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651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Correlation of Flow Cytometric BCL2 and MCL1 Expression with Cytogenetic Characteristics and Outcome in Multiple Myeloma

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Introduction:
Clonal plasma cells mediate apoptosis resistance through increased expression of anti-apoptotic proteins which increase survival and mediate resistance to intrinsic mitochondrial pathway. The key anti-apoptotic members are BCL-2, MCL-1, BCL-XL, BCL-B and BCL-W. High BCL2 expression or t(11;14) is shown to be predictive of response to Venetoclax in Multiple Myeloma (MM). Venetoclax may only be effective in a subset of patients, namely those who have relatively high BCL2 and low MCL1. Resistance to Venetoclax can be mediated by mutation in BCL2 or gain in copy number of MCL1, which maps to 1q21. The relationship between cytogenetic abnormalities and expression of BCL2 and MCL1 is not clear except for an occasional study which has shown MM with *CCDN1* translocation has high BCL2 and low MCL1 expression and MM with *MMSET* and *MAF* translocation has high MCL1 and low BCL2 expression. Also, previous studies have used either immunohistochemistry or real time quantitative PCR for studying BCL2 and MCL1 expression, both of which are met with challenges in interpretation. We aimed to correlate the intracellular expression of BCL2 and MCL1 by flow-cytometry (FCM) with cytogenetic characteristics and outcome.
Methodology:
FCM was performed in bone-marrow of 143 consecutive MM cases with ≥10% plasma cells (PCs) and 20 bone marrow samples with non-clonal PCs by lyse-stain-wash method followed by intracellular staining of BCL2 (Alexa Fluor 647 conjugated anti-human BCL2 antibody) and MCL-1 (PE-conjugated anti-human MCL1 antibody). The sequential gating strategy used in the analysis of intracellular BCL2 and MCL1 expression in PCs and T lymphocytes is shown in Figure 1. The percentage of PCs positive for BCL2 and MCL1 compared to the tube without these antibodies [Fluorescence Minus One (FMO) cutoff threshold], the mean fluorescence intensity (MFI) expression of BCL2 and MCL1 in PCs and T cells, and the ratio of expression of BCL2 and MCL1 on PCs and T cells (BCL2 $^{PC/T}$, MCL1 $^{PC/T}$), MFI BCL-2 ^{PC}/MFI MCL1 ^{PC} were studied and further correlated with 5-7 panel FISH cytogenetics by using Kruskal Wallis test or Mann-Whitney U test. The overall survival/OS and progression-free survival/PFS was calculated using Kaplan-Meir Survival Curve analysis.
Result and discussion:
During the study, FCM was performed in 143 MM patients (103 Newly Diagnosed MM and 40 Relapsed Refractory MM). The cytogenetic findings includes *IGH*:: *FGFR3* (n=29), *IGH*:: *CCND1* (n=8), *IGH*:: *MAF* (n=3), gain1q21 (n=76), 1q21-amplification (n=30), 1p32-deletion (n=17) and *TP53*-deletion (n=21). A median of 38,779 PCs was acquired by FCM. The BCL2 and MCL1 expression was seen in 4-100% (median-96.9%) and 0-100% (median-78.8%) of clonal PCs, respectively. The median BCL2 MFI and MCL1 MFI were 43,289 and 6,481 respectively. The median BCL2 $^{PC/T}$, MCL1 $^{PC/T}$ and BCL2 ^{PC}/MCL1 ^{PC} in clonal PCs were 1.90, 4.35, and 07.1, respectively, whereas in non-clonal PCs they were 0.87, 3.02 and 3.21, respectively.BCL2 $^{PC/T}$, MCL1 $^{PC/T}$ and BCL2 ^{PC}/MCL1 ^{P} were significantly higher in clonal PCs than non-clonal PCs, with a *p*-value of 0.002, 0.001, and 0.009, respectively. The median MCL1 MFI was significantly lower (3,825 vs. 6,559; p=0.020) and median BCL2 ^{PC}/MCL-1 ^{PC} (12 vs 6.7; p=0.012) was significantly higher in *IGH*:: *CCND1* compared to others; while an opposite pattern was seen in gain1q21 (7,420 vs 5,525; p=0.032 and 5.5 vs 8.6; p=0.046). The dim expression (<25-percentile) of BCL2 and MCL1 was noted in 25% (n=36) MM cases. Survival analysis did not yield a significant difference in OS or PFS between patients with dim, moderate or strong expression of BCL2 or MCL1.

Conclusions:
Our study demonstrates an objective assessment of BCL2 and MCL1 expression by FCM. BCL2 $^{PC/T}$,MCL1 $^{PC/T}$ and BCL2 ^{PC} /MCL1 ^{PC} were significantly higher in myeloma cells compared to non-clonal PCs. Low MCL1 expression or high BCL2/MCL1 ratio was associated with *IGH*:: *CCND1*; while strong MCL1 and low BCL2/MCL1 ratio was most frequent in 1q21 amplification. We identified dim-expression (< 25-percentile) of BCL2 or MCL1 in 25% of cases of MM, where the benefit of anti-BCL2/MCL1 targeted therapy needs to be studied. Routine testing of BCL2 and MCL1 expression by FCM may be beneficial before starting Venetoclax/similar targeted therapy.

