Supplementary methods

A.

Flow cytometry

Flow cytometric immunophenotyping was performed on LN aspirates, BM and PB at T0 and at T1. LN aspirates were obtained with 22 G needles, collected into tubes containing RPMI 1640 (Sigma Aldrich), refrigerated and processed within 24 h of sampling. BM aspirates were obtained with 16 or 18 G Illinois needles from the iliac crest. BM and PB samples were placed in tubes containing ethylene diamine tetraacetic acid (EDTA) and treated with erythrocyte lysis buffer containing 8% ammonium chloride. Erythrocyte lysis of LN samples was not necessary, because haemodilution from this site was minimal. Multi-colour labelling using CD45 was used to exclude from the analysis debris and erythrocytes. A panel of antibodies against the following markers was used to label cells using a multi-colour approach (30): CD45 conjugated to allophycocyanin (CD45-APC; AbD Serotec), CD3 conjugated to fluorescein isothiocyanate (CD3-FITC; AbD Serotec), CD5-FITC (AbD Serotec), CD4-FITC (AbD Serotec), CD8 conjugated to phycoerythrin (CD8-PE; AbD Serotec), CD21-PE (AbD Serotec), CD79a-PE (Dako) and CD34-PE (AbD Serotec). The extent of PB and BM infiltration by large B-cells was evaluated by FC and reported as the percentage of large (i.e. FSC > 400, CD21) positive cells out of the total CD45 positive cells (leucocytes and their precursors).

B.

Pathology

Three μm thickness serial sections mounted on Superfrost® Plus slides were used for immunohistochemistry analysis. A panel of four antibodies was used, including anti-CD3 (Dako), anti-CD5 (Dako), anti-CD79acy (Dako) and anti-CD20 (Thermo Fisher Scientific). Immunohistochemical staining was performed using the Ventana Benchmark XT (Roche).

C.

Detection of minimal residual disease by polymerase chain reaction for antigen receptor rearrangements

Briefly, 40 ng of genomic DNA was amplified by 3 sets of primers. Cη primers were used as an internal control for PCR, whereas IgH primers (IgH-major and IgH-minor) and TCRγ primers were used to amplify the rearranged fragments of these antigen receptor genes (33). PCR mixture contained 400 nM of each primer, 250 μM dNTP, 2 mM MgCl2, 5X GoTaq® Flexi Buffer and 2

units GoTaq® DNA polymerase (Promega Corp.). Amplification conditions employed a two-step, modified touchdown protocol to increase specificity of reactions (36). Briefly, an initial activation step of 94 °C for 2 min was followed by 10 cycles of 94°C for 30 sec, 65°C for 40 sec and 72°C for 30 sec with a decrease of annealing temperature of 0.5°C at every cycle; then 27 cycles of 94°C for 30 sec, 60°C for 40 sec and 72°C for 30 sec. A final extension of 72°C for 5 min was performed. All PCR reactions were run in duplicate. PCR products were analysed by 3% agarose gel electrophoresis. The gels were then stained with SYBR® Safe DNA Gel Stain (Invitrogen, Life Technologies) and visualized with a UV transilluminator (GelDoc 2000, Bio-Rad). Specific amplicon size was expected to occur within a target range of about 90–130 bp. A reaction was considered positive, if one dominant and discrete band was present. A reaction was considered negative, if no bands, a diffuse smears, or a ladder of bands was observed. The PCR product was denatured at high temperature (95°C for 4 min) and then immediately renatured at low temperature (4°C for 1 h) in case of ambiguous results. The sensitivity was at least 1%.