SUPPLEMENTARY APPENDIX

Plasma cell proliferative index predicts outcome in immunoglobulin light chain amyloidosis treated with stem cell transplantation

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Supplementary Material

Methods

From May 2012 onwards, the BrdU method was replaced by the DNA content measurement using flow cytometry. Briefly, the bone marrow specimen is spun down and the pellet is lysed using 14 mL of ACK lysing buffer (Thermo Fisher Scientific), followed by two washes with PBS. The cell pellet is then re-suspended in 0.2% BSA/PBS with Azide (BD Pharmingen) and stained with the following antibodies: CD138 PerCPcy5.5, CD19 PE-cy7, CD38 FITC, CD45 APC-H7 (all BD Biosciences) for 15 minutes. Following the wash in Caltag A reagent (Thermo Fisher Scientific), the pellet is re-suspended in Caltag B reagent for permeabilization. Antibodies for cytoplasmic staining are added (Kappa APC and Lambda PE – both from Dako North America Inc.), and the specimen is incubated for 20 minutes. This is followed by the wash step and the incubation in the 1000 units/mL RNAse in PBS (Worthington Biochemical Corporation). 21.4 µM working dilution of DAPI (Life Technologies) is added to the cell suspension and incubated at 4°C for 30 minutes. Cell pellet is then re-suspended in 500 µL of PBS. The flow cytometry FCS files are obtained on BD FACSCanto™ II instruments (500,000 events per specimen). The files are analyzed using Kaluza software (Beckman Coulter). Initial broad gates are set on $CD138^{+}CD38^{+}$ events. The clonal (abnormal) plasma cells are separated from the normal plasma cells using differential expression of CD38, CD19, CD45, kappa, and lambda. DAPI staining on polyclonal plasma cells is used to determine ploidy. S-phase of clonal plasma cells is calculated by manually gating on G_0G_1 and G_2M peaks and dividing the number of events in the S-window by the total number of abnormal plasma cells. Minimum 300 abnormal plasma cells were needed to reliably calculate S-phase.