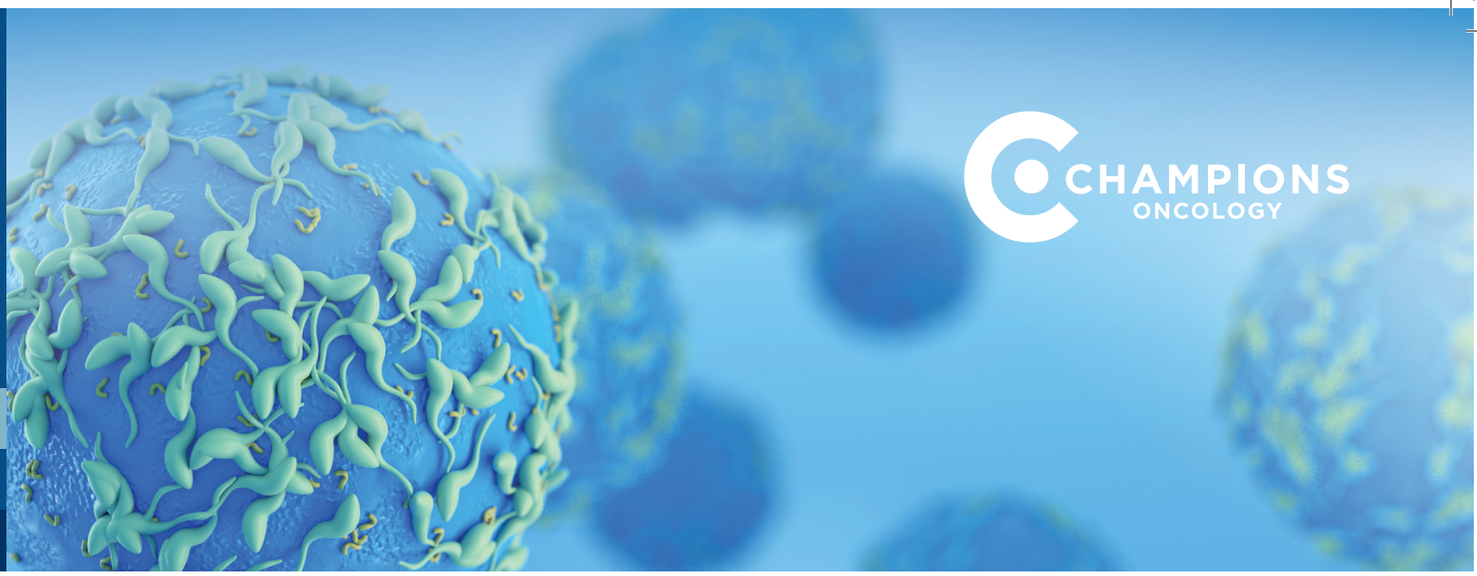
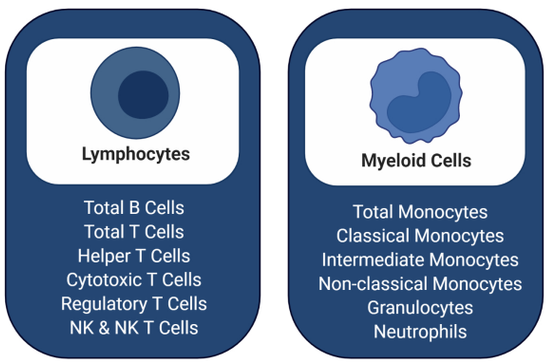
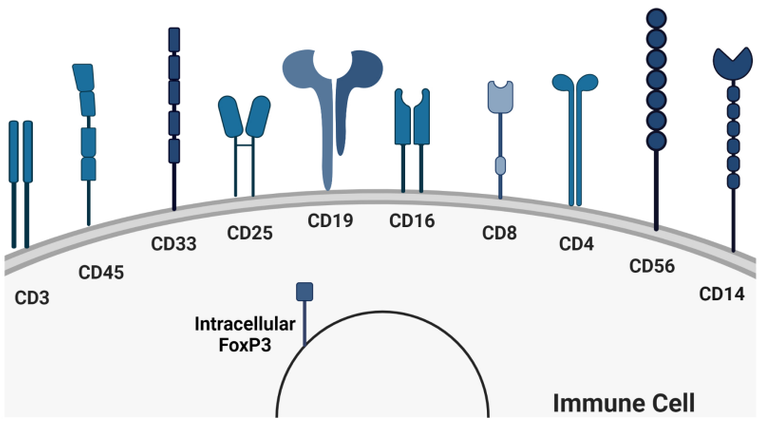


