Supplementary data document

Materials and methods

Patients' data was analyzed using the Registry of Monoclonal Gammopathies (RMG) of the Czech Myeloma Group. All patients met the diagnostic criteria for symptomatic MM (1) and were treated by best physician decision according to institutional guidelines. All participants provided written informed consent approved by institutional ethics boards in accordance with the latest Helsinki declaration.

Imaging methods and tissue sampling

Patients were evaluated for the presence of EMM by modern imaging methods whole body low-dose computed tomography (LD-CT), focused/whole body magnetic resonance imaging (MRI/WB-MRI), or positron-emission tomography/computed tomography (PET/CT).

Plasmacytoma tissue was obtained for the evaluation of TPCs exclusively when clinically necessary for other reasons and deemed safe for the patient, either through surgical sampling or CT-guided biopsy.

Cytogenetics studies on bone marrow plasma cells and plasmacytoma tissue plasma cells

The I-FISH analyses of commonly found aberrations were performed on separated BMPCs or imprints of plasmacytoma tissue plasma cells (TPCs) (2). At the time of MM diagnosis, BMPCs were assessed in NDMM patients without plasmacytoma, as well as BMPCs or TPCs of primary EMM patients. For patients with secondary EMM, BMPCs or TPCs were evaluated at the time of plasmacytoma development.

The probes were hybridized according to the instructions of manufacturers. For detection of IGH, t(4;14), t(11;14), t(14;16), TP53, 13q14, 5p15/9q22/15q22 hyperdiploidy (HDR) and 1p32/1q21 aberrations, the following probes were used: XL IGH Break Apart Probe, XL t(4;14) FGFR3/IGH Translocation/Dual Fusion Probe, XL t(11;14) MYEOV/IGH Translocation/Dual Fusion Probe, XL t(14;16) IGH/MAF Translocation/Dual Fusion Probe, XL TP53/17cen Deletion Probe, XL RB1/DLEU/LAMP Deletion Probe, XL 5p15/9q22/15q22 Hyperdiploidy, XL CDKN2C/CKS1B Enumeration Probe (MetaSystems GmbH, Altlussheim, Germany). Hybridization signals in at least 100 nuclei were scored on a Nikon Eclipse Niu fluorescence microscope at magnification 1000x (Nikon Instruments Europe BV, Amsterdam, Netherlands). For the IGH, t(4;14), t(11;14), t(14;16), hyperdiploidy, del17p, 13q14 and 1p32/1q21-detecting probes, the threshold for the positivity was set to 5%. FISH as a single-cell analysis enables identification of nuclei with different number of signals in one patient and therefore individual clones can be detected. Signals were documented using LUCIA Cytogenetics software (Laboratory Imaging s.r.o, Prague, the Czech Republic).

Response Assessment and Survival Intervals

Treatment response was assessed according to the current International Myeloma Working Group (IMWG) criteria. Survival intervals (progression-free survival, PFS and overall survival, OS) were assessed from the NDMM diagnosis in control NDMM patients without EMM, as well as in primary EMM patients. In secondary EMM patients, survival intervals were assessed from the time of plasmacytoma onset. Survival intervals were assessed to the event (PFS/OS) or patient's last follow-up.

Statistics

Descriptive statistical analysis respected the type of data and the distribution of values. Continuous parameters were described using the mean with standard deviation (SD) and the median with 5^{th} and 95^{th} percentile, together with the total number of non-missing observations. A summary of categorical parameters was done using absolute and relative frequencies. Relative frequencies were calculated based on the number of patients in the relevant subgroup. In accordance with data type (categorical x continuous), Pearson Chi-Square (resp. Fisher's exact test in case of non-meeting criteria) or Mann-Whitney U test (also known as Wilcoxon rank sum test) was used to examine the association between selected variables. Survival curves between the two categories were compared via Wald Test. Cox univariate models (in case of time-event type of response variables) was used to assess the appropriate relationship.

All statistical tests were performed at a significance level of $\alpha=0.05$ (all tests two-sided). Statistical analysis was performed using IBM SPSS, Statistics (version 29.0) and R software (version 4.1.2 and 4.2.3).

1) Rajkumar SV. Multiple myeloma: 2022 update on diagnosis, risk stratification, and management. Am J Hematol. 2022; 97(8):1086-1107. doi: 10.1002/ajh.26590. Epub 2022 May 23.

2) Besse L, Sedlarikova L, Greslikova H, Kupska R, Almasi M, Penka M et al. Cytogenetics in multiple myeloma patients progressing into extramedullary disease. Eur. J. Haematol. 2016; 97:93-100