Multiparametric Flow Cytometry

PRECISION for medicine®

Unlocking Potential: Flow Cytometry for Breakthrough Research

Flow cytometry is a crucial technology for scientific research and therapeutic development, providing detailed insights into cellular dynamics and biomarker exploration.

Precision for Medicine offers a suite of flow cytometry services tailored to the intricate demands of modern scientific research and therapeutic development.

Tailored Solutions for Diverse Research Needs

Understanding and meeting your specific requirements is our priority. We offer finely tuned multiparameter flow cytometry assays, designed in close collaboration with you to ensure your research objectives are seamlessly met.

Customization at Its Best

Precision provides custom assay development in addition to a range of readily available panels. We specialize in creating intricate multicolor panels with up to 40 parameters, which can be tailored to align with your specific research needs.

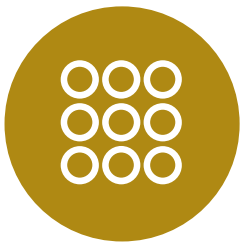
Advanced Techniques and Technology

Our range of techniques, including magnetic bead enrichment and the use of both conventional and spectral flow cytometers, are designed to deliver reliable and reproducible results. Assays can be validated to CLIA and used for patient selection.

Comprehensive Services

Our comprehensive flow cytometry services encompass analyses that support exploratory, secondary, and primary endpoints. Assays can be validated under CLIA for novel therapeutic research. All of these are performed using advanced flow cytometry technology to ensure the highest quality results.

Established experience in cell therapy, immuno-oncology, hematology, and rare disease therapies, with validations up to CLIA standards



Validated panels for faster turnaround times, catering to urgent research needs

Global flow cytometry supported by Precision's central lab services, ensuring sample integrity with real-time quality control

The Power of Flow Cytometry Applications

Through the characterization of the immune response, flow cytometry as a tool for immune surveillance can enable the identification of biomarkers and exploration of immune correlates in immunotherapy trials as well as facilitate an understanding of pharmacokinetics (PK) and pharmacodynamics (PD) through receptor occupancy.

Our experts specialize in developing complex multicolor panels through both conventional and spectral flow cytometry techniques. These advanced methodologies empower clinical trial advancements by enabling precise cell identification and characterization.

This approach facilitates the comprehensive analysis of multiple targets within cell subsets from limited samples, streamlining the process of simultaneous interrogation.

We offer a wide range of **techniques (blue)** with **applications (gold)**, including, but not limited to, the following:

Receptor Occupancy (RO) Assays	**Immunophenotyping**	**Phospho-Flow Analysis**	**Intracellular Cytokine Staining (ICS)**
Cell Therapy Flow Cytometry	**Cellular Function and Activation**	**Peptide and Antigen-Specific Flow Cytometry**	**Exploratory, Primary/Secondary Endpoint, CLIA**
Real-Time Whole Blood Flow Cytometry Assays	**Tumor Microenvironment Analysis (TILs)**	**Cell Enumeration Panels**	**Fluorescence-Activated Cell Sorting (FACS)**

Flow Cytometry Services Overview

Three Precision specialty laboratories offer flow cytometry services and are supported by 13 sample processing locations worldwide to handle global programs.

	Texas, United States	Maryland, United States	Berlin, Germany
Available Instruments	• BD FACSCanto™ —8-color • Cytek® Aurora —64-color spectral flow	• BD LSRFortessa™ —18-color • BD FACSymphony™ A5—31-color • BD FACSCanto™—10-color	• BD LSRFortessa™ —18-color • BD FACSAria™ Fusion Cell Sorter—16- to 18-color
Lab Certifications	GCP, CLIA	GCP, CAP, CLIA, ISO-13485, ISO-15189, ISO-9001	GCP/ICH, ISO-17025

BD FACSCanto™— 8- to 10-color

BD LSRFortessa™— 18-color

BD FACSymphony™ A5—31-color

Cytek® Aurora — 64-color spectral flow

BD FACSAria™ Fusion Cell Sorter— 16- to 18-color

Overcoming Assay Challenges With Precision

At Precision, standardization across all sites is key. We address the challenges posed by multicenter immune monitoring trials by ensuring harmonized and reproducible sample testing across our labs, via standard operating procedures (SOP).

