

# Allogeneic HSCT Improves Prognosis in Advanced Extranodal NK/T-Cell Lymphoma

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## Research Article

**Keywords:** ENKTCL, allo-HSCT, HBV, Prognostic study

**Posted Date:** August 19th, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-9109416/v1>

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**Additional Declarations:** No competing interests reported.

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# Abstract

Extranodal NK/T-cell lymphoma (ENKTCL) is a rare and aggressive lymphoid malignancy highly associated with Epstein-Barr virus (EBV) infection. Patients with stage III or higher disease often have a poor prognosis. This study retrospectively reviewed the treatment courses of 34 patients with relapsed/refractory (R/R) ENKTCL who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) at our center between January 2019 and July 2024.

The results showed that in this study, the use of PD-1 inhibitors before transplantation, the number of treatment lines, and whether the plasma EBV was negative or not had no significant impact on OS and PFS. Patients achieving complete response (CR) or partial response (PR) prior to allo-HSCT exhibited a more favorable prognosis. Conversely, a history of chronic active EBV infection (CAEBV) before transplantation and EBV reactivation post-transplantation were significantly associated with inferior survival, suggesting poor prognostic outcomes.

Genetic susceptibility analysis revealed that patients with advanced ENKTCL frequently harbored hemophagocytic lymphohistiocytosis (HLH)-associated gene variants. LYST gene variants were the most commonly identified HLH-related alterations, and patients developing HLH experienced significantly higher non-relapse mortality (NRM). Furthermore, TP53 gene variants were strongly correlated with post-allo-HSCT relapse.

In conclusion, Allogeneic hematopoietic stem cell transplantation can improve survival rates in high-risk, advanced-stage, relapsed, and refractory ENKTCL. Further studies with larger sample sizes and extended follow-up periods are warranted to better guide the clinical management of this disease.

## Background

**Extranodal NK/T-cell lymphoma (ENKTCL)** is an aggressive malignancy. Expression of P-glycoprotein in tumor cells confers a multidrug-resistant phenotype, rendering CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) and other anthracycline-containing combination chemotherapy regimens are both of poor efficacy<sup>1</sup>.

In recent years, with advances in the treatment and understanding of ENKTCL, a consensus on treatment strategies has gradually emerged. For low-risk, localized-stage disease (stage I/II), the preferred treatment combines chemotherapy (incorporating non-anthracycline agents, platinum compounds, or L-asparaginase) with radiotherapy. Conversely, advanced-stage ENKTCL (stage III/IV) is typically treated with L-asparaginase-based systemic chemotherapy alone. These approaches enable long-term survival in approximately 40-50% of patients<sup>2,3</sup>. Patients achieving complete remission (CR) with chemotherapy may receive consolidation chemotherapy or proceed to other intensive chemotherapy regimens<sup>4-6</sup>.

For patients with refractory or relapsed disease, outcomes with conventional salvage therapy remain poor, with reported median overall survival (OS) and progression-free survival (PFS) of 6.4 months and

4.1 months, respectively<sup>2</sup>. Recently, programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) inhibitors have demonstrated promising response rates in this population; however, robust data on long-term survival outcomes are still lacking<sup>7-9</sup>.

Both autologous hematopoietic stem cell transplantation (auto-HSCT) and allogeneic hematopoietic stem cell transplantation (allo-HSCT) have shown encouraging results in patients with advanced ENKTCL. Nevertheless, studies indicate that upfront auto-HSCT consolidation following first-line therapy yields OS and PFS rates comparable to those achieved with L-asparaginase-based regimens alone<sup>10,11</sup>. This suggests that early auto-HSCT does not translate into a survival benefit. Consequently, auto-HSCT may be more suitable for advanced-stage NK/T-cell lymphoma in first complete remission, whereas allo-HSCT appears better suited for relapsed/refractory (R/R) ENKTCL<sup>12</sup>.

Leveraging its associated graft-versus-lymphoma (GVL) effect, allogeneic hematopoietic stem cell transplantation (allo-HSCT) has emerged as a potential therapeutic approach for high-risk, relapsed/refractory ENKTCL. This study retrospectively analyzed the clinical data of 34 R/R ENKTCL patients who underwent allo-HSCT at our center between January 2019 and July 2024, summarizing their clinical characteristics and analyzing various prognostic factors.

## Materials and Methods

### Patients

This study retrospectively included 34 patients with ENKTCL who underwent hematopoietic stem cell transplantation (HSCT) at Beijing Gaobo Boren Hospital from January 2019 to July 2024 (Figure 1). All patients were diagnosed based on pathological cytology and immunohistochemical data. The median age of the patients was 30.77 years (range: 3.84-57.2 years), with 29 adults (87.9%) and 4 children (12.1%). Thirty-three patients had extramedullary disease (EMD) (97.1%), and 6 had central nervous system lymphoma (CNSL) (17.6%). Twenty-six patients had a PINK score of 3 (76.6%), 7 had a score of 2 (20.6%), and 1 had a score of 4 (2.9%). The median number of treatment lines was 3 (IQR: 2-4), with 29 patients having more than one treatment line (87.9%). Fifteen patients had relapsed before this transplant (44.1%), and 3 had a second transplant (8.8%). Twelve patients had hemophagocytic syndrome before the transplant (35.3%). Twenty-three patients had received at least one anti-PD-1 treatment. Nine patients achieved complete remission (CR) before allo-HSCT.

### Detection of Germline Susceptibility Genes and Somatic Gene Mutations

Prior to the transplant, peripheral blood mononuclear cells (PBMC) were collected from the patients to detect mutations in susceptibility genes associated with hematologic malignancies or immune system disorders. Bone marrow (BM) samples were also collected from the patients to analyze somatic gene mutations related to hematologic malignancies. Genomic DNA was extracted from the PBMC and BM samples using the TIANamp Blood Genomic DNA Kit (Tiangen Biotech). Subsequently, capture hybridization was performed using the NimbleGen SeqCap EZ Library SR (Roche Diagnostics) targeting

the exonic regions of 339 genes associated with hematologic malignancies, or the Agilent SureSelect XT Human All Exon v5 kit for germline susceptibility gene mutations. The captured libraries were sequenced on the Illumina NextSeq 550 platform, generating paired-end 150bp (PE 150 bp) reads. Candidate genetic risk variants were validated in the patient's family members by Sanger sequencing. Variants were interpreted based on the Human Gene Mutation Database (HGMD) and the guidelines of the American College of Medical Genetics and Genomics (ACMG). Somatic variant calling was performed using the Genome Analysis Toolkit (GATK) v4.0.4.0 and VarDict v1.12 from the Broad Institute, and annotated using Annovar v2018-04-16. Selected pathogenic variants were manually reviewed using the Integrative Genomics Viewer (IGV) software v2.8.0.

