Excellent Sensitivity for Immune Cell Detection by the MiSelect R System

Background

Flow cytometry, the fluorescence-based measurement of cells and particles within a flow, is widely used in drug discovery for screening compound effects at the single-cell level. Flow cytometry represents the gold standard for accurate determination of specific immune cells. However, as the rarity of the target cells falls to less than 1 in 100,000 background cells, it becomes more difficult to get accurate and absolute sorting and analysis using flow cytometry.

The MiSelect R System was developed specifically for rare cell isolation and identification, including rare immune cell profiling. By using the eDAR (ensemble-decision aliquot ranking) process, the MiSelect R System is able to sort and enrich rare immune cells from a background of whole blood.

Whole blood is labelled with the staining reagents in our SelectKit or other fluorescent cell surface antibodies. Once the initial labeling is completed, the blood is loaded into the instrument, and the rest of the process runs automatically. As an aliquot of blood passes through the detection region in the microfluidic channel, a laser illuminates and excites any fluorescent labels present in the aliquot. If a signal is detected from the target cell, it along with the rest of the cells in the aliquot, are sorted into a separate area of the chip. Here blood cells, especially RBCs, are removed from the target cells by an on-chip filtration system. After enrichment of the cells, staining reagents from the SelectKit are automatically added for imaging, identification, and enumeration of the target cells.

Methods

1. Sample Preparation

Blood samples from 12 healthy donors (HD) are collected into separate EDTA tubes and used to detect immune cells using both Flow cytometry and the MiSelect R System. For flow cytometry analysis, blood samples are processed in Ficoll gradient. The buffy coat is stained with PE-labeled PD-1, FITC-labeled CD8, APC-labeled TIM3, PerCP-labeled IFN-γ, PE-Cy7-labeled LAG3, and APC-Cy7-labeled CD3 antibodies.

For the MiSelect R System, whole blood samples are incubated with PE-labeled PD-1 antibody according to MiCareo's blood sample preparation instructions. Isolated PD-1 positive cells are further incubated by the MiSelect R system with FITC-labeled CD8, APC-labeled TIM3, PerCP-labeled IFN-g, QDot 625-labeled CD3, and QDot 800-labeled LAG3 for immune cell identification. All tests are performed blind and in duplicate.

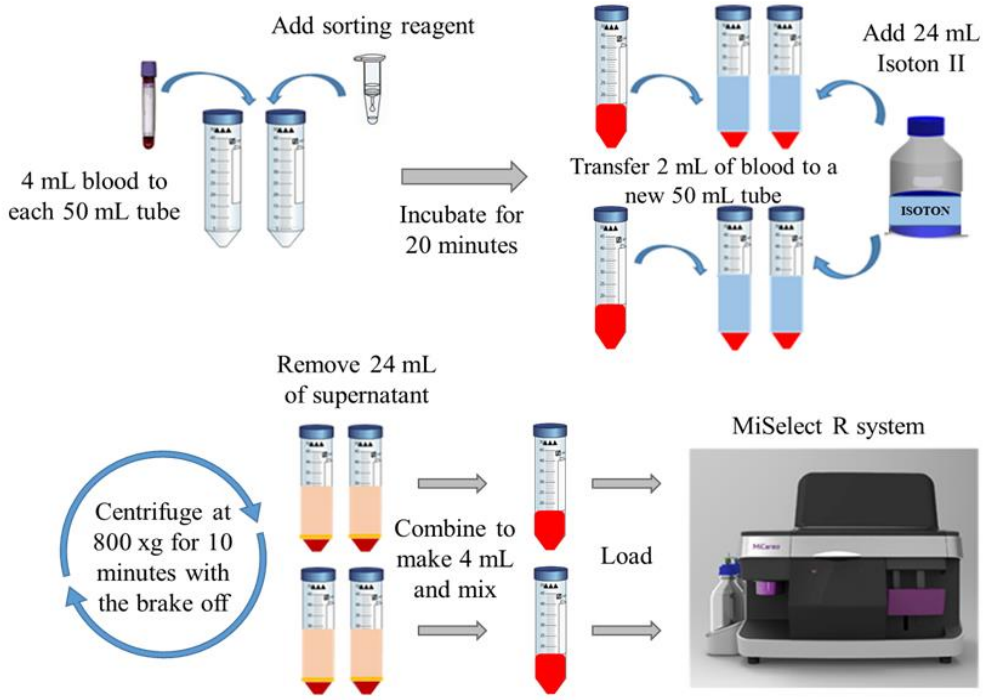

Figure1. Whole blood sample preparation

2. Immune cell identification with the MiSelect R

The MiSelect R System automatically labels and images the isolated cells with a panel of fluorescent antibodies for phenotypic profiling. Each cell is classified as a particular immune cell when its morphological features are consistent and it exhibits the target phenotype.