Custom Validation

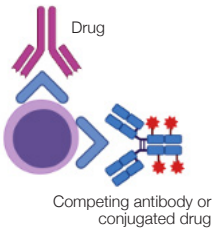
Our fit-for-purpose validation approach aligns with regulatory expectations and clinical objectives, incorporating extensive characterization to guarantee reagent specificity.

Receptor Occupancy: Understanding Therapeutic Engagement With Disease Targets

Receptor occupancy (RO) assays are a cornerstone in validating therapeutic antibody engagement and function and are pivotal for delineating PK/PD relationships. These assays are instrumental from preclinical selection through to late-stage clinical trials, shaping compound selection and informing dosing strategies.

Precision's offerings include:

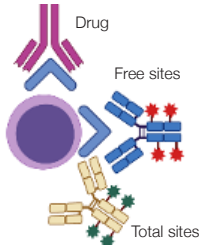
- Target Engagement Validation: Our RO assays rigorously confirm therapeutic engagement with disease targets, guiding compound selection and verification.
- Dosing Strategies: We specialize in the quantification of drug-receptor interactions to refine dosing regimens for early-phase trials and beyond.
- Efficacy and Safety Correlations: In late-stage development, our RO flow cytometry data support the correlation of therapeutic efficacy with population pharmacokinetics.



Common RO Assay Formats

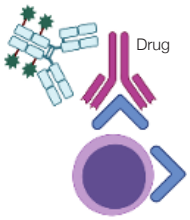
Free Receptor Assay

- Measures unbound or unoccupied receptors on a cell using an unlabeled antibody and a competing therapeutic antibody or drug-conjugated antibody
- Typically used for antagonistic therapeutic drugs that block ligand binding to the receptor



Total Receptor Assay

- Measures binding of a therapeutic antibody to its receptor site and couples with binding of an alternative antibody that binds to a different site of the same receptor
- Useful where receptor level or cell numbers can change over time



Direct Assessment of Bound Receptor

- Measures therapeutic antibody bound to the receptor with an antitherapeutic antibody detection reagent
- Often applied when monoclonal antibodies to the receptor are not available or conjugation of therapeutic antibody compromises binding to target

Immunophenotyping: Precision-Driven Cellular Analysis

Unveiling Immune Complexity With Advanced Immunophenotyping

Immunophenotyping stands as a pillar of flow cytometry, offering detailed insights into the composition, as well as the activation and exhaustion status of immune cells.

This sophisticated analysis is pivotal for deciphering the immune landscape in health and disease, from research and development to monitoring immunological changes induced during therapeutic treatment.

Our Immunophenotyping Expertise

Precision for Medicine brings exceptional depth to immunophenotyping with a range of comprehensive services:

- **Multicolor Analysis:** We employ advanced techniques to provide rich, multicolor analysis, capturing a detailed picture of immune cell populations.
- **Custom Panel Development:** Tailoring our approach to each study, we design custom panels that meet the specific needs of your research, from standard markers to specialized probes.
- **Quantitative Precision:** Our protocols are fine-tuned for high sensitivity and specificity, ensuring precise quantification of immune cell subsets for robust data interpretation.