Validated Human Complex Immunophenotyping Panel II

In this Complex Immunophenotyping Panel II, Champions Oncology can interrogate multiple immune cell subset populations in Whole Blood. This 12 color panel builds on the Complex Immunophenotyping Panel I by including two additional markers to evaluate Regulatory T Cells. A Live/Dead staining component is included in this panel, which allows for clean separation of the subsets without risking dead cell contamination. Champions Oncology has the capacity to execute up to 24-color fully optimized and validated flow cytometry markers in a single tube, therefore maximizing the value of your precious human clinical trial samples. This panel has been validated by our GCLP-trained flow cytometrists and is ready for off-the-shelf use.





Flow Cytometry Methodology

For the Complex Immunophenotyping Panel II, Champions Oncology scientists took 3 fresh naïve whole blood human samples. Samples were measured at 6 timepoints (3 replicates/sample/timepoint) to evaluate post-collection sample stability. At each timepoint, fluorescent antibodies were added to each sample for the staining of surface and intracellular markers. A Live/Dead staining component was used to discriminate dead cells contamination. FMX controls were included in the analysis. Beads were added to each tube to measure absolute cell number (number of cells/µL blood). Samples were collected on our BD Symphony instrument and analysis was completed using FlowJo Software.

CHAMPIONS ONCOLOGY

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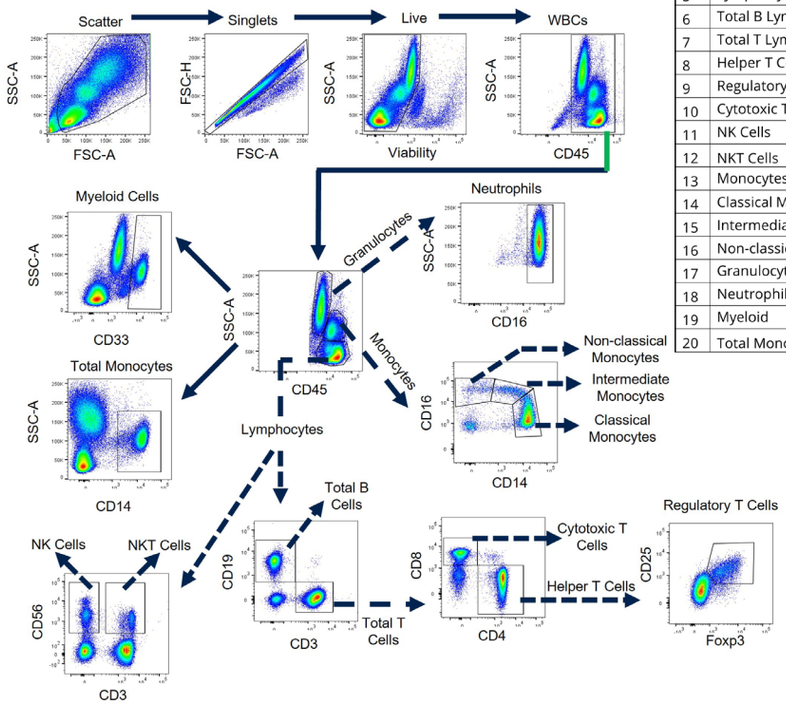
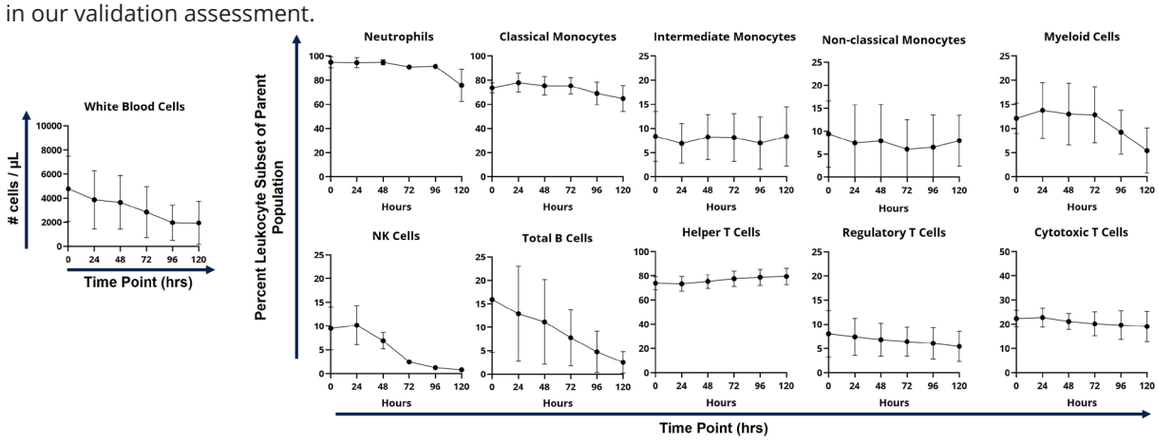
Complex Immunophenotyping Panel II: Gating Strategy



