Appendix S1

METHODS

Paraffin blocks of 13 PCSM-TCL cases were retrieved from the files of Laboratory of Tumor Pathology and Molecular Diagnostics, Szeged, Hungary and the Department of Pathology, County Hospital, Kecskemét, Hungary. All cases fulfilled the previously published diagnostic criteria of PCSM-TCL (1–5). Five hyperplastic tonsils, 1 primary cutaneous T_{FH}-cell lymphoma case, and 2 angioimmunoblastic T-cell lymphoma (AITL) cases were used as controls. This study was conducted in accordance with the Declaration of Helsinki, and was based exclusively on archival material.

Immunohistochemical stainings were performed on heat-treated sections using BCL6/LN22 (1:100), CD3/LN10 (1:500), CD4/4B12 (1:500), CD20/L26 (1:200), CD21/2G9 (1:100), CD30/JCM182 (1:1.000) (each from Leica Biosystems/Novocastra), CXCR5/51505 (1:500; R&D Systems, Minneapolis, MN, USA), PD-1/NAT-105 (1:10; kindly provided by Giovanna Roncador, Monoclonal antibodies Core Unit, Spanish National Cancer Centre, Madrid, Spain) monoclonal antibodies and CXCL13 (1:1.000), ICOS (1:500), PD-1 (1:500; R&D Systems), CXCR5 (1:1.000), MEF2B (1:500) (Atlas Antibodies, Stockholm, Sweden) polyclonal antibodies. For single immunoreactions, Novolink™ DAB (Leica Biosystems) detection system was used.

To further evaluate the phenotype of atypical cells, sequential double immunostainings (CXCR5+PD-1, CXCL13+PD-1, and ICOS+PD-1) were also performed. Briefly, immunoreactions of the first primary antibodies were detected as above. Subsequently, the slides were incubated with PD-1/NAT105C antibody, for which BOND™ Refine Red Polymer (Leica Biosystems/Novocastra, Newcastle, UK) was applied. For better colour contrast, the alkaline phosphatase reaction was developed with Permanent Red Substrate-Chromogen (DAKO, Glostrup, Denmark). For negative control stainings, each primary antibody was substituted with relevant non-immune serum.

PCR-based T-cell receptor gamma (TCRγ) clonality analysis was performed as decribed previously (8, 9). DNA samples extracted both from whole tissue sections (in 13 cases) and microdissected PD-1+ T-cell clusters collected using ultraviolet (UV)-Laser Microbeam Pressure Catapulting System (PALM Microlaser Technologies, Bernried, Germany) (in 10 cases) were used.