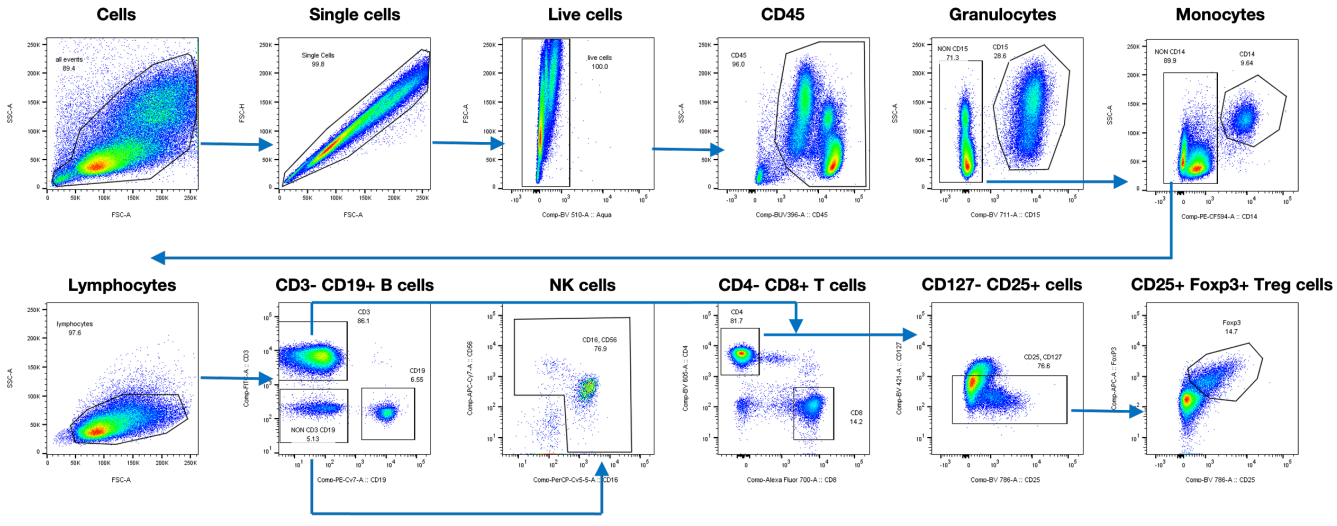


Figure 1. Immune cell subsets identified with an immunophenotyping panel include CD15+ granulocytes, CD14+ monocytes, CD3+ T cells, CD4+ T cells, CD8+ T cells, CD19+ B cells, CD16+ CD56+ NK cells, and CD127-CD25+ FoxP3+ Treg cells.

Phospho-Flow Cytometry: Deciphering Cell Signaling Pathways

Phospho-flow analysis is a technique used to measure the activation state of intracellular signaling molecules, such as phosphorylated ERK or STAT. By quantifying phosphorylated proteins, this method provides a snapshot of cellular function in response to stimuli, crucial for understanding disease mechanisms and therapeutic effects.

Precision's Expertise in Phospho-Flow:

- **Targeted Detection:** Precise measurement of the phosphorylation status of proteins within individual cells delivers insights into the activation state of cellular signaling pathways.
- **Multiplex Capability:** Simultaneous detection of multiple phosphorylated targets allows a comprehensive view of complex cellular signaling cascades.
- **Dynamic Response Measurement:** Monitoring of cellular responses provides a dynamic picture of cell signaling in real time or after specific treatments.

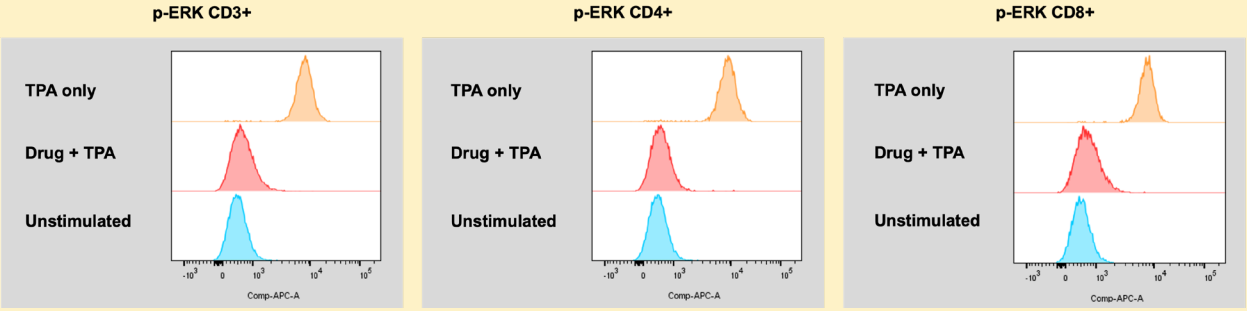
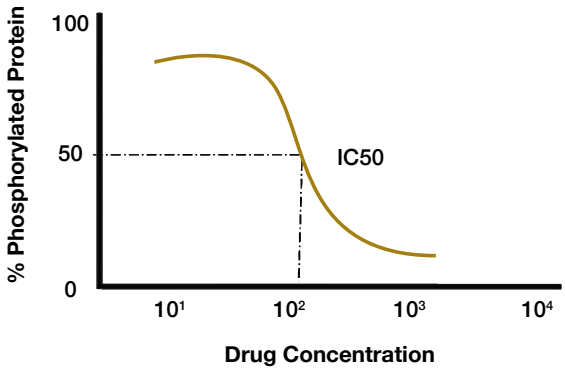


