#P026

MDSC: Inhibiting Immune Therapy

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INTRODUCTION

Certain cell subsets have been identified to have a negative impact on cancer immunotherapies by promoting angiogenesis and immunosuppression in the tumor microenvironment. One of these cell subsets is a heterogeneous population of immature myeloid cells that have been named Myeloid Derived Suppressor Cells (MDSC). MDSC are increased in states of cancer and their numbers have been shown to inversely correlate with a positive clinical outcome. These findings have prompted the measurement of MDSC in order to predict clinical outcome during treatment with immunotherapies. However, MDSC are a heterogeneous population defined by their immunosuppressive function that can be separated into two main lineages in humans, granulocytic MDSC (PMN-MDSC), which are polymorphonuclear, and monocytic-MDSC (M-MDSC) which are mononuclear. In this study, flow cytometry was used to measure M-MDSC in frozen PBMC samples and hence predict medical outcome in melanoma patients treated with an anti-CTLA-4 drug (ipilimumab).

RELIABLE IDENTIFICATION OF MDSC CAN BE PROBLEMATIC

Lack of markers exclusively expressed on MDSC

- MDSC are a heterogeneous population defined by their immunosuppressive function that can be separated into two main lineages in humans, polymorphonuclear- or granulocytic- MDSC (PMN-MDSC or G-MDSC), which are polymorphonuclear, and monocytic-MDSC (M-MDSC) which are mononuclear.
- M-MDSC are derived from monocytic/dendritic cells precursors (MDP), whereas PMN-MDSC are derived from myeloblasts (MB) (**Figure 1**).

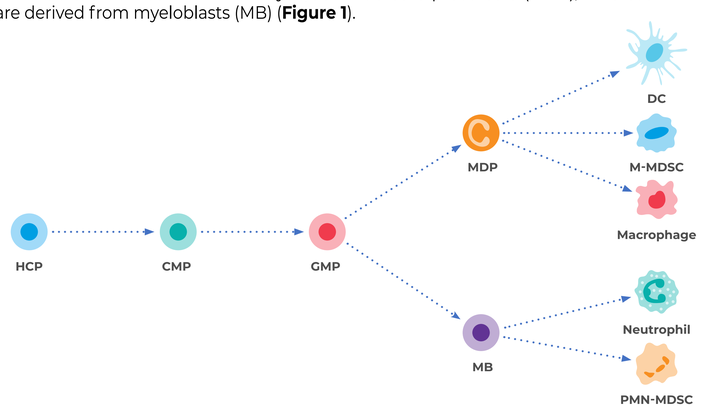

Figure 1. MDSC Lineage. Hematopoietic progenitor cells (HPC) differentiate via common myeloid progenitors (CMP) into granulocyte/macrophage progenitor cells (GMP). These cells can then differentiate into either monocytic/dendritic progenitor cells (MDP) or myeloblasts (MB). MDP cells will give rise to dendritic cells (DCs), macrophages or M-MDSC. MB cells will give rise to neutrophils and PMN-MDSC.

- Markers presented by MDSC are also shared by other subsets.
- Phenotypic markers used to identify different MDSC subsets are also shared by other cells. As their name suggests, M-MDSC share a strong phenotypic overlap with monocytes, whereas PMN-MDSC are closely related to neutrophils (**Table 1**).
- Without the ability to test function, one must rely on the lower expression of markers, such as HLA-DR for M-MDSC and CD33 for PMN-MDSC, to differentiate them from other subsets.

Table 1. Phenotypic similarities between MDSC and other cell subsets.

	CD11b	CD14	CD15	HLA-DR	CD33	Lineage markers*
M-MDSC	+	+	-	-/lo	+	-
Monocytes	+	+	-	+	+	-
PMN-MDSC	+	-	+	N/Ap	lo	-
Neutrophils	+	-	+	N/Ap	+	-

*CD3, CD19, CD20, CD16 and CD56

- Difficulty in standardizing HLA-DR -/lo expression gating on M-MDSC.
- HLA-DR expression on monocytes and M-MDSC does not show a bi-modal expression when analyzed through flow cytometry. The observed profile is represented as a cloud of spots (**Figure 2 A**).
- A shift in HLA-DR expression can be observed depending on the subject analyzed (**Figure 2B**). Although a FMO control for HLA-DR can be used to identify the left edge of the positive population (meaning that no positive cells can exist beyond that limit), it is not very helpful when it comes to defining what is a low expression versus a mid or high expression of HLA-DR.

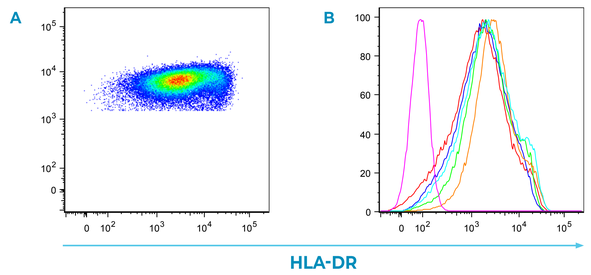

Figure 2. Flow Cytometry profiles of HLA-DR. A) HLA-DR expression on CD14+ Lin- cells shows a lack of bi-modal distribution with no clear separation between the positive and negative populations. **B)** Histogram representation of HLA-DR expression on six subjects and a FMO control sample (in purple) lacking the HLA-DR antibody. The dotted line represents the cut-off between negative and positive HLA-DR expression according to the FMO control.

