



MM – 329 Evaluation of Immunophenotype of the Non-Neoplastic Plasma Cell Compartment and Post-Therapy Immunomodulation to Understand Nuances in MRD Assessment of Multiple Myeloma Using Next- Generation Flow Cytometry.

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Abstract

CONTEXT: A deeper understanding of the normal plasma cell (PC) compartment and changes in immunophenotypic profile post treatment is required to assess low levels of residual disease in multiple myeloma (MM) using multiparametric flowcytometry. **OBJECTIVE:** The expression profile of selective antigens on normal and aberrant plasma cells prior to and following anti-myeloma therapy was assessed to ascertain its relevance in MRD assessment. **DESIGN:** This is a prospective analysis of 702 patients with the diagnosis of MM (January 2015 to June 2020) **SETTING:** The study was conducted at a tertiary care cancer center. **PATIENTS:** A total of 702 cases (321 treatment naïve;321 post-therapy; 60 control samples) were evaluated. 60 samples from non- MM staging marrow were assessed to study antigenic profile of normal PC. **INTERVENTIONS:** Bone marrow samples were collected in EDTA and processed within 6 hours of collection as per the consensus recommendation from Euro-flow protocol using two tube 8-color/single tube 10-color panel. An aberrant plasma cell cluster was defined by presence of >30 plasma cells with light chain restriction and/or aberrant immunophenotypes. **MAIN OUTCOMES MEASURES:** 1. Expression profile of antigens on polyclonal PC compartment 2. Post-therapy immunomodulation in MM. **RESULTS:** Polyclonal PC were observed in 70.63% of treatment naïve samples compared to 89.01 % post - treatment samples (p= 0.001). Frequency of aberrant expression of markers in polyclonal PC was higher at follow-up time point i.e. CD45 (38% vs 36%; p=0.6), CD19(31% vs 39%; p=0.04), CD56(29% vs 40%; p=0.009), CD27(26% vs 36%; p=0.01), CD38 (0% vs 31%; p<0.001) CD81(12% vs 21%; p=0.005), CD117(<1% vs 5%; p=0.003) and CD138 (0% vs 5%; p=0.002).Comparison of baseline phenotype with MRD positive samples showed loss of CD45 (23.11%), CD38 (20.96%), CD27=CD81 (15.05%), CD19 (12.90%) and CD56 (9.67%). Post-therapy gain of expression was most marked in CD56 (22.58%) followed by CD81 (18.81%), CD38 (17.20%), CD27=CD45 (15.05%) and CD19 (13.97%). **CONCLUSIONS:** Flowcytometry based MRD assessment in MM is challenged by wide immunophenotypic variation in non-neoplastic PCs and changes in immunophenotype after chemotherapy and thus, simultaneous analysis of multiple immunophenotypic markers including light chain expression is required for reliable MRD assessment.

Background

- Multiparametric Flow cytometry (MFC) has become the gold standard investigation to monitor measurable residual disease (MRD) and evaluate the depth of response in patients diagnosed with multiple myeloma (MM).
- Studies have shown overlap of benign and malignant plasma cells' (PC) compartment which may cause hindrance in accurate determination of MRD.
- Post-therapy change in expression profile of various markers is another aspect of MRD evaluation which has not been adequately addressed so far.

Objective

- To study the expression profile of antigens, commonly used to characterize plasma cells, in the non-neoplastic PC compartment.
- To evaluate the post-therapy immunomodulation in PC compartments in MM patients and to explore its impact on MRD assessment.

Materials & Methods

The study was conducted at a tertiary care cancer center. A total of 702 cases (321 treatment naïve; 321 post-therapy; 60 control samples) were evaluated. 60 samples from non- MM staging marrow were assessed to study antigenic profile of normal PC. Bone marrow samples were collected in EDTA and processed as per the consensus recommendation from Euro-flow protocol. An aberrant PC cluster was defined by presence of >30 PC with light chain restriction and/or aberrant immunophenotypes. Flow cytometric dot plots below shows the analysis strategy for MRD evaluation (Figure 1).