Figure 2. Tetradecanoyl Phorbol Acetate (TPA) stimulation-induced phospho-ERK (p-ERK) expression was inhibited by drug in CD3+, CD4+, and CD8+ T-cell populations.

Impact on Research and Drug Development:

- **Mechanistic Insights:** Offer critical data for elucidating drug action mechanisms and identifying potential therapeutic targets.
- **Biomarker Identification:** Assists in discovering predictive biomarkers of drug response.
- **Clinical Trial Support:** Enhances the understanding of therapeutic impacts on signal transduction pathways, supporting both exploratory and confirmatory phases of clinical trials.



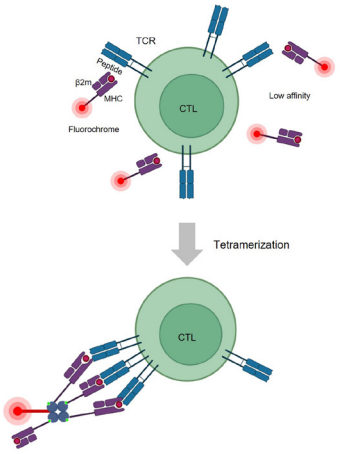
Measuring Antigen-Specific T-Cell by Flow Cytometry: Insights Into T-Cell Functionality

Antigen-specific flow cytometry is a technique used to measure the specific immune response to a particular antigen, such as a virus or a tumor antigen.

Precision has expertise in complex flow cytometry assays for the analysis of antigen-specific T cells using MHC I/II tetramers or dextramers or measurement of the induction of peptide-specific cellular responses through intracellular cytokine secretion (ICS).

Functional responsiveness by measuring cytokine production within cells by ICS offers invaluable insights into the cellular mechanisms underpinning immune responses, disease progression, and the efficacy of therapeutic interventions.

These techniques have been used to study immune responses to vaccination, inflammation, infection, and cancer immunotherapy.



Binding of MHC-peptide complexes to TCRs can be stabilized by increasing the avidity through the use of the multivalent MHC-peptide complex, called tetramer.

