Precision in the Detection of Myeloid-derived Suppressor Cells (MDSCs)

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ABSTRACT

Myeloid-derived suppressor cells (MDSCs) are considered a major regulator of the immune response and are a known biomarker associated with responsiveness to CTLA-4- and PD-1-targeting therapies, as well as, a potential target for therapeutic intervention. While there are publications dating back to the early 1970s that describe immune suppressive myeloid cells, MDSCs have only recently been widely appreciated due to a greater understanding of the phenotype of this heterogeneous population of cells. Recent efforts to harmonize flow cytometric detection of MDSCs across labs internationally led to the following suggestion on the human MDSC phenotype for monocyte-MDCSs (M-MDSCs) as $CD11b^{+}CD14^{+}HLA\text{-}DR^{-/lo}CD15^{-}$, polymorphonuclear-MDSCs (PMN-MDSCs) as $CD11b^{+}CD14^{-}CD15^{+}$, and early-MDSCs (eMDSCs) as $Lin^{-}CD14^{-}HLA\text{-}DR^{-/lo}CD33^{+}$. The objective of this study was to develop and validate a CLIA 7-color flow cytometry assay performed on a BD FACSCanto IVD to reliably detect these MDSC populations in peripheral blood mononuclear cell (PBMC) preparations for use in clinical studies. Evaluation of PBMCs isolated using density gradient separation and SepMate™ tubes was chosen, as this is currently the best method to enrich for PMN-MDSCs, which are part of the low density granulocyte (LDG) population, over normal granulocytes that share surface markers with MDSCs and will confound the analysis.

The MDSC flow cytometry assay met validation criteria for intra-assay, inter-assay, and inter-operator precision, in addition to, specificity, sensitivity/ linearity, and stability. Importantly, this assay clearly demonstrated that the predominant population of MDSCs in stage IV colorectal cancer patients is the PMN-MDSC population and that this PMN-MDSC population is heterogeneous in CD14 expression, with low to negative levels of CD14 expression. Low density granulocytes reportedly can express low levels of CD14; however, it is unknown if this differentiates the PMN-MDSCs from mature pro-tumor neutrophils that are both part of the low density granulocyte population. Monitoring MDSC populations in the clinic with this CLIA assay will support the elucidation of the role MDSCs play in a variety of disease states. We present this biomarker assay as a robust method to stratify patients for multiple immuno-therapies.

PHENOTYPES OF MDSCs: 3 TYPES

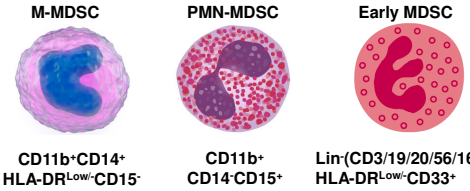
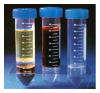


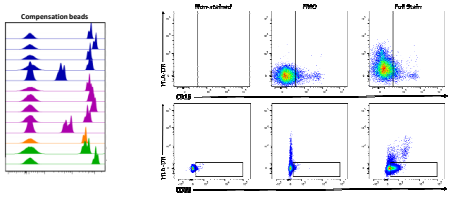
Figure 1.
Phenotype of the three major populations of MDSCs: Monocyte-MDSC (M-MDSC), Polymorphonuclear-MDSCs (PMN-MDSC), and Early MDSC (eMDSC) as reviewed by Bronte *et al* 2016 *Nat Comm.*

EXPERIMENTAL PROCEDURE

Step 1. Separation of PBMCs
SepMate™ tubes- best method to enrich for PMN-MDSCs



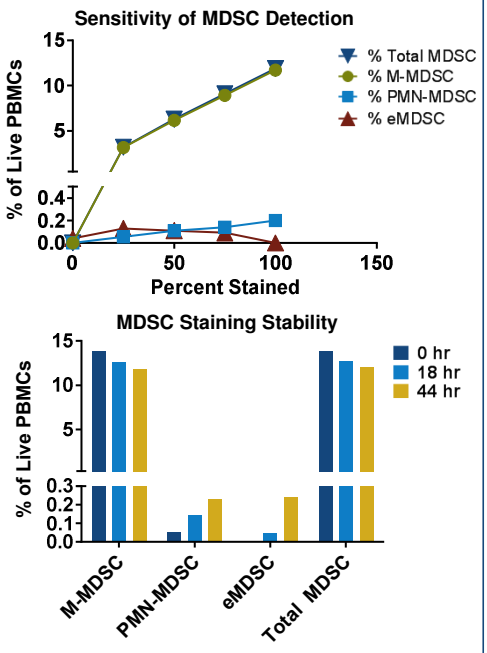
Step 2. Stain Freshly Isolated PBMCs
Single color, non-stained, FMOs, and full stain for all samples



Step 3. Detection with BD FACSCanto IVD

Figure 2.
The first step in this assay is gradient separation of PBMCs from whole blood using the SepMate™ procedure. This is a key step, as gradient separation is currently the only method to distinguish PMN-MDSCs, found in the low density granulocyte population, from normal granulocytes. Freshly isolated PBMCs either non-stained, stained as an FMO, or full panel stained, as well as, singly stained One Comp Beads™, were acquired on a BD FACSCanto IVD instrument and the data was analyzed using FlowJo software.