Disclosures No relevant conflicts of interest to declare.

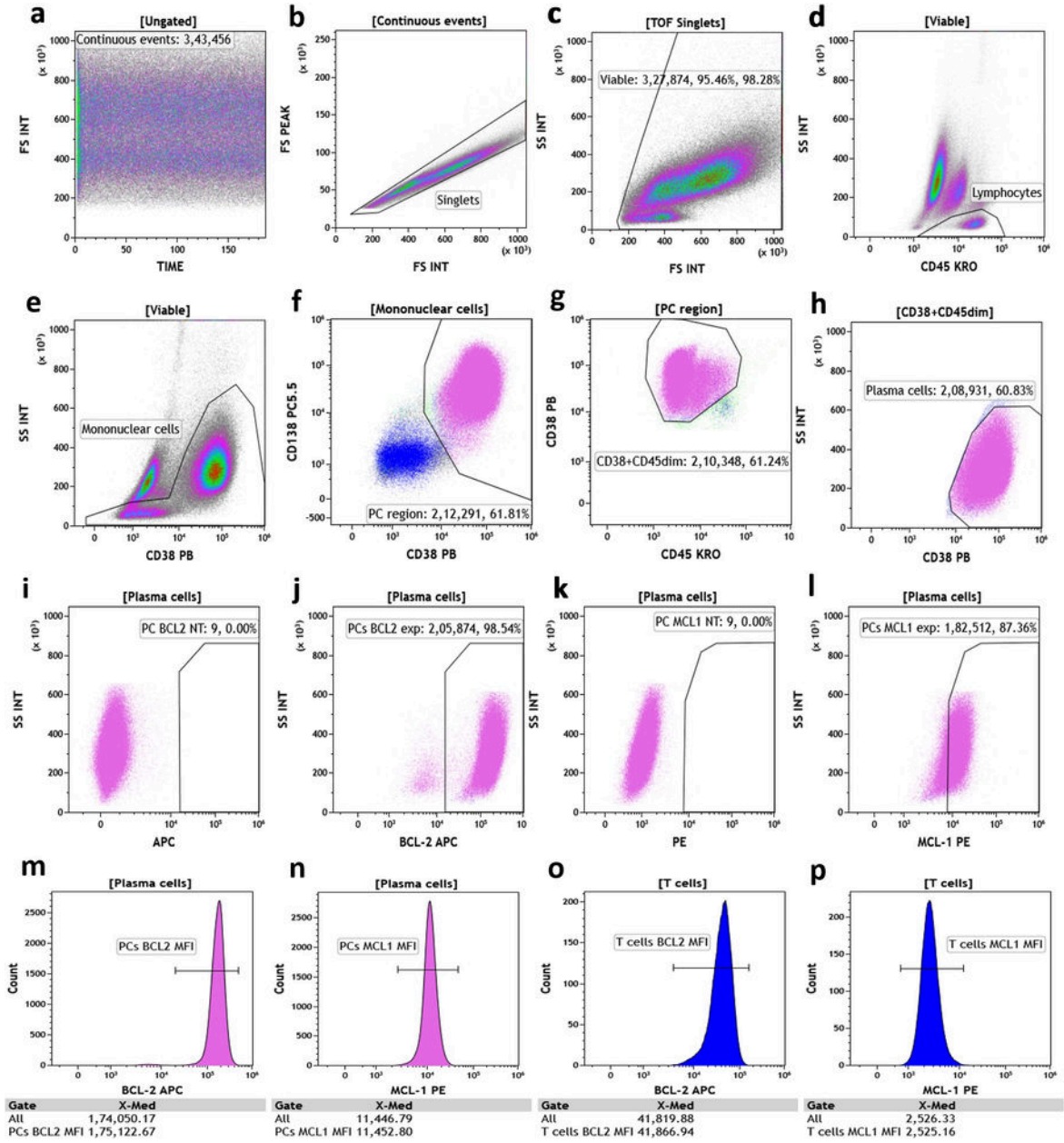

Figure 1: Flow cytometric analysis showing sequential gating strategy used in the analysis of intracellular BCL2 and MCL1 expression in plasma cells and T lymphocytes. Bivariate dot plots "a" to "h" demonstrate the initial gating strategy to identify the purified plasma cells based on the CD45, CD38, CD138 and side scatter. T lymphocytes are identified based on bright expression of CD3 and CD45; negativity for CD19 and low side scatter (dot plots not shown). In bivariate dot plots "i" and "k" FMO threshold is applied on plasma cells for BCL2 and MCL1. Dot plots "j" and "l" show expression of BCL2 and MCL1 on plasma cells on the basis of previously applied thresholds. Histograms "m" to "p" represent MFI of expression of BCL2 and MCL1 on plasma cells and T-cells.

Figure 1

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