View the scientific poster

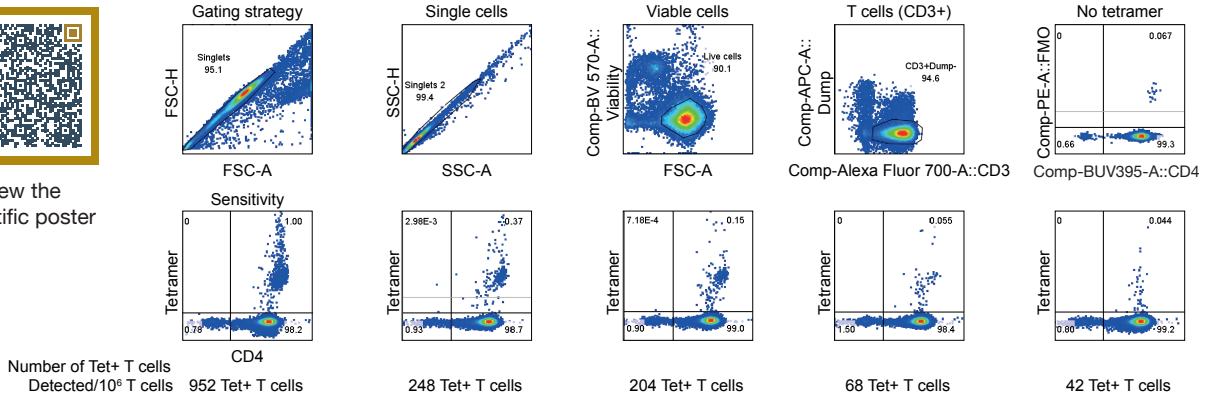


Figure 3. Titration of gliadin α-II tetramer-positive (Tet+) T cells in PBMCs measured by tetramer flow cytometry.

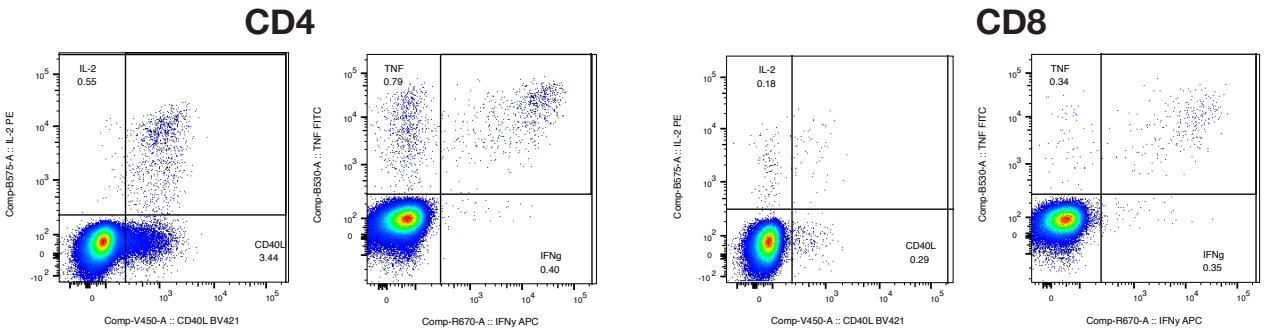


Figure 4. Intracellular cytokine (IFN-γ, TNF-α, IL-2, CD40L) secretion (ICS) by CD4+ and CD8+ T cells in response to CMV peptide stimulation.

Real-Time Whole Blood Flow Cytometry: Rapid Insights, Immediate Impact

Real-time flow cytometry assays that are run on whole blood provide swift and accurate insights into blood cell dynamics, essential for immediate clinical decisions and research observations.

- **Real-Time Analysis:** Our techniques enable rapid processing and analysis of blood samples, capturing cellular events at the time of collection.
- **Whole Blood Analysis:** We directly analyze whole blood samples, preserving the natural cellular state and interactions, crucial for accurate immunological assessments and determination of receptor occupancy.

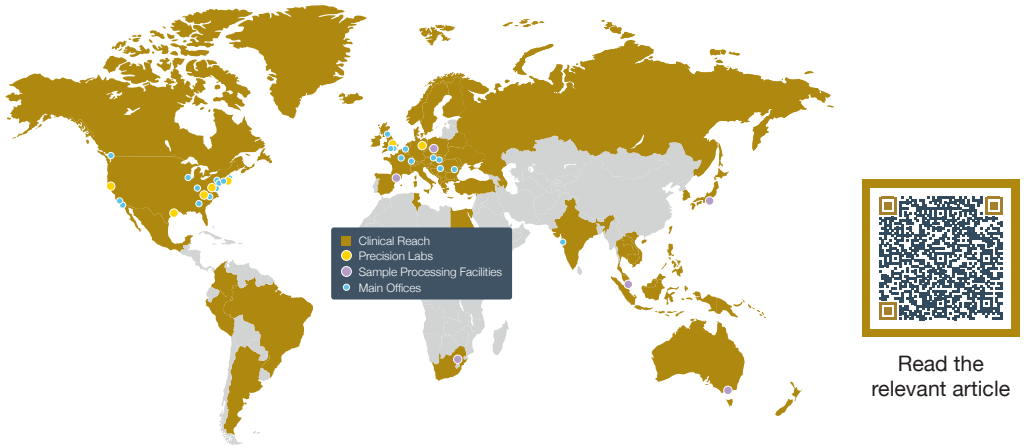


Figure 5. A global network of sample processing labs, specialty labs, and logistics services ensures reliable and robust assay results.

