Myeloid-derived suppressor cells (MDSC) assessment using an automated sequential chromogenic multiplex assay (Brightplex)

Anna Martirosyan, Assil Benchabane, Aurélie Collignon, Emilie Bonzom, Matthieu Duval, Alboukadel Kassambara, Felipe Guimaraes, Emmanuel Prestat, Christophe Haond, Jacques Fieschi | HalioDx, Marseille, France.

**Background**

The detection and quantification of MDSC populations is part of the Cancer Immunogram. Deciphering the complexity of tumor immune contexture is required to understand, predict and overcome resistance to anti-PD(1) immunotherapy of cancer.

In the context of NSCLC, MDSC accumulation in the tumor environment is associated with unfavorable prognosis and correlates with:
- The progression-free survival,
- The response to chemotherapy,
- The metabolic burden in patients.

MDSC are a heterogeneous population of cells:
- Characterised by their myeloid origin and immature state
- Endowed with highly suppressive machinery
- Hamper both innate and adaptive immune responses
- M-MDSC are suspected to be more immunosuppressive than PMN-MDSC

Like all immune cells, phenotyping MDSC requires a highly complex combination of biomarkers. **Brightplex**, a multiplex technology initially developed to characterize T cell exhaustion, was successfully applied to the detection and quantification of MDSC on a single section of NSCLC FFPE tissue.

**Method**

**STEP 1: Sequential Multiplex IHC Workflow**
- **Module 1**: Virtual slide alignment
- **Module 2**: Creation of pseudo-color image
- **Module 3**: Detection of positive cells for each biomarker
- **Module 4**: Identification of regions of interest (ROIs: core tumor, invasive margin, tumor parenchyma/struma)

**STEP 2: Digital Pathology**
- Virtual slide with immunostaining
- Calculation of tumor-infiltrating myeloid cell densities
- Map reconstruction to locate cells of interest within tumor

**A) Unique combination of biomarkers to detect different MDSC populations on a single tissue section**

Monocytic MDSC (M-MDSC) and polymorphonuclear MDSC (PMN-MDSC) are two major subpopulations of MDSC described by their phenotypical, morphological and functional characteristics. Both subsets are found in many cancers. PMN-MDSC generally represent more than 80% of all MDSC.

**B) Hierarchical gating based on staining intensity to identify populations of Tumor-Infiltrating Myeloid Cells**

**C) Assessing the abundance of major myeloid cell populations within NSCLC microenvironment**

MDSC and other tumor infiltrating myeloid cell types were analyzed within different regions of interest.

**D) Myeloid cells distribution within NSCLC landscape**

Reconstructed maps showing MDSC spatial distribution within tumor context.

One color corresponds to one phenotype:
- TMI (Tumor-infiltrating myeloid cells)
- M-MDSC
- PMN-MDSC
- Granulocytes
- Neutrophils
- Monocytes

**Conclusion**

Here we present Brightplex, an integrative solution developed by HalioDx for sequential chromogenic multiplex IHC together with whole slide digital pathology analysis. This tool allows to:
- Evaluate multiple biomarkers specific to tumor infiltrating myeloid cells and spatial relationship between cells
- Maximize data harvesting per sample (tissue section)
- Make educated decisions and design new treatment strategies

**Future Directions**

The detection and quantification of MDSC, exhausted T cells (CD3, CD8, FOI, LAG3, TIM3 Brightplex panel) and other tests from the Cancer Immunogram could be key in Lung cancer to:
- Predict patient response to immunotherapy
- Analyze the mechanisms of anticancer drug action
- Evaluate the immunosuppressive landscape of NSCLC tumors