## **Preparative Regimen and Graft**

Patients underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) at Beijing Gaobo Boren Hospital. The myeloablative preparative regimens included TBI/FLU/VM-6, Bu/FLU/VM-6, and TBI/CY. The TBI/FLU/VM-6 regimen (n=6, 17.6%) consisted of fractionated total body irradiation (total dose 1000 cGy, 800 cGy for second transplant patients) + fludarabine (30 mg/m<sup>2</sup>/day, administered from day -6 to -2 before transplant) + VM26 (100 mg/m<sup>2</sup>/day, once daily for 3 days). The Bu/FLU/VM-6 regimen (n=26, 76.5%) included VM26 (100 mg/m<sup>2</sup>/day, once daily for 3 days)+busulfan (adults: intravenous injection, 0.8 mg/kg, every 6 hours for 3 days; pediatric patients' doses adjusted according to weight)+fludarabine (30 mg/m<sup>2</sup>/day, administered from day -6 to -2 before transplant). The TBI/CY regimen (n=2, 5.9%) consisted of fractionated total body irradiation (total dose 1000 cGy, 800 cGy for second transplant patients) + cyclophosphamide (adults: intravenous injection, 60 mg/kg/day × 2 days; children: 50-60 mg/kg/day × 2 days intravenous infusion + adequate hydration/mesna to protect the bladder). cyclophosphamide (250 mg/m<sup>2</sup>, administered on day -3 before transplant). For unrelated or haploidentical transplants, rabbit anti-human T-cell immunoglobulin (ATG-F; 100 mg/5 mL; Astellas Pharma Japan) was administered, with a total dose of 20 mg/kg over 4 days (from day -5 to -2 before transplant). Donor types included haploidentical donors (n=18, 52.9%) and unrelated donors (n=16, 47.1%). For haploidentical transplants, the graft consisted of a combination of granulocyte colony-stimulating factor (G-CSF)-mobilized bone marrow (BM) and peripheral blood stem cells (PBSCs). For matched sibling and unrelated donor transplants, only PBSCs were used.

## **Prevention of GVHD and Infections**

**GVHD Prevention** A combination of cyclosporine, mycophenolate mofetil, and short-term methotrexate was used for GVHD prophylaxis. Cyclosporine: Intravenous injection (i.v.), 1.25 mg/kg every 12 hours, starting on day -9 before transplant; switched to oral administration after 1 month. Methotrexate: Intravenous injection (i.v.), 15 mg/m<sup>2</sup> on day +1 after transplant, and 10 mg/m<sup>2</sup> on days +3, +6, and +11. Acute GVHD (aGVHD) was graded using the modified Glucksberg criteria by Przepiorka et al., with grades III and IV considered severe aGVHD. Chronic GVHD (cGVHD) was assessed according to the National Institutes of Health (NIH) consensus criteria<sup>13</sup>.

**Infection Prevention** Antifungal prophylaxis was administered based on the patient's history before transplant, using echinocandins or triazoles for primary or secondary prophylaxis. Antiviral prophylaxis included acyclovir to prevent herpes simplex virus infection. Trimethoprim-sulfamethoxazole was used to prevent *Pneumocystis pneumonia* (PCP). Viral monitoring was conducted using quantitative polymerase chain reaction (qPCR) to detect human herpesvirus types 1-8 (HHV-1 to 8), BK virus (BKV), and JC virus (JCV). Plasma levels of cytomegalovirus (CMV) DNA and Epstein-Barr virus (EBV) DNA were routinely monitored 1-2 times per week after transplant. Viremia was defined as the presence of CMV/EBV infection in the blood, based on guidelines. Viral disease referred to viral infections in tissues and organs (i.e., with clinical symptoms or organ damage), such as viral cystitis caused by BK virus, JCV, and/or adenovirus after allo-HCT.

### **Post-Transplant Efficacy Evaluation**

Definitions of complete remission (CR), CR with incomplete hematologic recovery (Cri), and no remission (NR) followed the National Comprehensive Cancer Network (NCCN) guidelines<sup>14</sup>. Post-transplant relapse was defined according to the Chinese Society of Hematology (2016) criteria<sup>15</sup>, referring to hematologic relapse or extramedullary disease (EMD). EMD was assessed using imaging methods including computed tomography (CT), positron emission tomography-CT (PET-CT), and magnetic resonance imaging (MRI). Monitoring time points were as follows: monthly for the first 3 months after transplant, every 1.5 months from 3 to 12 months after transplant, every 3 months from 1 to 2 years after transplant, and every 6 months from 2 to 5 years after transplant.

### **Follow-Up**

The follow-up cut-off date was January 3, 2025. Follow-up was conducted by phone with the patients or their family members. Overall survival (OS) was defined as the time from hematopoietic stem cell infusion to death from any cause or last follow-up. Progression-free survival (PFS) was defined as the time from achieving CR after hematopoietic stem cell infusion to disease relapse or last follow-up for those without relapse.

### **Statistical Analysis**

Statistical analyses were conducted using R v. 3.6.2. Data are expressed as the median and interquartile range (IQR). The Wilcoxon rank-sum test was applied to assess differences between two groups. Pearson's chi-squared test or Fisher's exact test was employed to compare differences between categorical variables. Gray's test or Log-rank test, and Kaplan-Meier curves were used to compare relapse, transplant-related mortality (TRM), PFS and OS. The Cox proportional hazards model was applied for survival analysis. All survival analyses were performed using the "survminer" and "survival" packages in R.  $P < 0.05$  was considered significant for two-sided tests.

## **Results**

## Patient Cohort Information

We conducted a retrospective study on 34 ENKTCL patients who underwent hematopoietic stem cell transplantation at Beijing Gaobo Boren Hospital from January 2019 to July 2024. The median follow-up time was 20.8 months (95% CI: 19.3-34.4 months), with the last follow-up date being January 3, 2025. The median age of the patients was 30.77 years (range: 3.84-57.2 years). PINK prognostic score: 4 (n=1, 2.9%), 3 (n=26, 76.6%), 2 (n=7, 20.6%). The median number of treatment lines was 3 (IQR: 2-4), with 29 patients having more than one treatment line (87.9%). Pre-transplant status: relapse before transplant (n=15), CR before transplant (n=9), NR before transplant (n=14), PR before transplant (n=11). Three patients had a second transplant (8.8%). Twelve patients had hemophagocytic syndrome before transplant (35.3%). All patients received myeloablative conditioning regimens, including TBI/FLU/VM-6 (n=6, 17.6%), Bu/FLU/VM-6 (n=26, 76.5%), and TBI/CY (n=2, 5.9%). Transplant types included haploidentical (n=18, 52.9%) and unrelated (n=16, 47.1%), with 16 patients receiving ABO-matched transplants (47.1%). Post-transplant engraftment occurred in 33 patients (n=33, 97.1%), while one patient died of multiple organ failure before stem cell infusion.