METHOD

M-MDSC were measured in frozen PBMC from 20 healthy donors and 68 patients with melanoma treated with ipilimumab. M-MDSC were enumerated using a $Lin^{-}CD14^{+}CD11b^{+}HLA\text{-}DR^{low/-}$ phenotype. In order to prevent subjectivity during gating, caused by the lack of bi-modality with HLA-DR staining, a computational algorithm was used. As distinct HLA-DR spread can be observed in the different subjects, measuring the CV (a ratio between GMFI and SD) of this spread on healthy donors and applying the upper limit of CVs (defined as the 99th percentile) as a cut-off in a generated nomogram allows to calculate an ad hoc quantitative measure of MDSC frequency in cancer patients. This measurement enables identification of M-MDSC in an objective manner and was used to determine whether the percentage of M-MDSC in patients could be linked with overall survival.

CONVERTING THE HLA-DR GMFI INTO A CV STANDARDISES M-MDSC IDENTIFICATION

The HLA-DR CV is higher in melanoma subjects when compared to healthy donors.

- The geometric mean fluorescence intensity (GMFI) can be subject to inter-replicate variability.
- A coefficient of variation (CV) calculated from the GMFI is used to assess HLA-DR spread on CD14+Lin- cells, largely minimising inter-replicate variability (**Figure 3A**).
- When using the $CV_{HLA\text{-}DR}$ to compare HLA-DR spread in samples from 20 healthy donors with whole blood samples from 60 melanoma patients, we observed that the spread is higher in a subset of patients (as defined by a $CV_{HLA\text{-}DR}$ higher than the 99th percentile observed among healthy donors (**Figure 3B**).

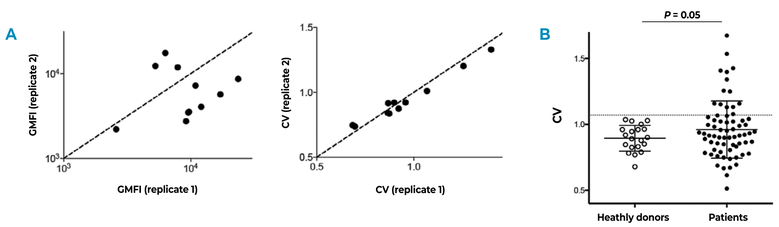

Figure 3. Applying CV to assess HLA-DR spread. A) Deriving the CV from the HLA-DR GMFI reduces inter-replicate variability when compared to the GMFI. **B)** The $CV_{HLA\text{-}DR}$ shows a higher spread in melanoma subjects (n=60) when compared to healthy donors (n=20).

The $CV_{HLA\text{-}DR}$ values can be converted into a M-MDSC frequency.

- The mean-normalized variance in the data is translated to a percentage of the MDSC population using a proprietary algorithm licensed from Memorial Sloan Kettering Cancer Center.
- A "cut-off" can be applied between what is considered a normal expression of MDSC (as defined by the expression in healthy donors) vs an abnormal expression of MDSC (as defined in the subset of melanoma patient with a higher $CV_{HLA\text{-}DR}$) (**Figure 4**).
- This "cut-off" was set at 14.9% to delimit between "low" and "high" M-MDSC expressers in the study.

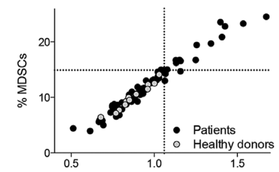

Figure 4. Normogram plot representing relationship between CV values and M-MDSC frequencies in both melanoma patients and healthy donors.

RESULTS

The relative frequency of M-MDSC was determined in 68 melanoma patients treated with two different doses of ipilimumab. By comparing the percentage of M-MDSC at baseline (pre-treatment) and after two doses of ipilimumab with the overall survival data and applying log-rank statistics, a cut-off was defined allowing the separation of "high" and "low" M-MDSC expressers. Patients with "low" M-MDSC were associated with improved overall survival with a hazard ratio of 0.35.

OUR M-MDSC PHENOTYPING ASSAY ALLOWS IDENTIFICATION OF A CELL SUBSET WITH STRONG CLINICAL IMPACT

Using the "cut-off" to validate the M-MDSC phenotyping method with clinical data

- The "cut-off" was used to separate a cohort of 64 melanoma patients treated with Ipilimumab, in order to compare overall survival (OS) between "low" and "high" M-MDSC expressers (**Figure 5**).
- The comparison shows a better OS in subjects showing a "low" level of M-MDSC expression with a hazard ratio of 0.35.

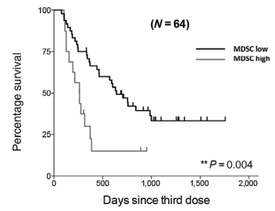

Figure 5. Comparison of OS from subjects samples 6 weeks after start of ipilimumab between "low" and "high" MDSC expressers.

The clear observed differences in OS when using our proprietary algorithm to measure the $CV_{HLA\text{-}DR}$ and translate the value into a M-MDSC frequency demonstrates that the cells being measured have an impact on clinical outcome.

CONCLUSION

- The use of an algorithm to determine the CV of HLA-DR expression on CD14-Lin- cells and translate that into a % of M-MDSC allows reliable and standardized profiling of these cells.
- A 'cut-off' of normal expression of M-MDSC was set by comparing M-MDSC frequency between healthy donors and melanoma patients, based on the 99th percentile of $CV_{HLA\text{-}DR}$ amongst CD14-Lin- cells from healthy donors.
- Applying this "cut-off" to separate a cohort of 68 melanoma patients treated with ipilimumab between "low" and "high" M-MDSC expressers showed a clear difference in OS, with the "low" M-MDSC expressers displaying improved overall survival with a hazard ratio of 0.35.
- This proof-of-concept study shows that the M-MDSC measured are clinically relevant and have a strong impact on immunotherapy.

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