rapidly expand, and $CD8^+$T cells in patients with secondary HLH express a higher level of the functional failure marker PD-1 when stimulated. PD-1 monoclonal antibody can restore the degranulation and costimulatory gene expression of $CD8^+$T cells. Furthermore, it can achieve a high response rate in refractory relapsed EBV-HLH (25). Studies have shown that multiple genes involved in T cell activation failed to be upregulated in active EBV-infected $CD8^+$T cells. Moreover, nivolumab treatment expanded the subpopulation of cytotoxic T cells with high PD-1 levels, allowing such cells to reactivate and proliferate from previously exhausted populations (26, 27). After our patient was discharged from the hospital, we continued examining the whole blood EBV copy number and EBV DNA copy number in each lymphocyte line to assess her condition. We believe that the number of single lymphocytes in blood routine examinations is not sufficiently sensitive to predict EBV infection in patients. This may be because the EBV can be reactivated at any time in the memory B cell pool receiving an appropriate stimulus (28), which is also a period where relapse can more likely occur. Rapid identification of infected cells in the blood during EBV infection is critical. PrimeFlow EB encoding region assay is more reliable in identifying EBV-infected cells, because the purity of sorted cells is never 100% in our method, and residual cell EBV DNA may remain (29). Our patient experienced fatigue and anorexia 5 months after discharge, and the re-examination

results showed increased EBV DNA copy and B lymphocyte number. Therefore, we administered rituximab targeted therapy to curb the recurrence at an early stage, and the patient has not reported other complications so far. Although the patient has not yet achieved a complete molecular response, the level of plasma EBV DNA continued to decline.

In conclusion, we report a case of EBV-HLH that was successfully treated with a multischeme combination. The patient achieved complete clinical remission after salvage therapy combined with an anti-CD20 monoclonal antibody and PD-1 inhibitor. Thus, PD-1 inhibitors may help eradicate potential EBV virus by activating the normal immunity of patients. PD-1 inhibitor maintenance therapy can help prevent the recurrence of EBV-HLH. Furthermore, during maintenance therapy, in addition to detecting the level of IL2Rα (30), we believe that the number of each lineage of lymphocytes needs to be dynamically reviewed to prevent recurrence. This study suggests a new combination therapy for EBV-HLH with T and B lymphocyte involvement and presents a potential therapeutic approach for HLH patients without transplantation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Jiangsu Subei People's Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

ACKNOWLEDGMENTS

The authors would like to thank the hematology department, hematology laboratory, respiratory department, digestive department, and other brother departments of our center for their intervention in this case. The author(s) reported there is no funding associated with the work featured in this article.

AUTHORS' CONTRIBUTION

MZ and JZ contributed to the conception and design of the study. QS and XS performed the HLH-related experiments. YL and MS provided technical guidance and patient management. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Imashuku S, Morimoto A, Ishii E. Virus-triggered secondary hemophagocytic lymphohistiocytosis. Acta Paediatr. 2021;110(10):2729-2736.
2. Maakaroun NR, Moanna A, Jacob JT, Albrecht H. Viral infections associated with haemophagocytic syndrome. Rev Med Virol. 2010;20(2):93-105.
3. Mottaghipisheh H, Kalantar K, Amanati A, Shokripour M, Shahriari M, Zekavat OR, et al. Comparison of the clinical features and outcome of children with hemophagocytic lymphohistiocytosis (HLH) secondary to visceral leishmaniasis and primary HLH: a single-center study. BMC Infect Dis. 2021;21(1):732.
4. Cui X, Snapper CM. Epstein Barr Virus: Development of Vaccines and Immune Cell Therapy for EBV-Associated Diseases. Front Immunol. 2021;12:734471.
5. Ressing ME, van Gent M, Gram AM, Hooykaas MJ, Piersma SJ, Wiertz EJ. Immune Evasion by Epstein-Barr Virus. Curr Top Microbiol Immunol. 2015;391:355-81.
6. JankaGE, LehmbergK. Hemophagocytic lymphohistiocytosis: pathogenesis and treatment. Hematology Am Soc Hematol Educ Program, 2013, 2013:605-611.
7. Maakaroun NR, Moanna A, Jacob JT, Albrecht H. Viral infections associated with haemophagocytic syndrome. Rev Med Virol. 2010;20(2):93-105.