## Post-Transplant Complications

### GVHD After Transplantation

The incidence of grade II-IV aGVHD after transplantation was 36.4% (n=12 cases), with grade IV aGVHD occurring in 5 individuals (n=5 cases, all with gastrointestinal aGVHD). Among the organs affected by aGVHD, gastrointestinal aGVHD had the highest incidence at 27.3% (n=9 cases), followed by skin aGVHD at 15.2% (n=5 cases) and liver aGVHD at 9.1% (n=3 cases). The incidence of cGVHD after transplantation was 27.3% (n=9 cases), with skin cGVHD being the most common at 12.1% (n=4 cases). The specific details of aGVHD and cGVHD are described in Table 2.

### Infections After Transplantation

The incidence of bacterial infections was 51.5% (n=17 cases), viremia occurred in 48.5% (n=16 cases), viral diseases in 76.5% (n=26 cases), fungal infections in 36.4% (n=12 cases), and infections with unidentified pathogens in 12.1% (n=4 cases). Among the pathogens, CMV infection was the most common (n=14 cases, 42.4%), followed by EBV infection (n=13 cases, 39.4%).

## Post-Transplant Survival

The overall survival after allo-HSCT is shown in Figure 2A. The 1-year cumulative incidence of relapse (CIR) was 21.2% (95% CI: 10.7-39.4%), and the 2-year CIR was 40.8% (95% CI: 25.3-61.1%); the 1-year transplant-related mortality (TRM) was 3.03% (95% CI: 0.43-19.6%), and the 2-year TRM was 7.04% (95% CI: 1.78-25.7%) (Figure 2A left). The 1-year progression-free survival (PFS) was 75.8% (95% CI: 62.5-91.9%), and the 2-year PFS was 52.1% (95% CI: 36.5-74.6%) (Figure 2A middle). The 1-year overall survival (OS) was 80.9% (95% CI: 68.1-96.0%), and the 2-year OS was 57.6% (95% CI: 40.5-82.1%) (Figure 2A right).

## Impact of Clinical Factors on Prognosis

Analysis of clinical factors revealed that donor type significantly impacted prognosis. Patients receiving haploidentical transplants had significantly superior OS compared to those receiving MUD transplants (HR = 0.235, 95% CI: 0.066–0.966,  $p = 0.031$ ; Figure 2B). Similar trends were observed for PFS (HR = 0.387, 95% CI: 0.128–1.17,  $p = 0.085$ ) and CIR ( $p = 0.11$ ). No significant associations were found between prognosis and gender, age, EMD, or CNSL (data not shown). The relationship between survival and the number of prior treatment lines, prior anti-PD-1 therapy, and pre-transplant disease status is shown in Figures 2C-E.

Although not statistically significant, patients with  $\geq 2$  prior treatment lines (1-year OS), those receiving anti-PD-1 therapy, and those not achieving CR before allo-HSCT exhibited trends toward poorer outcomes, suggesting unmet therapeutic needs. Notably, patients receiving anti-PD-1 therapy had OS and PFS curves below those of untreated patients. This may be attributed to the anti-PD-1 group being older (median = 33.01 years, IQR: 28.26–38.83 vs. Non-anti-PD-1 patient group median = 24.45 years, IQR: 22.16–29.77;  $p = 0.06$ ) and having received more treatment lines (median = 3, IQR: 2.5–4 vs. median = 1.5, IQR: 1–2;  $p < 0.001$ ). Importantly, no increase in aGVHD, cGVHD, or infections was observed in anti-PD-1 treated patients (Table S1).

## Prognosis and EBV Infection

We further analyzed the relationship between EBV infection and the prognosis of NKTCL patients. Eleven patients (32.4%) had a history of chronic active EBV infection (CAEBV). Post-transplant EBV infection occurred in 4/11 CAEBV patients, but no significant association was found (4/11 vs. 9/23,  $p = 1.0$ ). CAEBV was associated with significantly inferior OS (HR = 3.97, 95% CI: 1.2–13.1,  $p = 0.015$ ; Figure 3A), with a 1-year OS of only 58.3% (95% CI: 34.0–100%). A similar trend for PFS did not reach statistical significance ( $p = 0.14$ ). While CIR was not higher in CAEBV patients, non-relapse mortality (NRM) was significantly increased ( $p = 0.025$ ), with a 1-year NRM of 10.0% (95% CI: 1.47–52.7%).

Post-transplant EBV infection was also a significant risk factor for poor prognosis (OS: HR = 6.53, 95% CI: 1.67–25.6,  $p = 0.00089$ ; PFS: HR = 8.93, 95% CI: 2.47–32.3,  $p = 0.00032$ ; Figure 3B). Patients with EBV infection had a 1-year OS of 60.6% (95% CI: 38.7–94.7%) and a 1-year PFS of 53.8% (95% CI: 32.6–89.1%). EBV infection was also associated with increased CIR ( $p = 0.00035$ ); the 1-year CIR was 46.2% (95% CI: 24.0–75.2%), significantly higher than in patients without EBV infection (1-year CIR: 5.0%, 95% CI: 0.72–30.5%). CAEBV patients who developed post-transplant EBV infection had an extremely poor prognosis: median OS was only 7.12 months (95% CI: 1.58–NA,  $p = 0.00012$ ), median PFS was 3.34 months (95% CI: 1.45–NA,  $p = 0.0016$ ), and 1-year CIR was 75% (95% CI: 33.5–99.1%,  $p = 0.0041$ ) (Figure 3C).

