a schedule with IMiDs pre-treatment may improve the depth and duration of response of MM patients both as upfront therapy and in the relapsed/refractory setting.

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FLOW CYTOMETRY REMISSION BY IG LIGHT CHAINS RATIO ON PLASMA CELL SUBPOPULATIONS PREDICTS PROGRESSION-FREE SURVIVAL IN ELDERLY MULTIPLE MYELOMA PATIENTS TREATED WITH VMP

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Flow Cytometry (FC) is a powerful technique in both diagnosis and Minimal Residual Disease (MRD) identification in multiple myeloma (MM) patients. However, most of data were obtained into controlled clinical trials and if MRD assessment by FC is routinely applicable to clinical practice is so far under consideration. From January 2011 to January 2015, 37 consecutive MM patients, median age 73 years (57-83) treated with a program containing Bortezomib, Melphalan and Prednisone (VMP) for a total of 9 courses entered the study. Plasma-cells (PC) FC characterization was performed on bone marrow (BM) samples using a 6-colors panel of antibodies (Fitc/PE/PerCP/PE-Cy7/APC/APC-Cy7) evaluating the cy-Ig lambda/cy-Ig kappa light chains expression on CD19 CD38 CD56/CD117 CD45 surface markers, for a total of 19 kappa/lambda ratio evaluations. BM B lymphocytes were used as internal control for cy-Ig light chains expression. The analysis was performed at diagnosis and after 30-60 days from the end of the last course of treatment for flow-MRD assessment. Patients were considered flow-MRD negative when pathological PC were undetected in the BM samples at the FC sensitivity limit of 10-5 cells. A mean of 15420 PC was analyzed for MRD assessment, allowing a maximum sensitivity of detection of 0.0001% in all cases. At the end of VMP, 18 patients (48%) achieved a standard CR, 10 a VGPR, 3 a PR and the remaining 6 a SD/PD (three of these did not complete the treatment for progression of disease). Among 18 patients in CR, 12 (67%) were flow MRD-negative and 6 (33%) flow MRD-positive. Overall, MRD negativity by FC assessment is the better marker of clinical outcome in terms of PFS (2y-PFS: 90% vs 52% for flow MRD negative and positive, respectively; P=0.003). Moreover, among patients in standard CR, 2y-PFS of flow-MRD negative patients was significantly better than MRD positive (P=0.012). As previously documented on MM patient after autologous hematopoietic stem cell transplant, FC remission through cy-Ig light ratio on PC subpopulations is a low-cost, sensitive and applicable MRD assay, a powerful tool in treatment response evaluation and a crucial marker of outcome in MM.

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A NOVEL IN-HOUSE DEEP-SEQUENCING METHOD FOR NON-INVASIVE DISEASE MONITORING IN MULTIPLE MYELOMA PATIENTS

