Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Rawstron AC, Bennett FL, O'Connor SJM, et al. Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. N Engl J Med 2008;359:575-83.

Supplement

Cases with a clonal B-cell excess (kappa:lambda ratio < 1:1 or > 2.1:1) were assessed with an extended panel as follows. For cases with a lymphocytosis, $5x10^5$ cells were incubated for 30 minutes with 5µl each of anti-CD19 Phycoerythrin (PE)-Cyanin5.5 (Cy5.5)*, and FITC/PE: CD3/CD3 (control), CD20‡/CD5, CD10†/CD38†, kappa/lambda, FMC7ʃ/CD22‡, CD11a/CD23, IgM†/IgD†, and IgG†/CD79b‡. For cases with a normal blood count, $5x10^5$ cells were incubated for 30 minutes with 5µl each of anti-CD19 Phycoerythrin (PE)-Cyanin5.5 (Cy5.5)*, anti-CD5 Allophyocyanin (APC)†, and FITC/PE: CD20‡/CD79b‡, FMC7ʃ/CD23. Antibody suppliers were: * Invitrogen, Paisley, UK; † BD Biosciences, Oxford, UK; ‡ Beckman Coulter, High Wycombe, UK; ʃ Chemicon Europe Ltd., Chandlers Ford, UK; all other reagents were prepared in-house.

Cells were washed, acquired using a FACSort cytometer and analyzed using CELLQuest software (BD Biosciences) as reported previously [5].