## HLHand Germline Genetic Susceptibility Mutations in Patients

All 34 patients underwent pre-transplant next-generation sequencing (NGS) for germline susceptibility variants. Somatic mutation testing was also performed in 24 patients at diagnosis. Germline and somatic mutation profiles are shown in Figures S1 (A-B). Somatic mutations were heterogeneous; recurrently mutated genes included *BCOR* (n=4, 17%), *KMT2D* (n=4, 17%), *TP53* (n=4, 17%), *ARID1A* (n=3, 12%), *STAT3* (n=3, 12%), *DNMT3A* (n=2, 12%), *JAK3* (n=2, 12%), and *KDM6A* (n=2, 12%) (Figure S1B). Patients with *DNMT3A* variants (n=2) had poor outcomes, with both dying from relapse (PFS: median PFS = 8.75 months, 95% CI: 4.41–NA, p = 0.029; OS: median OS = 13.3 months, 95% CI: 10.5–NA, p = 0.0033; CIR: p = 0.036; NRM: p = 1). To investigate the intrinsic link between gene mutations and HLH, we leveraged the Genmain platform to construct a gene network (Figure 4A) and performed a corresponding clustering analysis (Figure 4B). The analysis uncovered a critical connection: these highly variable genes not only interact closely with genes like *E2F5* but are also collectively involved in core biological activities such as hematopoietic stem cell proliferation and cellular metabolism. This finding is particularly significant, as dysregulation of hematopoietic stem cells has been recognized as a key step in the HLH disease process. Therefore, we propose the scientific hypothesis that these gene mutations likely exert a negative impact on patient health by disrupting the normal function of hematopoietic stem cells.

Secondary HLH has been linked to germline immunodeficiency variants. Twelve patients (35.3%) had secondary HLH. Allo-HSCT improved outcomes in these patients; no significant differences in OS or PFS were observed compared to non-HLH patients (OS: p = 0.72; PFS: p = 0.65; CIR: p = 0.17; Figure 3A). NRM was slightly higher in HLH patients (1-year NRM = 9.1%, 95% CI: 1.3–49.2%, p = 0.043). Further analysis revealed an association between HLH and germline variants in immunodeficiency susceptibility genes (Figure 5B). Germline variants in HLH-related genes were detected in 8/12 HLH patients: *LYST* variants (n=5, 41.7%, all heterozygous), *MPEG1* variants (n=3, 25%, all heterozygous), and a homozygous *XIAP* variant (n=1, 8.3%). Germline *LYST* variants were significantly associated with HLH (p = 0.037; Figure 5C). The recurrent *LYST* variant identified was p.Lys2790Gln (p.K2790Q) (n=2; Figure 5D).

## Discussion

Our study retrospectively analyzed the clinical data of 34 patients with R/R ENKTCL who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) in our center from January 2019 to July 2024. The results show that the 1-year progression-free survival (PFS) was 75.8% (95% CI: 62.5–91.9%), and the 2-year PFS was 52.1% (95% CI: 36.5–74.6%) (Figure 2A middle). The 1-year overall survival (OS) was 80.9% (95% CI: 68.1–96.0%), and the 2-year OS was 57.6% (95% CI: 40.5–82.1%), with a median follow-up time of 20.8 months. In previous multicenter clinical studies for advanced ENKTCL, patients treated with chemotherapy, chemotherapy combined with radiotherapy, or HSCT had a 1-year OS rate of 59.5%, significantly lower than the results of this study<sup>16</sup>. This suggests that allo-HSCT can significantly improve the prognosis of patients with R/R ENKTCL, with haploidentical transplantation showing the best outcomes.

The status before HSCT is a key factor affecting the efficacy of allo-HSCT in patients with relapsed or refractory ENKTCL. In this study, patients who achieved CR or PR before transplantation had significantly better overall survival (OS) and PFS compared to those who did not achieve CR, consistent with previous research findings<sup>6,12,17</sup>. A French study showed that auto-HSCT or allo-HSCT did not demonstrate significant benefits for patients who responded well to asparaginase-based chemotherapy with or without local radiotherapy. However, it often provided survival benefits for relapsed patients with initial high-risk clinical features who achieved a second remission before transplantation<sup>17</sup>. For patients with r/r ENKTCL, making efforts to achieve CR or PR through chemotherapy, radiotherapy, or other means before HSCT to improve sustained remission rate. Previous real-world studies have suggested that the use of asparaginase and gemcitabine alone is beneficial for improving PFS and OS<sup>18</sup>. Combining use of asparaginase and gemcitabine chemotherapy to minimize tumor burden before HSCT can be considered in subsequent treatment, in order to achieve CR or PR of the primary disease to optimize therapeutic efficacy, duration of response, and survival outcomes.

In previous retrospective studies, upfront (first-line or second-line) allogeneic HSCT provided extranodal PTCL patients with longer PFS, a lower 3-year CIR (16.7% vs. 56.0%), and comparable NRM (10.4% vs. 11.0%). The 3-year NRM for extranodal PTCL patients receiving allogeneic HSCT after  $\geq 3$  lines of treatment (37.5%) were higher than that after first-line (10.4%) or second-line (8.5%) treatment<sup>18</sup>. In this study, univariate analysis of the number of treatment lines and prognosis showed no significant correlation between the number of pre-transplant treatment lines and patients' OS or PFS, which is inconsistent with previous studies. However, considering that all patients in this study had r/r ENKTCL, with a median of 3 treatment lines and a minimum of 2 lines, it is suggested that among patients receiving  $\geq 3$  lines of treatment, there may be no significant difference in HSCT prognosis. For such patients, early allogeneic hematopoietic stem cell transplantation following first- or second-line treatment may lead to higher OS and PFS, reducing the 3-year cumulative relapse rate and non-relapse mortality rate.

Plasma EBV viral load can serve as a tumor burden marker for ENKTCL, useful for assessing patient prognosis and disease status<sup>19</sup>. This study shows that whether plasma EBV viral load turns negative before HSCT has no significant impact on patient survival, consistent with previous studies on autologous transplantation for ENKTCL<sup>20</sup>. This study also found that a history of chronic active EBV infection is a significant risk factor affecting OS and PFS. Of the patients, 38.7% experienced hemophagocytic syndrome (HPS) before transplantation, identified as NK/T-cell lymphoma-associated hemophagocytic syndrome (T/NK-LAHLH). Studies suggest that its prognosis may be related to whole-blood EBV-DNA levels. Zhang et al. reported that in 162 cases of T/NK-LAHLH, a whole-blood EBV-DNA load  $>16,100$  copies/mL was significantly associated with reduced 60-day OS<sup>21</sup>; In Xiong's cohort, the median OS of EBV-positive T/NK-LAHLH patients was only 52 days, significantly shorter than that of EBV-negative patients<sup>22</sup>. Thus, peripheral blood EBV-DNA may also be considered a prognostic marker. However, in this study, the level of EBV copy number showed no significant correlation with prognosis, possibly due to the small sample size. Future studies could expand the sample size and evaluate both

plasma and peripheral blood EBV-DNA loads, particularly in patients with chronic active EBV infection, to better assess risk and prognosis.