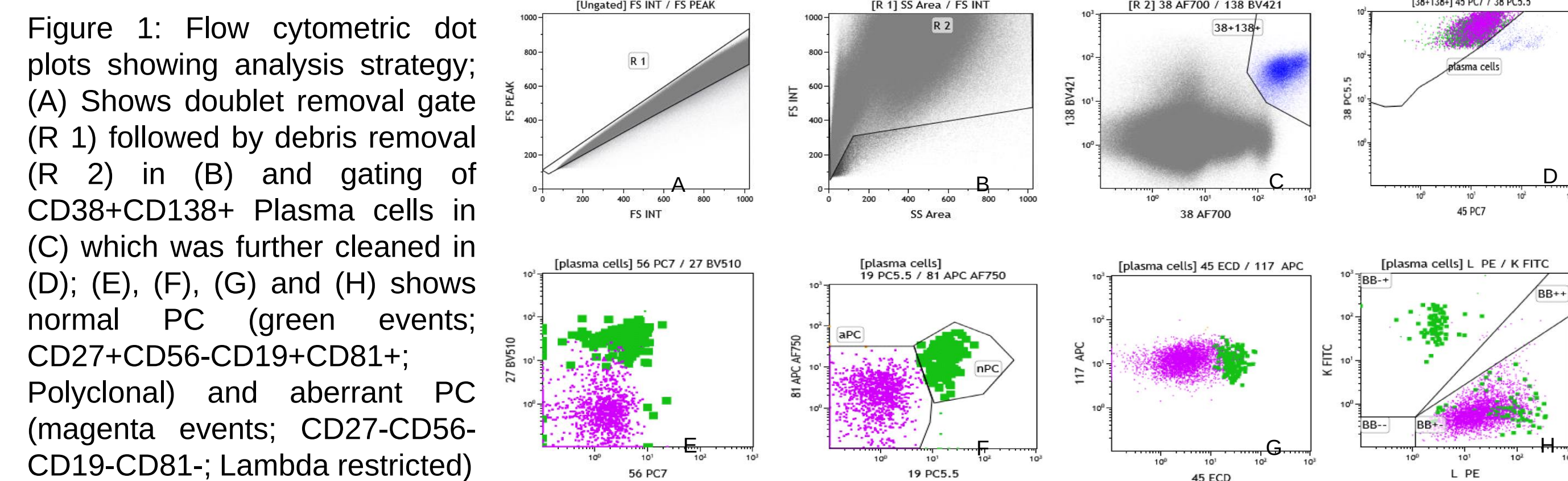


Figure 1: Flow cytometric dot plots showing analysis strategy; (A) Shows doublet removal gate (R 1) followed by debris removal (R 2) in (B) and gating of CD38+CD138+ Plasma cells in (C) which was further cleaned in (D); (E), (F), (G) and (H) shows normal PC (green events; CD27+CD56-CD19+CD81+; Polyclonal) and aberrant PC (magenta events; CD27-CD56-CD19-CD81-; Lambda restricted)

Results

Polyclonal PC were observed in 65.42% of treatment naïve samples compared to 88.16% post -treatment samples (p= 0.001). Frequency of aberrant expression of markers in polyclonal PC was higher at follow-up time point (Figure 2). A change in the immunophenotype of PC during treatment was seen in the majority of patients (147/186; 79%) when both loss and gain of antigen expression were taken into account (Figure -3).

