Clonally related duodenal-type follicular lymphoma and *in situ* follicular neoplasia

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doi:10.3324/haematol.2019.226142

Supplement to:

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Material and methods

Histology, immunohistochemistry and fluorescence in situ hybridization

All specimens were fixed in 4 % buffered formalin, and embedded in paraffin for histopathological analysis. Three µm thick sections were stained with hematoxylin and eosin (H&E). Immunohistochemical examination was performed on an automated immunostainer (Ventana Benchmark XT, Ventana, Tucson, AZ, USA). Antibodies were used against CD3 (1:100, DCS, Hamburg, Germany), CD5 (1:50, Medac, Wedel, Germany), CD10 (1:30, Novocastra, Berlin, Germany), CD20 (1:500, DAKO, Hamburg, Germany), CD23 (1:30, Novocastra), BCL2 (1:20, DCS), BCL6 (1:25, Zytomed, Berlin, Germany), Ki67 (1:200, DAKO), IgD (1:500, DAKO), and Cyclin D1 (1:40, DCS). Images were acquired with a Zeiss Axio Imager A1 microscope (Carl Zeiss Microscopy, Jena, Germany) equipped with a Jenoptik ProgRes C10 plus camera (Jenoptik, Jena, Germany) and software ImageAccess, Version 6 Release 07.4 (Imagic Bildverarbeitungs AG, Glattbrugg, Switzerland), as well as finished with Adobe Photoshop CS4 (Version 11.0, Adobe Systems, San José, CA, USA). Objectives Plan-Neofluar used were: x1.25/0.03, x2.5/0.075, x5/0.13, x10/0.30, x20/0.50, and x40/0.75. Fluorescence *in situ* hybridization (FISH) analysis was performed on formalin-fixed, paraffin-embedded tissue, using a break-apart probe for the BCL2 gene locus (Vysis LSI BCL2 Dual Color Break Apart Rearrangement Probe, Abbott Molecular, Wiesbaden, Germany). FISH images were acquired with a x100/1.40 oil immersion objective in a Zeiss Axio fluorescence microscope (Zeiss) equipped with the appropriate filters sets and were documented and processed using the AxioVision Rel. 4.8 system (Zeiss).

DNA extraction and clonality analysis

DNA was extracted from 5 µm paraffin sections after macrodissection, dewaxing and proteinase K digestion applying the QIAamp DNA FFPE Tissue Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. PCR for immunoglobulin heavy chain (IGH) gene rearrangements (FR1,

FR2 and FR3 primer sets) was performed as previously described.[1] For testing of IG kappa rearrangements, modified primer concentrations were used for individual primers: 20 µM of VK2f, VK4 and VK5; 50 µM of JK1-4, JK5 and Kde. Amplification conditions were carried out with an initial denaturation step of 95°C (7 minutes), 40 cycles (95°C 30 seconds, 60°C 45 seconds, 72°C 45 seconds) and a final step of 4 minutes. The JH primer was modified with D4 fluorescent dyes (Sigma-Aldrich, St. Louis, MO, USA). For Genescan analysis 0.5 µl of the PCR products were mixed with sample loading solution containing 0.24 µl DNA Size Standard 400 (Beckman Coulter, Brea, CA, USA). The products were separated by capillary electrophoresis on the GenomeLab GeXP Genetic Analysis System and analyzed by the GenomeLab GeXP software 10.2 (Beckman Coulter, Brea, CA, USA).

Next generation sequencing

NGS analysis was done on the Ion Torrent PGM from Thermo Fisher Scientific (Schwerte, Germany). NGS libraries were amplified using 4 primer pools of an Ion AmpliSeq custom panel covering 22 genes that have been shown to be frequently mutated in FL. The panel covered > 98 % of all exons of *TNFRSF14, KMT2D* (*MLL2*), *FOXO1, EP300, MEF2B, HIST1H1B-E, GNA13, TNFAIP3, STAT6, and SOCS1* as well as hotspot regions of *EZH2* (exon 16), *CREBBP* (exons 24-28 and 30), *XPO1* (exon 15), *NOTCH1* (exon 26,27,34), *NOTCH2* (exon 34), *MYD88* (exon 5), *MAP2K1* (exon 2, 3), *NOTCH2 3`UTR*, and *NOTCH1 3`UTR*. The custom panel was designed using the Ion AmpliSeq Designer from Thermo Fisher Scientific (version 3.4). For validation of the NGS results, the detected mutations were reanalyzed as single amplicons using a targeted resequencing approach on the Ion GeneStudio S5 Prime System from Thermo Fisher Scientific. Amplicons were prepared using the Ion Amplicon Library Preparation Fusion Method protocol from Thermo Fisher Scientific.

The study was approved by the local ethics committee (096/2016BO2).

Sequencing data are deposited in the European Nucleotide Archive under the accession number PRJEB31658.

References:

1. van Dongen JJ, Langerak AW, Bruggemann M, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia. 2003;17(12):2257-2317.