	Gate Description	Parent Gate	Gate Variables	Population Name	Reportables
1	Scatter	N/A	FSC-A vs SSC-A	Scatter	Abs, %
2	Singlets	Scatter	FSC-A vs FSC-H	Singlets	Abs, %
3	Live Cells	Singlets	Live/Dead vs SSC-A	Live Cells	Abs, %
4	White Blood Cells (WBCs)	Live Cells	CD45+ vs SSC-A	WBCs	Abs, %
5	Lymphocytes	WBCs	CD45+ vs SSC^{low}	Lymphocytes	Abs, %
6	Total B Lymphocytes	Lymphocytes	CD3- vs CD19+	Total B Cells	Abs, %
7	Total T Lymphocytes	Lymphocytes	CD3+ vs CD19-	Total T Cells	Abs, %
8	Helper T Cells	Total T Lymphocytes	CD4+ CD8-	Helper T Cells	Abs, %
9	Regulatory T Cells	Helper T Cells	FoxP3+ vs CD25+	Regulatory T Cells	Abs, %
10	Cytotoxic T Cells	Total T Lymphocytes	CD4- CD8+	Cytotoxic T Cells	Abs, %
11	NK Cells	Lymphocytes	CD3- vs CD56+	NK Cells	Abs, %
12	NKT Cells	Lymphocytes	CD3+ vs CD56+	NKT Cells	Abs, %
13	Monocytes	WBCs	CD45+ vs SSC^{med}	Monocytes	Abs, %
14	Classical Monocytes	Monocytes	CD14+ vs CD16-	Classical Monocytes	Abs, %
15	Intermediate Monocytes	Monocytes	CD14+ vs CD16+	Intermediate Monocytes	Abs, %
16	Non-classical Monocytes	Monocytes	CD14- vs CD16+	Non-classical Monocytes	Abs, %
17	Granulocytes	WBCs	CD45+ vs SSC^{high}	Granulocytes	Abs, %
18	Neutrophils	Granulocytes	CD16+ vs SSC-A	Neutrophils	Abs, %
19	Myeloid	WBCs	CD33+ vs SSC-A	Myeloid cells	Abs, %
20	Total Monocytes	WBCs	CD14+ vs SSC-A	Total Monocytes	Abs, %

Complex Immunophenotyping Panel II: Validation of Marker Stability

Each cell subset population was analyzed by timecourse (6 timepoints with 3 replicates/sample/timepoint) to reveal their stability in the panel. NK Cell, Myeloid Cell, and B Cell marker expression declines throughout the timecourse, while Neutrophil, Monocyte, Helper T Cell, Regulatory T Cell, and Cytotoxic T Cell marker expression remains stable through 120 hours in our validation assessment.



Our scientific experts in GCLP-Compliant flow cytometry can provide advice and guidance for all of your clinical trial needs. Reach out to your Business Development Representative to learn more about using this panel in your upcoming clinical trial.

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