LONGITUTIONAL ASSESSMENT OF MINIMAL RESIDUAL DISEASE DYNAMICS IN PATIENTS WITH MULTIPLE MYELOMA WHO ACHIEVE COMPLETE REMISSION AFTER FIRST LINE THERAPY

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Background

Minimal residual disease (MRD) negativity is the most reliable biomarker in Multiple Myeloma (MM) associated with a favorable outcome. However, MRD status at a single timepoint cannot guarantee for its sustainability overtime, hence relapses are also observed in MRDneg patients. The BM niche has a critical role in supporting the myeloma cells, but little is known about the BM dynamic changes during the disease progression

Objective

The prognostic evaluation of MRD at distinct timepoints, the frequency and prognostication of MRD dynamics overtime, and the highlight of possible associating alterations in the bone marrow (BM) microenvironment.

Patients & Methods

MRD and BM profiling were evaluated with Next-Generation Flow (NGF) according to Euroflow guidelines in 313 MM patients achieving complete remission (CR), divided in two groups based on the timing of their first MRD evaluation: Group A:240 patients examined for MRD at their first evidence of CR; Group B:73 patients having a minimum of 24-month CR period when first tested for MRD. The median follow-up monitoring of patients since the time of their first MRD evaluation was 35 months. A second, third and fourth consecutive MRD assessment was performed for 52%, 34% and 18% patients respectively (median time interval:6 months).

The BM niche profiling was examined for each patient with the surface panel of NGF. A total of 17 BM subsets were examined per patient, thus allowing for the construction of an individualized patient immune profile **(Figure 1).** These profiles were saved and compared for patients showing changes in their MRD status

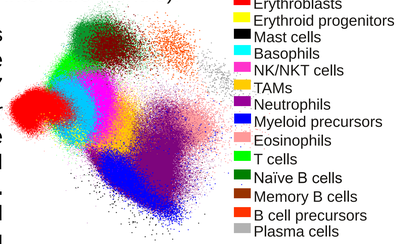


Figure 1: *Indicative BM profile of a MM patient based on the distribution of 17 immune substes*

Results

The prognostic value of MRD positivity

In total 106/313 (33.9%) patients were MRDpos in their first examination with a similar frequency of MRD positivity in both groups. MRDpos patients had a shorter PFS than the MRDneg counterpart in both Groups A and B (**Figures 2A,B**) with a cumulative median PFS of 60 months vs. not reached for the MRDneg patients of both Groups combined (HR:0.36, 95% CI:0.22-0.61, p<0.0001, **Figure 2C**). Of note MRDpos patients in Group B had a 2-fold higher risk of subsequent progression than those of Group A (HR:0.51, 95% CI: 0.24–1.05, p=0.03), probably due to their higher tumor burden (median: $3x10^{-4}$ vs. $3x10^{-5}$, p<0.001). Indeed, the differential tumor burden conferred a distinct prognostication among MRDpos patients of both groups **(Figure 2D).** On the other hand, the presence of high risk cytogenetics or the adverse ISS III stage could not affect the favorable prognosis of MRD negativity achievement (respectively).

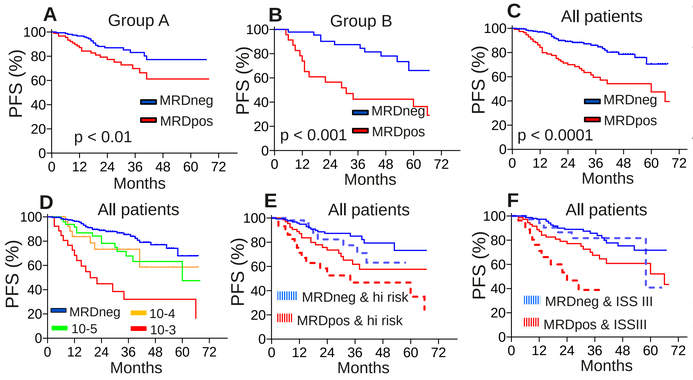


Figure 2: *Progression free survival (PFS) according to the MRD status. **A:** PFS since the first achievement of complete response (CR) according to the MRD status. **B:** PFS since the first MRD evaluation in long-term CR patients according to the MRD status. C: PFS according to MRD status for all patients enrolled. D: Patients with a differential log tumor burden show statistical different risk of progression (p<0.0001). **E,F:** PFS according to the MRD status combined with the presence of high-risk (dashed lines) or low-risk (solid lines) aberrations **(E)** or the presence (dashed lines) or absence (solid lines) of the adverse ISS stage III **(F).***

Frequency and prognostic impact of MRD dynamics

A total of 34% of patients changed their MRD status overtime. Those patients changing from MRDneg to MRDpos showed a 2.2-fold higher risk for subsequent relapse than those with persistent MRD. Surprisingly, patients changing from MRDpos to MRDneg had a more favorable prognosis when compared to continuously MRDneg patients (**Figure 3**).

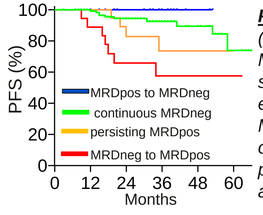


Figure 3: *Progression free survival (PFS) according to the different MRD dynamics. PFS was estimated since the time of the first MRD evaluation. Patients with a dynamic MRD status showed a different risk of progression than those with a persisting MRD profile. p<0.001 among groups*

The BM microenvironment composition is highly dynamic and associates with MRD status

We have previously shown that patients with the same MRD status share a similar BM immune profiling (**Figure 4A**). To eliminate bias among patients, we performed a BM profiling comparison for those patients changed from a MRDneg to a MRDpos status (n=27) between two consecutive MRD assessments and found that this alteration was accompanied with a relevant increase in the NK cells and the ratio of memory to naïve B cells (Figures 4B and C respectively).

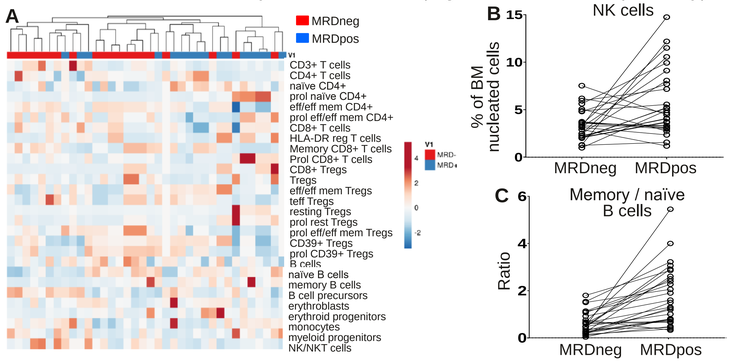


Figure 4: *Bone marrow (BM) profiling according to MRD status. **A:** Heatmap displaying the unsupervised hierarchical clustering of MRD patients based on their entire immune composition. Patients of the same MRD status are clustered together. **B,C:** Patients changing form a MRDneg to a MRDpos status show a statistical significant increase (p<0.001) in their NK **(B)** and memory B cell compartments **(C).***

Discussion

The prognostic value of MRD is irrespective of CR durability, while tumor burden can be used for an improved risk stratification of MRDpos patients. MRD should be periodically re-evaluated, since MRD dynamics confer a distinct prognostication than a persisting MRD status. MRD dynamics are accompanied by alterations in BM immune profiling, which may shed light in the underlying biology of MRD.

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