Appendix S1

Materials and methods

Cerebrospinal fluid (CSF) cell count, centrifugation and stains. The cell count in a 10-μl sample of fresh CSF was manually assessed using a Neubauer counting chamber. A 500-μl CSF sample was centrifuged at 800 x g for 5 min at room temperature (RT), and the cells thus obtained were placed on microscope slides and subjected to cytological analysis. The cells were air-dried, stained with Wright-Giemsa stain for 5 min at RT, washed under running water for 30 sec at RT, dried and then observed under a microscope (Axiolab 5 TL; Carl Zeiss AG). Peroxidase and Periodic acid-Schiff stains were used to further identify the cell type. For peroxidase staining, the cells were air-dried, and stained with 3-8 drops of benzidine and an equal volume of hydrogen peroxide for 5 min at RT, and then washed under running water for 30 sec at RT, dried and observed under a microscope. Periodic acid-Schiff staining was performed according to the manufacturer's instructions (Xi'an Google on a surface biotechnology co., Itd, Xi'an, Shaanxi)

FACScan analysis. The expression of monoclonal antibodies labeled with fluorescein and Fc antigen, the size of the cells and the number of particles in the cells were used to identify normal and abnormal cells in the CSF sample. The monoclonal antibodies, matching reagent (washing buffer) and the cell-sorting instrument were all obtained from Beckman Coulter. Analysis was conducted using the Kaluza software 2.1 (Beckman Coulter). After centrifugation of the sample (400 x g for 5 min at RT), the supernatant was discarded and red cell lysate was added to the cell pellet for 3 min. PBS was used to wash the cells at RT until the solution ran clear. Next, 20 μl of monoclonal antibody was reacted with the cells in the dark for 15 min at room temperature. PBS was used to adjust the total volume to 1 ml. All CSF samples were sent to the Hematopathology Laboratory of Shaanxi Provincial People's Hospital (Xi'an, China) for detection analysis. The antibodies used to assess the different lymphoma subtypes were as follows: κ-FITC (C73900), λ-PE (C73901), CD5-PC7 (A21690), CD3-PC5.5 (B49203), CD19-SNv428 (C69244), CD20-APC CD45-KO (C41157), CD4-APC (IM2468), CD8-PE (A07757), CD56-PC5.5 (B49189), CD2-FITC (C41166), CD30-PE (B329928; BioLegend, Inc.), CD16-PE (A7766), CD7-APC (A97050), HLA-DR-APC750 (B42021) and CD38-SNv428 (C69243). Using CD45/SSC as the gate, a total of 10,000 nucleated cells were analyzed.

CSF genetic mutation-detection for hematological diseases. The genetic tests were performed by the Institute of Hematology, Chinese Academy of Medical Sciences (Tianjing, China). The genomic DNA of the CSF was extracted, and the point mutations and short insertion/deletion mutations in the protein-coding regions of the tested genes were analyzed. For next-generation sequencing, the average sequencing depth was 2,000x. The sequencing depth of each detection site was different; at a sequencing depth of 200x, the detection sensitivity was ~10%. At a sequencing depth of 500x, the detection sensitivity was ~5%. At higher sequencing depths (≥1,000x), a lower frequency (1%) of mutations could be detected.

Statistical analysis. Comparison of CSF parameters among the three types of lymphoma was performed. As only one case of primary central nervous system natural killer/T-cell lymphoma was identified, CSF protein, glucose and chloride were detected three times for statistical analysis before chemotherapy. All data are presented as the mean ± SD. Statistical analysis was conducted using one-way ANOVA followed by Bonferroni's post hoc test using SPSS software (version, 17.0; SPSS Inc.) $P<0.05$ was considered to indicate a statistically significant difference.

Figure S1. Flow cytometry analysis of cerebrospinal fluid sample. Using CD45/SSC as the gate, a total of 10,000 nucleated cells were analyzed. Lymphocytes constituted ~95.33% of nucleated cells, with CD3-CD56+ NK cells accounting for ~98.96% of the lymphocyte population. The green population is positive cells and the grey population is negative cells. (A) FSC-H and FSC-A. (B) SSC-H and FSC-A. (C) C45 and SSC-A. (D) CD19 and CD20. (E) λ and κ light chains. (F) CD19 and CD5. (G) CD5 and CD56. (H) λ and κ light chains. (I) CD19 and CD45. (J) CD8 and CD3. (K) CD4 and CD3. (L) CD45 and CD3. (M) CD45 and CD56. (N) FSC-H and FSC-A. (O) SSC-H and FSC-A. (P) CD45 and SSC-A. (Q) CD2 and CD56. (R) CD30 and CD2. (S) CD38 and HLADR. (T) CD5 and CD2. (U) CD16 and CD56. (V) CD57 and CD56. (W) CD7 and CD2.