Results

The MiSelect R is found to be 2-fold more sensitive than flow cytometry at detecting immune cells, such as CD3(+), CD8(+) T cells, CD3(+), PD-1(+) T cells and CD8(+), PD-1(+) T cells.

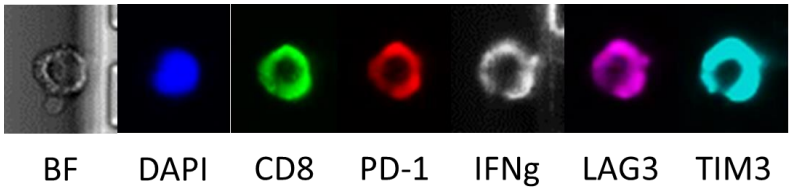

Figure 2. Images from the MiSelect R of a PD1(+), CD8(+), IFNγ(+), TIM3(+), LAG3(+) T cell.

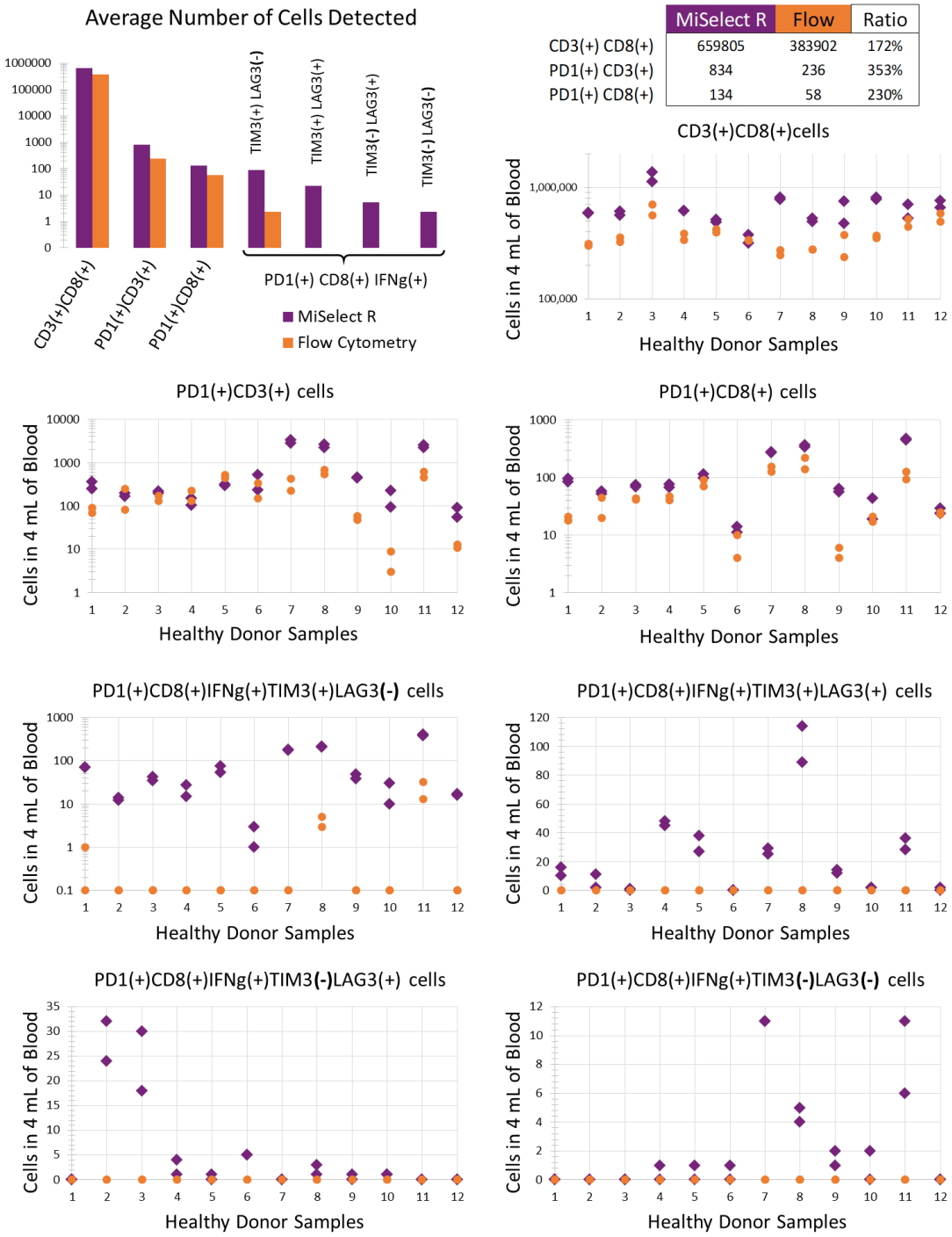

Figure 3. Side by side comparison of immune cells detected by the MiSelect R System and by flow cytometry from 12 healthy donors.

Conclusion

The MiSelect R System is able to isolate and quantify rare immune cells for profiling and subtyping with greater sensitivity than flow cytometry.