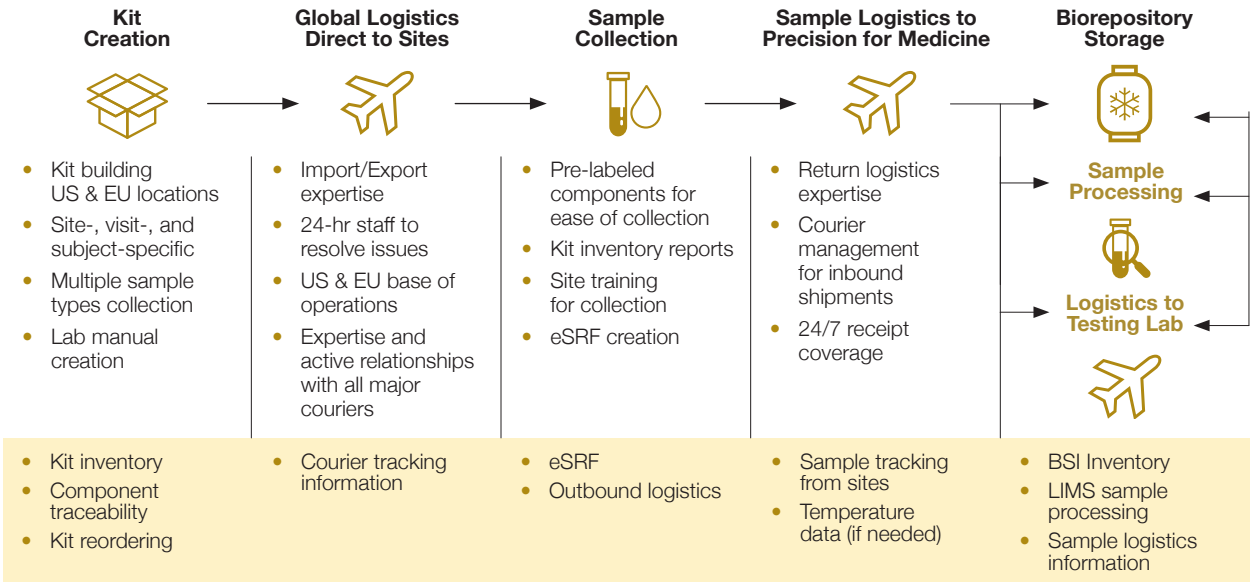


Figure 6. Customized kitting for sample collection and global logistic support ensure high-quality samples for specialty assays.

Sample Stabilization Solutions: Streamline Operational Execution

In situations where real-time flow cytometric analysis cannot be performed, Precision can advise on sample stabilization approaches. A variety of sample stabilization fixatives are now available, offering a key benefit of an extended sample testing window. Additionally, stabilizing fixatives may allow for operational execution of global studies to be streamlined, allowing for assays to be centralized in one lab, reducing costs and variability—critical for longitudinal samples or markers sensitive to time or temperature during transit.

Precision scientists carefully evaluate sample stabilization approaches and conduct feasibility studies to assess how fixatives affect flow cytometric staining of each antibody and drug target.

Our custom kits are also designed to simplify sample collection and stabilization, significantly reducing the burden on sites.

Case Study

Precision for Medicine was asked to develop a 10-color assay to assess engagement and modulation of cell-surface CD6 to evaluate pharmacodynamic properties of itolizumab treatment on T cells in patients with graft-versus-host disease (GVHD).

Whole blood collected at sites in New Zealand, Australia, and the United States was fixed and stabilized using SMART™ Tube proteomic stabilizer. This allowed for –80°C storage up to 3 months and subsequent batched flow cytometry testing.