VALIDATION OF MDSC FLOW ASSAY



Validation Parameters	Criteria
Intra-assay precision	%CV ≤25
Inter-assay precision	%CV ≤40
Inter-operator precision	%CV ≤40
Specificity	Full stain > negative control
Sensitivity	Linearity
Stability	0-44 hr

Figure 3.
Assay validation was carried out on cryopreserved healthy donor PBMCs. The table shows the validation parameters tested and the criteria required for passing validation. The top graph shows that upon dilution of fully stained PBMCs into non-stained PBMCs, the assay performs with excellent linearity at the highest dilution of stained cells (25% stained). For the %Total MDSC, % M-MDSC & % PMN-MDSC R^2 values are ≥ 0.991, % eMDSC R^2 value was 0.1297. The bottom graph shows the stained cells are stable prior to acquisition for at least 44 hours post-staining.

MDSCs IN HEALTHY VS. STAGE IV CRC DONORS

Healthy Donors

Population	M-MDSC	PMN-MDSC	eMDSC	Total circulating MDSC in PBMC
Donor ID	% PBMC	% PBMC	% PBMC	% Total circulating MDSC in PBMC
Healthy 1	2.09	0.058	1.67E-03	2.1
Healthy 2	1.83	0.11	0.49	1.7
Healthy 3	1.13	0.028	0.77	1.2
Healthy 4	4.79	0.19	1.46	5.0
Healthy 5	1.96	0.024	1.04	2.0

Stage IV- Colorectal Cancer (CRC) Donors

Population	M-MDSC	PMN-MDSC	eMDSC	Total circulating MDSC in PBMC
Donor ID	% PBMC	% PBMC	% PBMC	% Total circulating MDSC in PBMC
DD17	0.54	0.31	0.92	0.9
DD18	0.10	5.91	0.65	6.0
DD19	1.91	71.8	2.22	73.7
DD20	3.37	44.0	28.4	47.4

Figure 4.
The top table shows the results of the assay performed on 5 healthy donors and the bottom table shows the results of the assay performed on 4 stage IV colorectal cancer patients. All healthy donors had < 10% Total circulating MDSCs (M-MDSCs + PMN-MDSCs); whereas 2 of the 4 stage IV colorectal cancer patients had dramatically higher frequencies of Total circulating MDSCs, primarily driven by the PMN-MDSC population.

GATING HIERARCHY FOR MDSC POPULATIONS

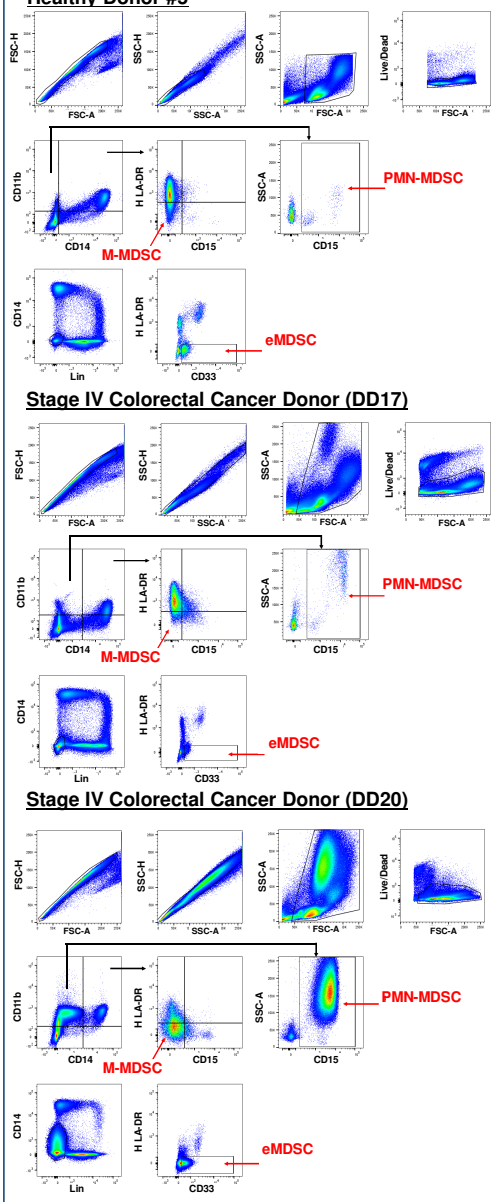


Figure 5. The gating strategy used for data analysis is shown for 1 healthy donor and 2 stage IV colorectal cancer donors, one with and one without elevated Total circulating MDSCs. Clear distinctions were observed in the FSC-A vs SSC-A, CD14 vs CD11b, and CD15 vs SSC-A plots for these donors. Of note, the stage IV CRC cancer donors with the elevated Total circulating MDSC population had an observable $CD11b^{+}CD14^{Lo}$ population.

CONCLUSION

This MDSC flow cytometry assay passed all Precision, Specificity, Sensitivity, and Stability validation criteria and therefore can be performed under CLIA regulation. This flow cytometry assay identifies all three MDSC populations and clearly distinguishes between healthy and stage IV colorectal cancer donors. The dominant MDSC population observed in stage IV colorectal cancer patients was the PMN-MDSC population, which was due to an increase in the $CD11b^{+}CD14^{Lo/-}$ population.

ACKNOWLEDGMENTS

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Bronte V. et.al., Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards, Nature Communications 7, 12150 (2016).