SUPPLEMENTARY APPENDIX

Inferior survival in high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements is not associated with *MYC/IG* gene rearrangements

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Supplementary Appendix. Case Identification and Case Criteria

Fifty-seven cases in this study were obtained during the time period (after July 6, 2012) when all B-cell lymphomas (BCLs) with either large-cell or high-grade histologic features were analyzed with fluorescence in situ hybridization (FISH) to exclude high-grade BCL with *MYC* and *BCL2* and/or *BCL6* rearrangements (aka double-hit/triple-hit lymphoma, or DH/THL). For specimens accessioned before that date (n=43), FISH to identify DH/THL was performed selectively at the discretion of the original pathologist. After this date, some DH/THLs may have been missed because in approximately 10% of cases, FISH could not be performed because of insufficient tissue or test failure, but specimens were not excluded from testing because of pathologist/hematologist preference. We excluded patients who 1) were younger than 18 years and 2) had primary central nervous system lymphoma, posttransplant lymphoproliferative disorder, human immunodeficiency virus–related lymphoma, primary cutaneous large BCL, or mediastinal large BCL. We also excluded patients whose lymphomas had an *MYC* rearrangement without *BCL2* or *BCL6* rearrangement (called *single-hit lymphoma*), *MYC* amplification, trisomy 8, or *MYC* expression by immunohistochemistry without documented *MYC* rearrangement.

We abstracted from health records the patient age, sex, timing of diagnosis (ie, de novo diagnosis, transformation of previously diagnosed low-grade BCL, or relapse of previously diagnosed BCL with large-cell or high-grade morphologic characteristics), and clinical outcomes.

High-grade histologic features were assigned as monotonous to slightly pleomorphic cells with medium-sized nuclei (nuclear diameter

equivalent to the nuclear diameter of a macrophage or an endothelial cell), round to slightly irregular nuclear contours, and finely dispersed chromatin. Large-cell histologic features were assigned as monotonous to pleomorphic cells with large nuclei (nuclear diameter greater than the nuclear diameter of a macrophage or an endothelial cell), round to irregular nuclear contours, variably sized nucleoli, and varying amounts of cytoplasm.

For images, we used an Olympus BX51 microscope with a 40× UPlanApo oil objective (Olympus Corp) with a numerical aperture of 1.0. The camera was an Olympus DP73. Olympus cellSens Standard software was used for image acquisition. The images began as TIFF raw files. The black, white, and midpoint levels were minimally adjusted referring to the digital image histograms using Photoshop (PC 2015; Adobe Systems Inc) to render final digital photographs that matched the original microscopic image.

Cell of origin was determined according to the Hans classifier[1] by paraffin section immunohistochemistry using antibodies directed against CD10, BCL6, and MUM1, with a cutoff value of 30% for all 3 antigens. Paraffin section immunohistochemistry using antibodies directed against *MYC* and *BCL2* was performed in tandem in 37 cases, with cutoff values of 40% for *MYC* and 50% for *BCL2*. Cases were considered to be *double expressers* when expression of both *MYC* and *BCL2* exceeded their respective cutoff values.[2]

The following interphase FISH probes were used: break-apart probes for *MYC*, *BCL2*, and *BCL6* (Abbott Molecular, Inc, Des Plaines, Illinois), dual-fusion probes for *IGH*/*MYC* and *IGH*/*BCL2* (Abbott

Molecular, Inc), and dual-fusion probes for *IGL*/*MYC* and *IGK*/*MYC* (homebrew).

Supplementary References

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