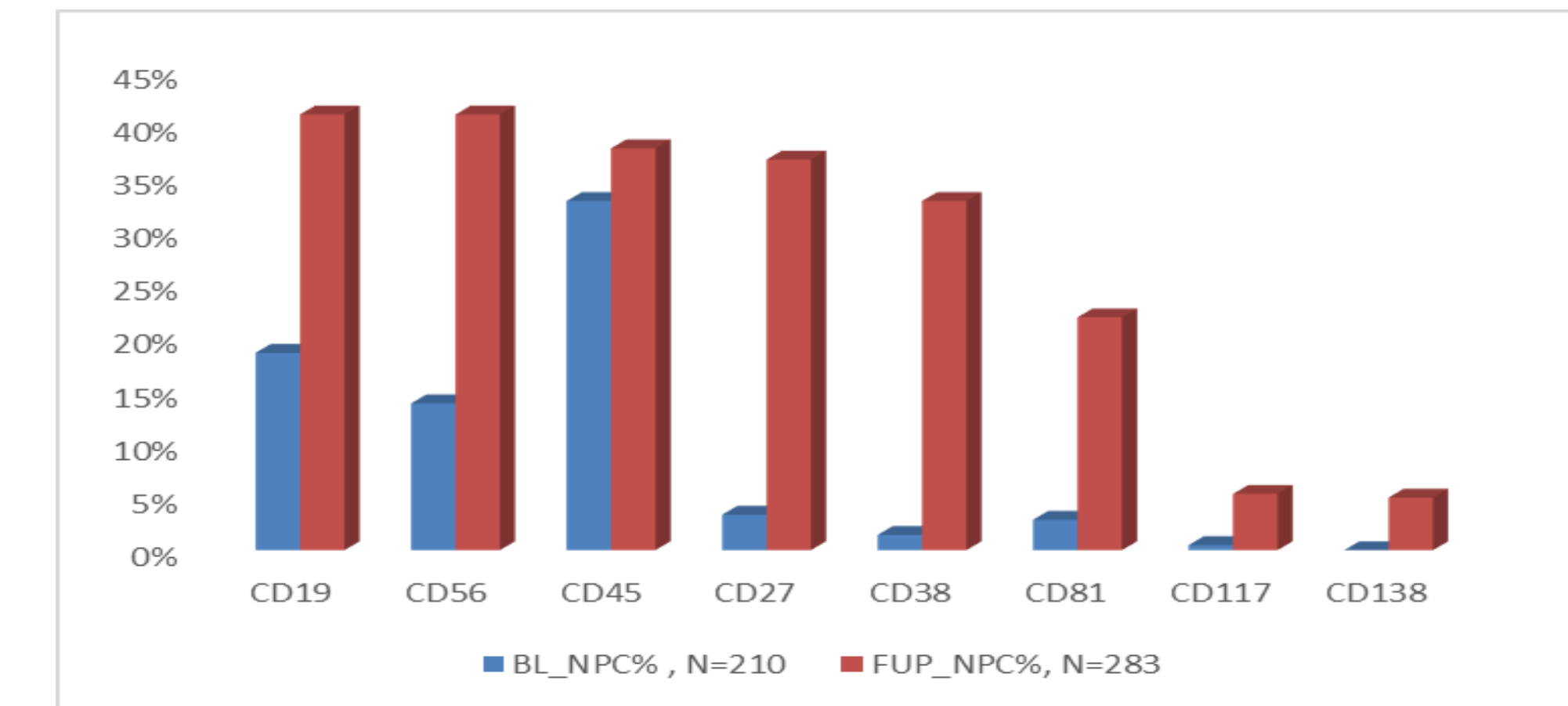


Figure 2: Frequency of aberrant expression of markers in polyclonal PC was higher at follow-up time point i.e.,CD45 (33% vs 38%; p=0.6), CD19(19% vs 41%; p=0.001), CD56(14% vs 41%;p<0.001), CD27(3% vs 37%; p<0.001), CD38 (0% vs 33%; p<0.001) CD81(3% vs 22%; p<0.001), CD117(<1% vs 5%; p=0.003) and CD138 (0% vs 5%; p=0.002).