All patients in this cohort underwent germline genetic susceptibility gene testing, with 19/34 (55.48%) showing HLH-related gene mutations. The most common Type I mutations were *MPEG1* (24%) and *PRF1* (9%); common Type II mutations included *LYST* (21%), *SLC7A7* (6%), *STXBP2* (6%), *UNC13D* (6%), *XIAP* (6%), *CTPS1* (3%), *LAMP1* (3%), and *PRF1* (3%). The presence of HLH-related genes, such as *MPEG1*, *LYST*, and *XIAP*, was associated with the prognosis of ENKTCL, but the molecular mechanisms require further investigation. Their prognostic impact is primarily related to HLH-induced NRM, with patients experiencing HLH showing significantly higher NRM than those without HLH (35.7% vs.  $P = 0.047$ ). Among HLH-related germline susceptibility genes, *LYST* mutations were the most prevalent and had the greatest impact on NRM. Guan YQ's study indicated that patients with germline mutations in CAEBV-T/NK and EBV-HLH had significantly lower 3-year OS compared to those without germline mutations ( $P = 0.0284$ ,  $P = 0.0137$ )<sup>23</sup>.

In recent years, PD-1/PD-L1 inhibitors, with or without histone deacetylase inhibitors, have been increasingly used in salvage therapy for r/r ENKTCL following asparaginase-based regimens, demonstrating certain efficacy<sup>24</sup>. Blocking the PD-1 receptor after HSCT can enhance the graft-versus-leukemia (GVL) effect, but it also promotes T-cell activation and proliferation, potentially leading to fatal aGVHD<sup>25,26</sup>. Bradley et al. have also reported that PD-1 inhibitors can induce severe aGVHD<sup>27</sup>. However, in this cohort, 67.7% of r/r ENKTCL patients received PD-1 inhibitors before allo-HSCT without an increased incidence of post-transplant aGVHD or improved prognosis. This may be related to the immune system reconstitution from high-intensity chemotherapy in the pre-transplant conditioning regimen. Administering PD-1 inhibitors post-transplant might yield different clinical outcomes.

In Ye Z's studies, high p53 protein expression was found in 33.3% (28/84) of NKTCL, high p53 protein expression is significantly associated with more advanced tumour stage ( $p=0.016$ ) and international prognostic index ( $p=0.026$ ) in patients with NKTCL, both P53 protein expression rate and the International Prognostic Index score are statistically significant independent factors for assessing the prognosis of ENKTCL patients<sup>28</sup>. Dysregulation of p53 in ENKTCL may be attributed to mutations, EBNA1-induced p53 degradation, and/or mutations in downstream apoptotic proteins such as FAS<sup>29-31</sup>. Most *TP53* mutations occur in functional domains and are found to have dominant-negative effects<sup>32</sup>. In this study, we also observed that *TP53* mutations were significantly associated with post-transplant relapse. Even in some patients with no P53 protein expression before allo-HSCT, high P53 protein expression emerged after relapse post-transplant<sup>33</sup>. However, for ENKTCL a patients with *TP53* mutations and high p53 protein positive expression, there are currently no clear targeted therapeutic interventions. In contrast, in diseases such as acute myeloid leukemia, PARP inhibitors (e.g., olaparib), either as monotherapy or in combination with decitabine or WEE1 inhibitors, exert anti-tumor effects by affecting the DNA damage response (DDR) pathway and modulating immune function<sup>34-36</sup>. Future studies could explore the application of these therapies in the treatment of ENKTCL.

CNS involvement in ENKTCL is relatively rare, with an incidence ranging from 1.37% to 16.70%<sup>37,38</sup>. Due to the relatively low incidence, large-scale, prospective, randomized controlled clinical studies on extranodal NK/T-cell lymphoma with CNS involvement are challenging to conduct. Additionally, the disease progresses rapidly, has a high mortality rate, and a very poor prognosis<sup>39</sup>. In this cohort, as all included patients had relapsed/refractory extranodal NK/T-cell lymphoma, 6 cases (17.6%) presented with CNS involvement. These patients had a poor prognosis and were prone to disease relapse. Further research targeting this specific subtype is warranted.

## Declarations

### Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Beijing GoBroad Boren Hospital. Written informed consent was obtained from all patients or their legal guardians before their inclusion in the study.

### Consent for publication

Consent for publication has been obtained from all authors.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

### Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

### Acknowledgements

Not applicable.

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## Tables

**Table1. Baseline Characteristics of the Patients and Subgroups**

| <b>Factor</b>                   | <b>level</b> | <b>NKTCL patients(n=34)</b> |
|---------------------------------|--------------|-----------------------------|
| Sex (%)                         | Male         | 19 (55.9)                   |
|                                 | Female       | 15 (44.1)                   |
| Age (median [range])            |              | 30.77 [3.84-57.2]           |
| EMD (%)                         | No           | 1 (2.9)                     |
|                                 | Yes          | 33 (97.1)                   |
| CNSL (%)                        | No           | 28 (82.4)                   |
|                                 | Yes          | 6 (17.6)                    |
| stage (%)                       | III          | 1 (2.9)                     |
|                                 | IV           | 33 (97.1)                   |
| PINK score (%)                  | II           | 7 (20.6)                    |
|                                 | III          | 26 (76.5)                   |
|                                 | IV           | 1 (2.9)                     |
| Chr status (%)                  | Abnormal     | 1 (2.9)                     |
|                                 | Normal       | 33 (97.1)                   |
| Lines (median [IQR])            |              | 3.00 [2.00, 4.00]           |
| Aspartate treatment (%)         | Yes          | 33 (97.1)                   |
| Radiation therapy (%)           | Yes          | 7 (20.6)                    |
| HLH (%)                         | Yes          | 12 (35.3)                   |
| anti-PD-1 (%)                   | No           | 11 (32.4)                   |
|                                 | Yes          | 23 (67.6)                   |
| anti-PD-1 cycles (median [IQR]) |              | 2.00 [0.00, 7.00]           |
| CAEBV (%)                       | Yes          | 11 (32.4)                   |
| Auto-HSCT before (%)            | Yes          | 4 (11.8)                    |
| Second HSCT (%)                 | Yes          | 3 (8.8)                     |
| Relapse before allo-HSCT (%)    | No           | 19 (55.9)                   |
|                                 | Yes          | 15 (44.1)                   |
| Status before HSCT (%)          | CR           | 9 (26.5)                    |
|                                 | Not CR       | 25 (73.5)                   |

|                                             |           |                     |
|---------------------------------------------|-----------|---------------------|
| Duration of illness (months) (median [IQR]) |           | 14.04 [9.12, 28.85] |
| Conditioning regimen (%)                    | BU/FLU    | 26 (76.5)           |
|                                             | TBI/CY    | 2 (5.9)             |
|                                             | TBI/FLU   | 6 (17.6)            |
| Donor type (%)                              | Haplo     | 18 (52.9)           |
|                                             | Unrelated | 16 (47.1)           |
| Blood match (%)                             | Yes       | 16 (47.1)           |