8. Ohga S, Nomura A, Hara T. Immunological aspects of Epstein-Barr virus infection. Crit Rev Oncol Hematol. 2002;44(3):203-15.
9. Diao B, Huang X, Guo S, Yang C, Chen Y, Wu Y. MAGT1-mediated disturbance of Mg2+ homeostasis lead to exhausted of HBV-infected NK and CD8+ T cells. Sci Rep. 2017;7(1):13594.
10. Elmore JP, McGee MC, Nidetz NF, Anannya O, Huang W, August A. Tuning T helper cell differentiation by ITK. Biochem Soc Trans. 2020;48(1):179–185.
11. Ghosh S, Bienemann K, Borkhardt A. Interleukin-2-inducible T-cell kinase (ITK) deficiency - clinical and molecular aspects. J Clin Immunol. 2014;34(8):892–9.
12. Moore EK, Strazza M, Mor A. Combination Approaches to Target PD-1 Signaling in Cancer. Front Immunol. 2022;13:927265.
13. Henter JI, Aricò M, Egeler RM, Elinder G, Favara BE, Filipovich AH, et al. HLH-94: a treatment protocol for hemophagocytic lymphohistiocytosis. HLH study Group of the Histiocyte Society. Med Pediatr Oncol. 1997;28(5):342-7.
14. Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124-31.
15. Bergsten E, Horne A, Aricó M, Astigarraga I, Egeler RM, Filipovich AH, et al. Confirmed efficacy of etoposide and dexamethasone in HLH treatment: long-term results of the cooperative HLH-2004 study. Blood. 2017;130(25):2728-2738.
16. Johnson TS, Terrell CE, Millen SH, Katz JD, Hildeman DA, Jordan MB. Etoposide selectively ablates activated T cells to control the immunoregulatory disorder hemophagocytic lymphohistiocytosis. J Immunol. 2014;192(1):84-91.
17. Keenan C, Nichols KE, Albeituni S. Use of the JAK Inhibitor Ruxolitinib in the Treatment of Hemophagocytic Lymphohistiocytosis. Front Immunol.2021;12:614704.
18. Wang H, Gu J, Liang X, Mao X, Wang Z, Huang W. Low dose ruxolitinib plus HLH-94 protocol: A potential choice for secondary HLH. Semin Hematol. 2020;57(1):26-30.
19. Zhang Q, Wei A, Ma HH, Zhang L, Lian HY, Wang D, et al. A pilot study of ruxolitinib as a front-line therapy for 12 children with secondary hemophagocytic lymphohistiocytosis. Haematologica. 2021;106(7):1892-1901.
20. Wang Y, Huang W, Hu L, Cen X, Li L, Wang J, et al. Multicenter study of combination DEP regimen as a salvage therapy for adult refractory hemophagocytic lymphohistiocytosis. Blood. 2015;126(19):2186-92.
21. Lai W, Wang Y, Wang J, Wu L, Jin Z, Wang Z. Epstein-Barr virusassociated hemophagocytic lymphohistiocytosis in adults and adolescents-a life-threatening disease: analysis of 133 cases from a single center. Hematology.2018;23(10):810-816.
22. Chatterjee B, Deng Y, Holler A, Nunez N, Azzi T, Vanoaica LD, et al. CD8+ T cells retain protective functions despite sustained inhibitory receptor expression during EpsteinBarr virus infection in vivo. PLoS Pathog. 2019;15(5):e1007748.
23. Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. Nat Med. 2018;24(9):1449-1458.
24. Meng GQ, Wang JS, Wang YN, Wei N, Wang Z. Rituximabcontaining immuno-chemotherapy regimens are effective for the elimination of EBV for EBV-HLH with only and mainly B lymphocytes of EBV infection. Int Immunopharmacol. 2021;96:107606.
25. Latour S, Fischer A. Signaling pathways involved in the T-cell-mediated immunity against EpsteinBarr virus: Lessons from genetic diseases. Immunol Rev. 2019;291(1):174-189.
26. Liu P, Pan X, Chen C, Niu T, Shuai X, Wang J, et al. Nivolumab treatment for relapsed/refractory Epstein-Barr virusassociated hemophagocytic lymphohistocytosis in adults. Blood. 2020;135 (11):826–833.
27. Young LS, Yap LF, Murray PG. Epstein-Barr virus: more than 50 years old and still providing surprises. Nat Rev Cancer. 2016;16(12):789-802.
28. Kerr JR. Epstein-Barr virus (EBV) reactivation and therapeutic inhibitors. J Clin Pathol. 2019;72(10):651-658.
29. Fournier B, Boutboul D, Bruneau J, Miot C, Boulanger C, Malphettes M, et al. Rapid identification and characterization of infected cells in blood during chronic active Epstein-Barr virus infection. J Exp Med. 2020;217(11):e20192262.
30. Shokripour M, Mokhtari M, Samizadeh B. Association of soluble interleukin-2 receptor alpha with laboratory parameters and clinical findings of hemophagocytic lymphohistiocytosis patients: The first report from South of Iran. J Educ Health Promot. 2020;9:48.