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 classifier1 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