**Table2. Patients and Transplant Characteristics**

| <b>Factor</b>                   | <b>level</b>      | <b>NKTCL patients(n=33)</b> |
|---------------------------------|-------------------|-----------------------------|
| aGVHD (%)                       | Yes               | 12 (36.36)                  |
|                                 | No                | 21 (63.63)                  |
| aGVHD level (%)                 | I                 | 2 (6.06)                    |
|                                 | II                | 3 (9.09)                    |
|                                 | III               | 2 (6.06)                    |
|                                 | IV                | 5 (15.15)                   |
| Acute cutaneous GVHD (%)        | I                 | 2 (6.06)                    |
|                                 | II                | 2 (6.06)                    |
|                                 | III               | 1 (3.03)                    |
| Acute hepatic GVHD (%)          | I                 | 3 (9.09)                    |
| Acute gastrointestinal GVHD (%) | I                 | 1 (3.03)                    |
|                                 | II                | 1 (3.03)                    |
|                                 | III               | 1 (3.03)                    |
|                                 | IV                | 6 (18.18)                   |
| cGVHD (%)                       | Yes               | 9 (27.27)                   |
|                                 | No                | 24 (72.72)                  |
| cGVHD sites (%)                 | Skin (cutaneous)  | 4 (12.12)                   |
|                                 | Eyes (ocular)     | 2 (6.06)                    |
|                                 | Lungs (pulmonary) | 1 (3.03)                    |
|                                 | Mouth (oral)      | 1 (3.03)                    |
|                                 | nail              | 1 (3.03)                    |
| Bacterial infection (%)         | Yes               | 17 (51.51)                  |
| Fungal infection (%)            | Yes               | 12 (36.36)                  |
| Viremia (%)                     | Yes               | 16 (48.48)                  |
| Viral disease (%)               | Yes               | 12 (36.36)                  |
| Viral cystitis (%)              | Yes               | 14 (42.42)                  |
| Other infections (%)            | Yes               | 4 (12.12)                   |

|         |     |            |
|---------|-----|------------|
| EBV (%) | Yes | 13 (39.39) |
| CMV (%) | Yes | 14 (42.42) |