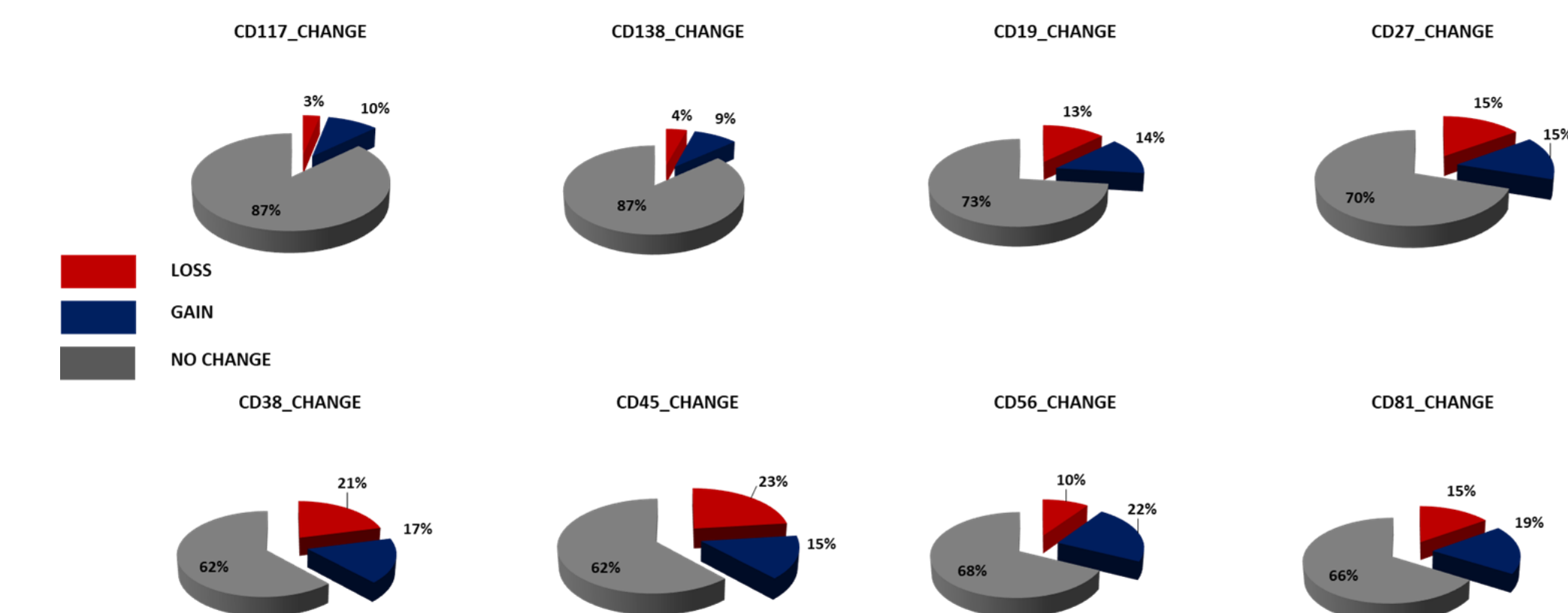


Figure 3: Comparison of baseline phenotype with MRD positive samples showed loss of CD45 (23%),CD38(21%), CD27=CD81(15%),CD19 (13%) and CD56 (10%). Post-therapy gain of expression was most marked in CD56 (23%) followed by CD81 (19%),CD38 (17%), CD27=CD45 (15%) and CD19 (14%).

Discussion

This study shows that the high frequency of IPT aberrations on polyclonal PCs is relevant in the context of MM-MRD assessment and signifies MRD assay validation on a large cohort of normal and reactive conditions. The discriminative power of various antigens for delineating neoplastic from non-neoplastic PC is variable due to rampant immunomodulation in polyclonal as well as clonal PC and warrants cautious interpretation. Nonetheless, data presented in the current study ascertains the fact that baseline immunophenotyping is not a prerequisite for MRD evaluation.

References

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