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Background: Novel and more effective treatment strategies have significantly prolonged multiple myeloma (MM) survival and raised interest in the depth of response. This implies the need of highly sensitive assays such as the determination of minimal residual disease (MRD) by multiparametric flow cytometry (MFC) and next generation sequencing (NGS) of immunoglobulin (IGH) gene rearrangements. Ongoing studies are examining circulating cell-free tumor DNA (cfDNA) as a sensitive measure of small amounts of residual cells. In the present study, we describe and analytically validate a simplified in-house deep-sequencing method to identify and quantify residual tumor burden in MM patients from plasma samples. Methods: We retrospectively analyzed 25 MM paired tumor (n=25) and plasma samples (n=48) obtained at diagnosis and at specified time points during treatment. Genomic DNA (gDNA) and cfDNA were extracted from selected CD138+ plasma cells (PC) and from plasma (Qiagen). IGH gene rearrangements were amplified, quality assessed (Agilent hsDNA kit) and sequenced on Ion Torrent PGM. Raw reads were filtered and aligned using IMGT germline database and aggregated into clonotypes. Post-processing analyses were performed using VDJtools and customized R scripts. Results: Our sequencing method successfully identified a IGH MM clonotype in 88% of tumor samples (22/25), subsequently detected in plasma of all 22 cases (median 4.7% of total filtered reads). Levels of the IGH clonotype in cfDNA distinguished between groups of patients with different prognosis: patients with levels >4.7% prior to therapy, had significantly shorter PFS than patients with levels<4.7% (median 268 vs 990 days; HR=3.532, P=0.04827, Log-rank). IGH cfDNA levels over the median were significantly associated with higher risk of disease progression (HR=7.9, P=0.0384). cfDNA levels reflected the number of PC enumerated by MFC (r=0.7249, P<0.0001, Pearson's correlation test). Accordingly, TTP was significantly longer (P<0.0001) for patients displaying frequencies lower than 10-5 (TTP 9±3 months, mean±SD, for frequencies>10-5 vs 15±5 months for frequencies=10-5 vs 37±4 months for frequencies<10-5). Those patients are in CR and characterized by PC frequencies <10-5 by MFC, and are therefore defined as MRD-negative. Conclusions: Results of this study support the clinical applicability of quantifying tumor levels by our in-house deep-sequencing of IGH gene rearrangements in plasma of MM patients.

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PTOLOMAIC PROJECT: A LOOK ON MYELOMA BEYOND THE DISEASE

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Aim: To outline a picture of the world of patients (pts) with myeloma and their caregivers (cgs) trying to identify their unmet needs and to explore how they face this distressing experience inside and outside the hospital. Methods: Pts and cgs filled in some questionnaires regarding their satisfaction, at the time of enrolment and after 6 months (t0 and t1). Pts were also evaluated for their fitness with: non-instrumental and Instrumental Activities of Daily Living (ADL and IADL), Study of Osteoporotic Fractures (SOF), Geriatric Depression Scale (GDS) and Cumulative Illness Rating Scale (CIRS). Cgs were evaluated with Caregiver Burden Scale (CBS). One hundred seventy two pts were included in the study,136 of which (79.1%) had a cgs. Results: Features of pts and cgs at t0 are reported in Table1. A quarter of pts were at disease onset (≤1year), 28% were on treatment. Basing on the questionnaires,in case of emergency about half of pts ask for help firstly to cgs, while most of cgs ask general practitioner. Median (mdn) satisfaction level for global hospital service was high in both groups. Mdn cgs burden scale was 10. Forty-six pts (27%) were defined depressed (GDS≥2). Most of pts reached showed a high score for ADL and IADL and IADL was higher in males vs females (85% vs 70%,p=0.025). Eighty-nine pts (52%) were defined frail (at least one SOF criteria) and these were older than the others (67 vs 63 years,p=0.020). Mdn score for psychiatric pathologies, severity and comorbidity were 2, 0.07 and 0. No difference in ADL, IADL, SOF scales and in severity and comorbidity indexes were found according to the duration and the phase of disease at t0 (all p>0.1). After 6 months,135 pts (78.5%) were evaluated again. Percentage of frail pts decreased among pts with a duration of disease >1year (t0:48% t1:33%,p=0.009) and among pts treatment-free at t0 (t0:47% t1:29%,p=0.004). Mdn severity and comorbidity indexes decreased (p<0.001) with no difference according to the duration of disease and to the disease phase at t0 (all p>0.4). Mdn score for psychiatric pathologies decreased in the whole cohort (t0:2 t1:0,p=0.012), in pts at disease onset (t0:2 t1:0,p=0.006) and in pts treatment-free at t0 (t0:1 t1:0,p=0.043). ADL and IADL did not change (p=0.5 and p=0.8). Conclusions: When dealing with a complex disease as myeloma, it is important to extent the health professional intervention to an accurate evaluation of the emotional and practical difficulties of people (pts and cgs) who have to face such a distressing experience.