Figures

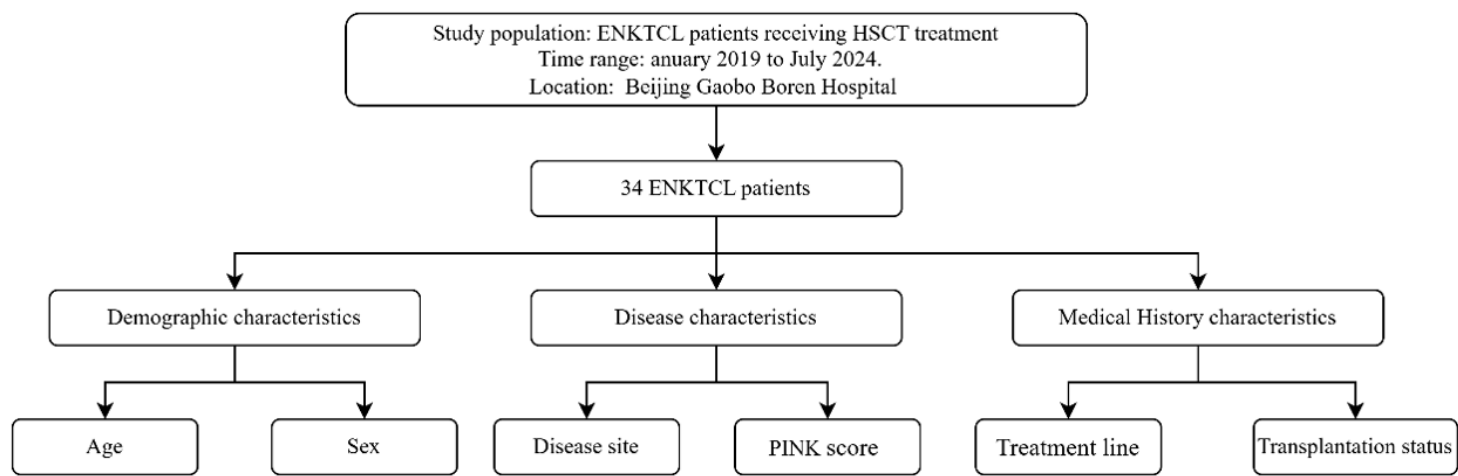


Figure 1

Flowchart of Case Analysis

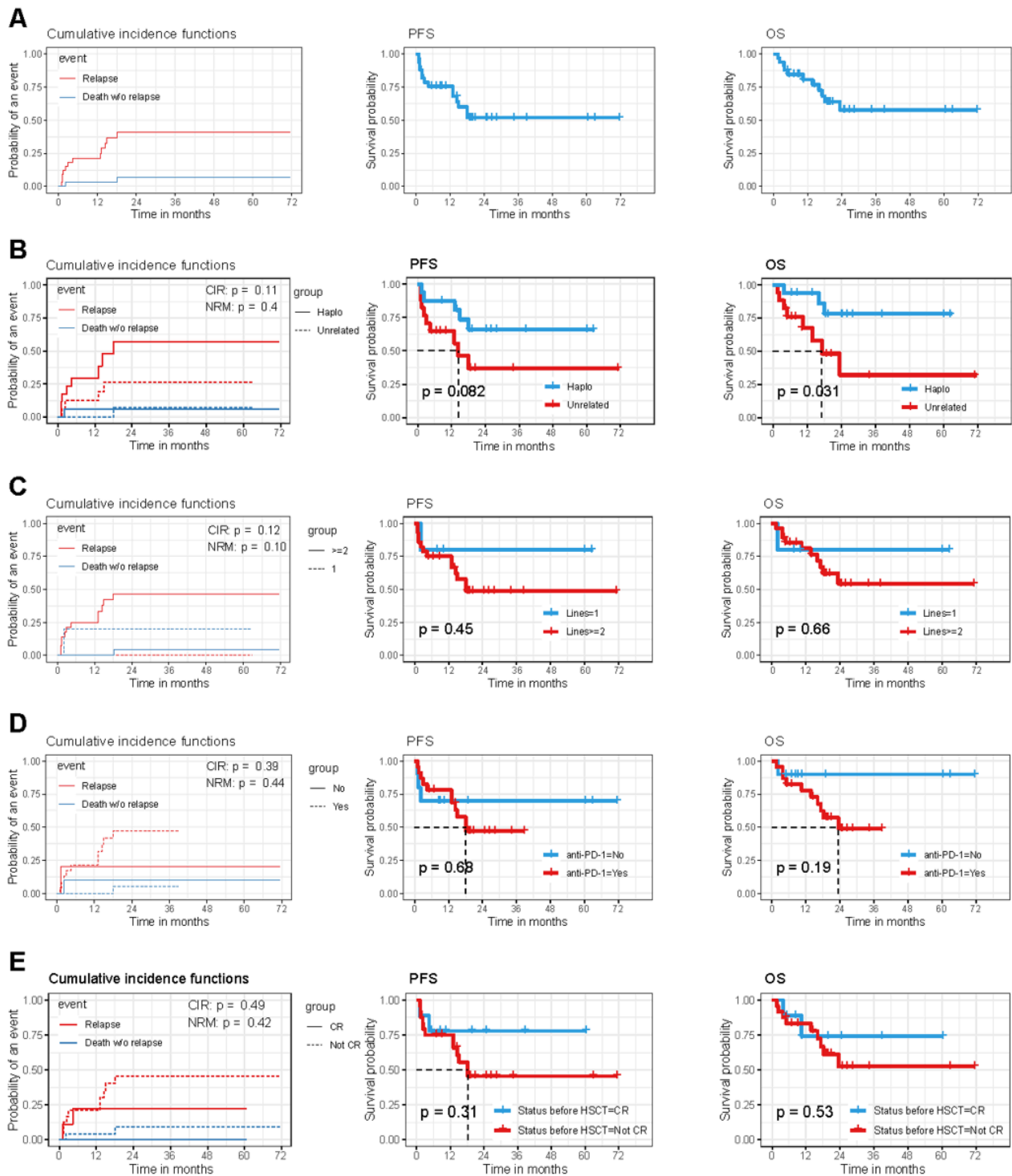


**Figure 2**

(A)Kaplan-Meier survival analysis OS (middle) and PFS (right) in advanced ENKTCL patients.  
 (B)haploidentical vs unrelated transplantation. (C)First-line and second-line allogeneic HSCT. (D)  
 Received or not received anti-PD-1 inhibitor before HSCT. (E)CR vs not CR before allogeneic HSCT

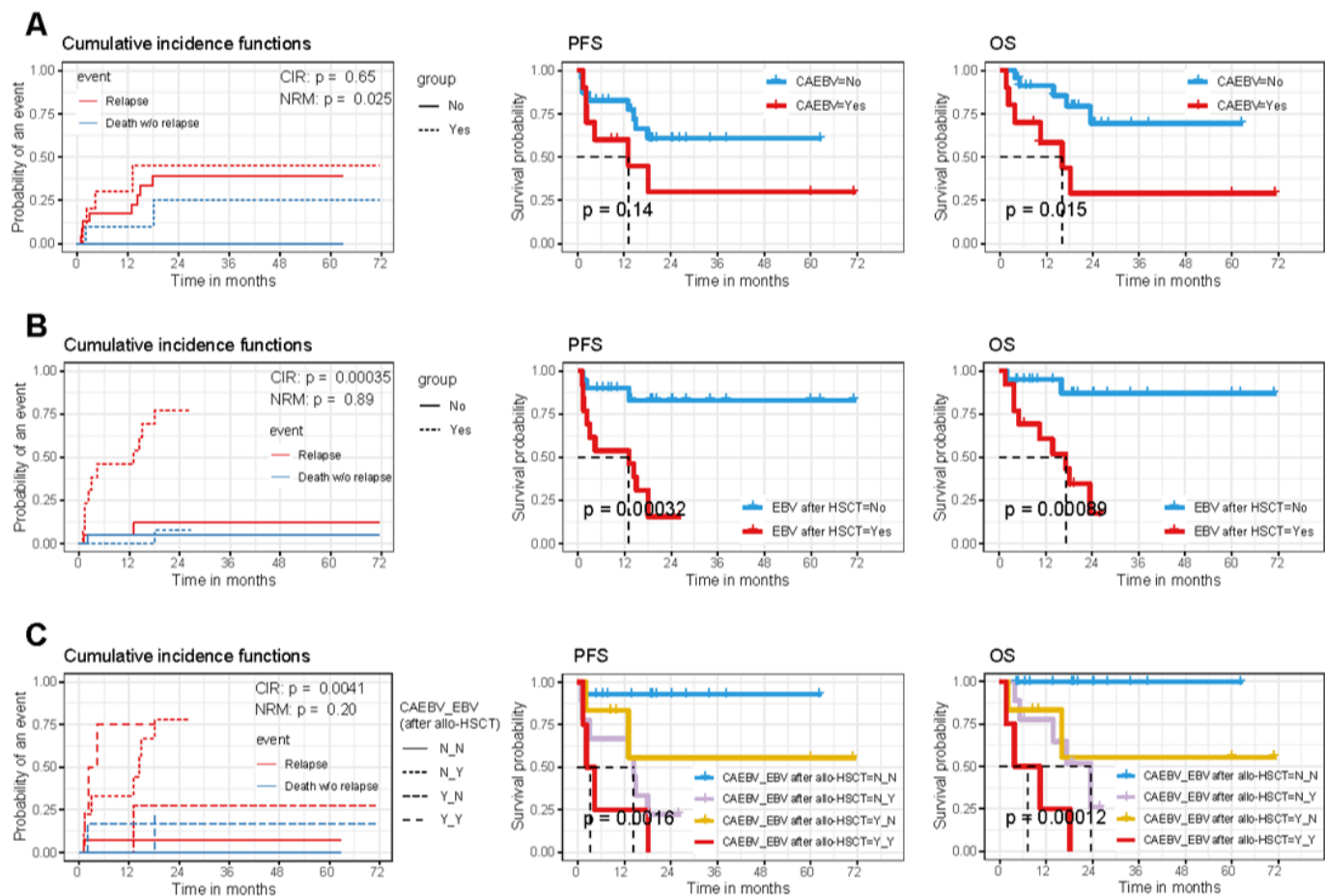


**Figure 3**

(A) Kaplan-Meier survival analysis comparing OS (middle) and PFS (right) in ENKTCL patients with history of CAEBV and without history of CAEBV. (B) EBV positive vs EBV negative after HSCT. (C) Not CAEBV keeping EBV negative after HSCT vs not CAEBV turning EBV positive after HSCT vs CAEBV keeping EBV negative after HSCT vs CAEBV turning EBV positive after HSCT

