Assessment of Monocytes, MDSCs and Myeloid Cell Populations in Immuno-Oncology Clinical Trials by a Standardized High Complexity Flow Cytometry Approach

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Abstract
Advances in novel immunotherapies including checkpoint inhibitors, bispecific antibodies and cytokines have demonstrated exceptional clinical responses. However, their clinical benefit is restricted to only a subset of patients and thus identification of predictive biomarkers that can differentiate patients most likely to respond to a given therapy could be greatly beneficial.

Various myeloid populations including MDSCs (both G-MDSC, M-MDSC), inflammatory (CD14+CD16-) or proangiogenic monocytes (CD14+CD16+) and macrophages were suggested to be potentially predictive of anti-PD1 responses as they affect pathophysiology of cancer through inflammation, suppression of anti-tumor immune response or through pro-angiogenesis.

To enable simultaneous detection of MDSCs and multiple myeloid cell subsets (a process regarded as technically challenging) in a single tube, we developed a 13-parameter high complexity flow cytometry assay and explored it’s utility in understanding the correlates of efficacy in metastatic melanoma patients treated with immunotherapies.

Method Development

**Validation Parameters**

<table>
<thead>
<tr>
<th>Validation Parameters</th>
<th>Specificity (antibody and assay)</th>
<th>Precision (intra-assay, inter-assay, inter-operator, inter-instrument)</th>
<th>Sensitivity (LLOQ)</th>
</tr>
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**Specimens**

Normal donor PB, BM (as well as PBMCs/BMMCs) spiked with different levels (high, medium, low) of cell lines were used for development and validation.

**Staining Procedure**

Specimens were incubated with Fc receptor blocker, stained with fixable viability dye (eF506) followed by antibody panel for 20 minutes at RT.

**Acquisition/Analysis**

Sample data was acquired on a Fortessa X-20 Flow cytometry (BD Biosciences) and analyzed using FlowJo software.

**Gating Strategy**

Population                   | Phenotype                |
-----------------------------|--------------------------|
MDSC                        | LIN-HLA-DR-CD11b+CD33+²  |
M1 Monocytes                | HLA-DR+CD14+CD16-²       |
M2 Monocytes                | HLA-DR+CD14+CD16+²       |
M3 Monocytes                | HLA-DR+CD14lowCD16+²     |

**Validation Summary**

Analytical Sensitivity

- Cell frequencies < 0.1% of parent and/or < 100 events exhibited > 25% CV
- LLOQ is 1% of parent with > 100 clustered events

Precision (Acceptance Criteria: CV ≤ 25% above LLOQ)

- Intra-assay: Pass
- Inter-instrument: Pass
- Inter-operator: Pass
- Inter-assay: Pass

**PD Biomarker Analysis**

The study group consisted of 25 PBMC samples of metastatic melanoma cases treated with ipilimumab and high dose INFα2b evaluated at baseline and at defined timepoints post treatment.

**Down Modulation of MDSC by Immunotherapies**

9 out 18 patients showed increase in CD14+CD16+ population at 6-weeks time-point.

**Differential Modulation of Monocyte Subsets by I-O**

8 out 18 patients showed decrease in CD14+CD16+ population at 6-weeks time-point.

**Conclusions**

- This assay is reproducible and robust enough to detect CD33+CD11b+ MDSC cells and distinct myeloid cell subsets.
- It enables monitoring of dynamic changes in various myeloid populations after cancer treatment.
- Measurement of different myeloid subsets can provide deeper insights into activity of next generation immunotherapies targeting myeloid suppression pathways.

**References**

2. Picot T et al., Front Oncol, 8:1, 2018

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