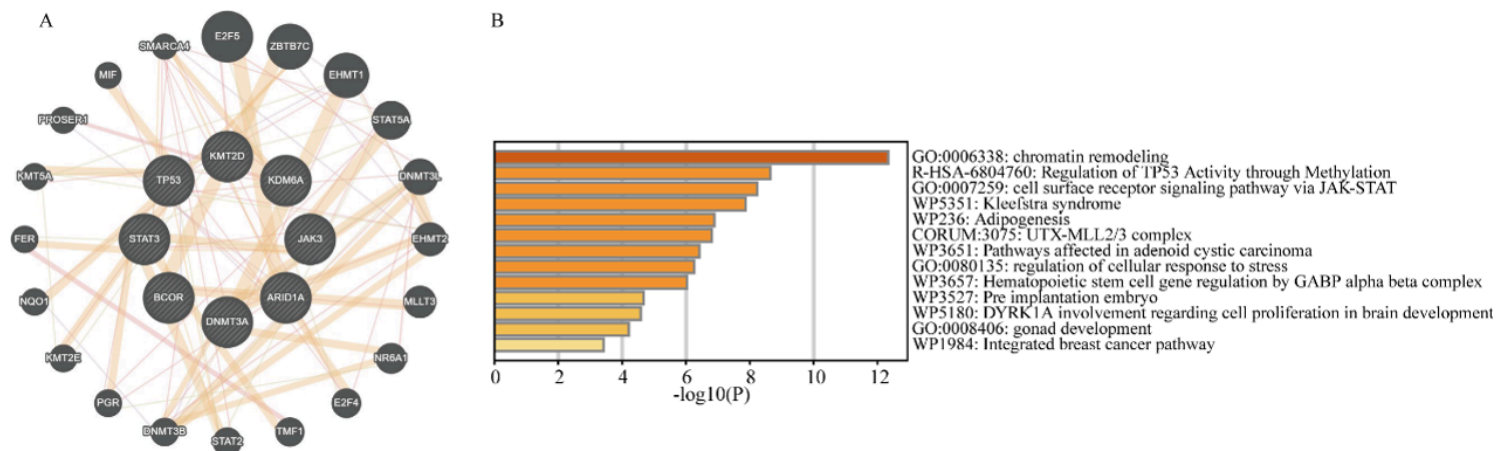


**Figure 4**

Characterization of functional features of high-variability genes in HLH. (A) Construction of the gene regulatory network; (B) Hierarchical clustering analysis.

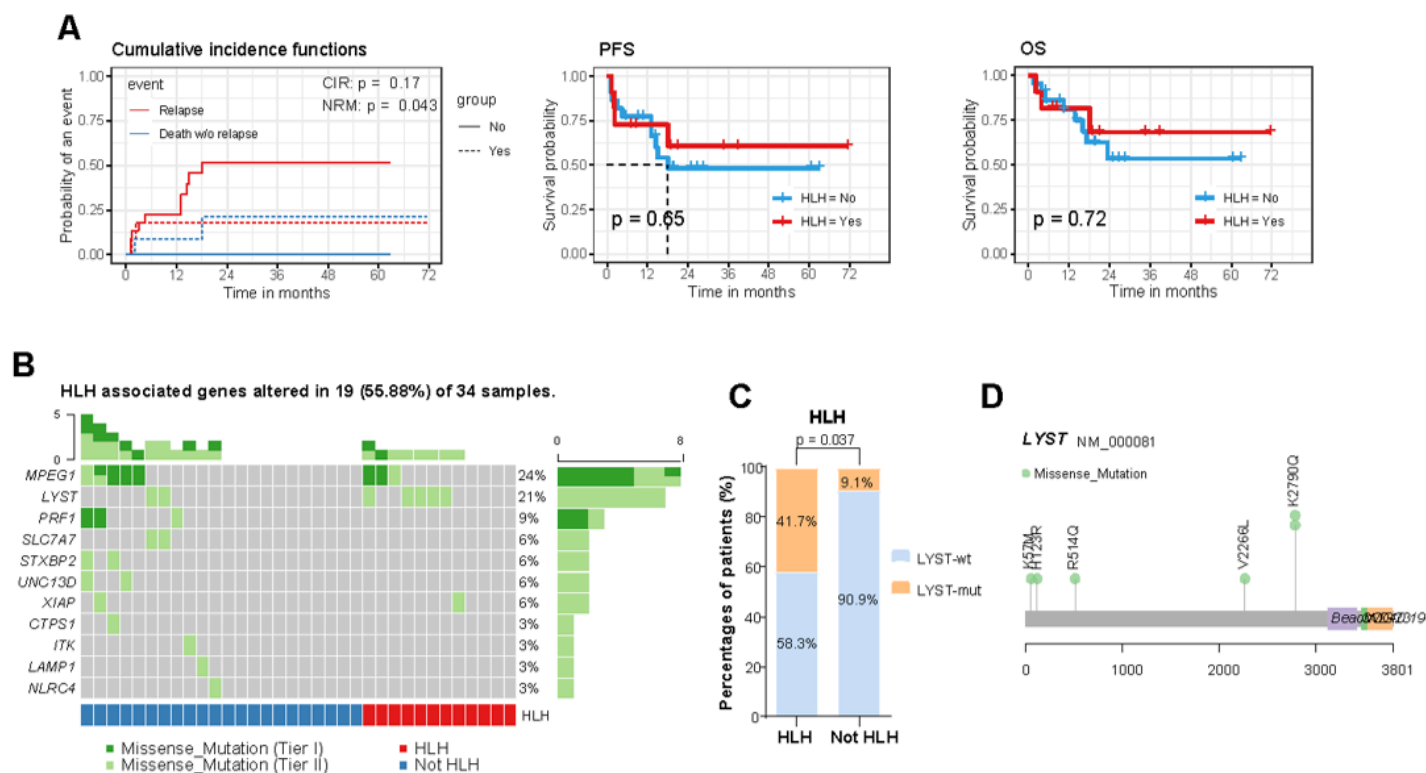


Figure 5

(A) Kaplan-Meier survival analysis comparing OS (middle) and PFS (right) in ENKTCL patients with HLH and without HLH. (B) HLH associated gene germline mutations oncoplot. (C) HLH and not HLH with LYST-wt or LYST-mut. (D) LYST mutation sites.

## Supplementary Files

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