View the scientific poster

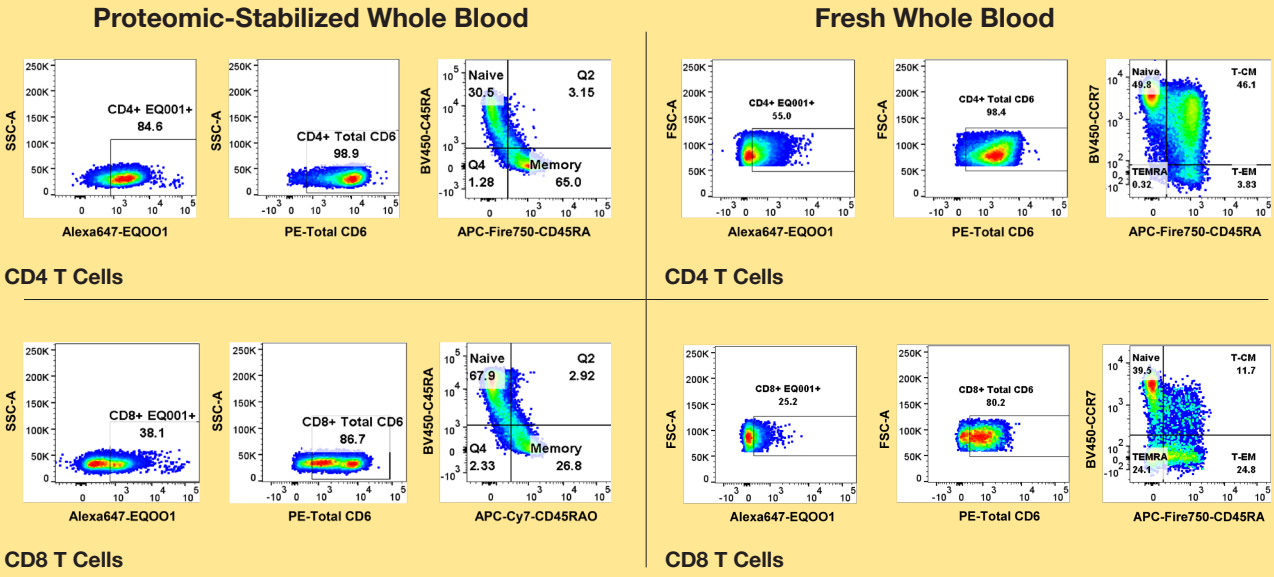
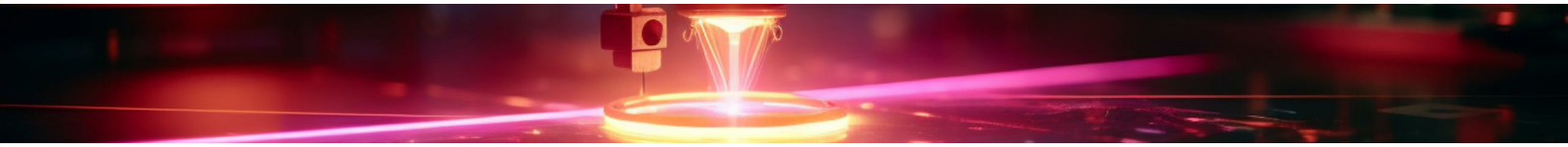


Figure 7. Assessment of CD6 engagement on CD4+ and CD8+ T cells. Memory cell phenotyping was compatible in whole blood fixed with SMART™ Tube proteomic stabilizer (left) when compared to fresh whole blood (right).

Investigating the Tumor Microenvironment by Flow Cytometry

The tumor microenvironment is a complex network of cells and molecules that play a critical role in cancer progression and treatment response. Our optimized sample collection and shipment kits, sample dissociation protocols, and multiparameter flow cytometry technology allow for the analysis of tumor-infiltrating lymphocytes (TILs) within the tumor microenvironment.

This technique has been utilized in studies examining the immune response to cancer immunotherapy, providing information on the type, activation, or exhaustion status of cells infiltrating the tumor. Additionally, the use of flow cytometry allows immunophenotypic comparisons between the tumor and the periphery.



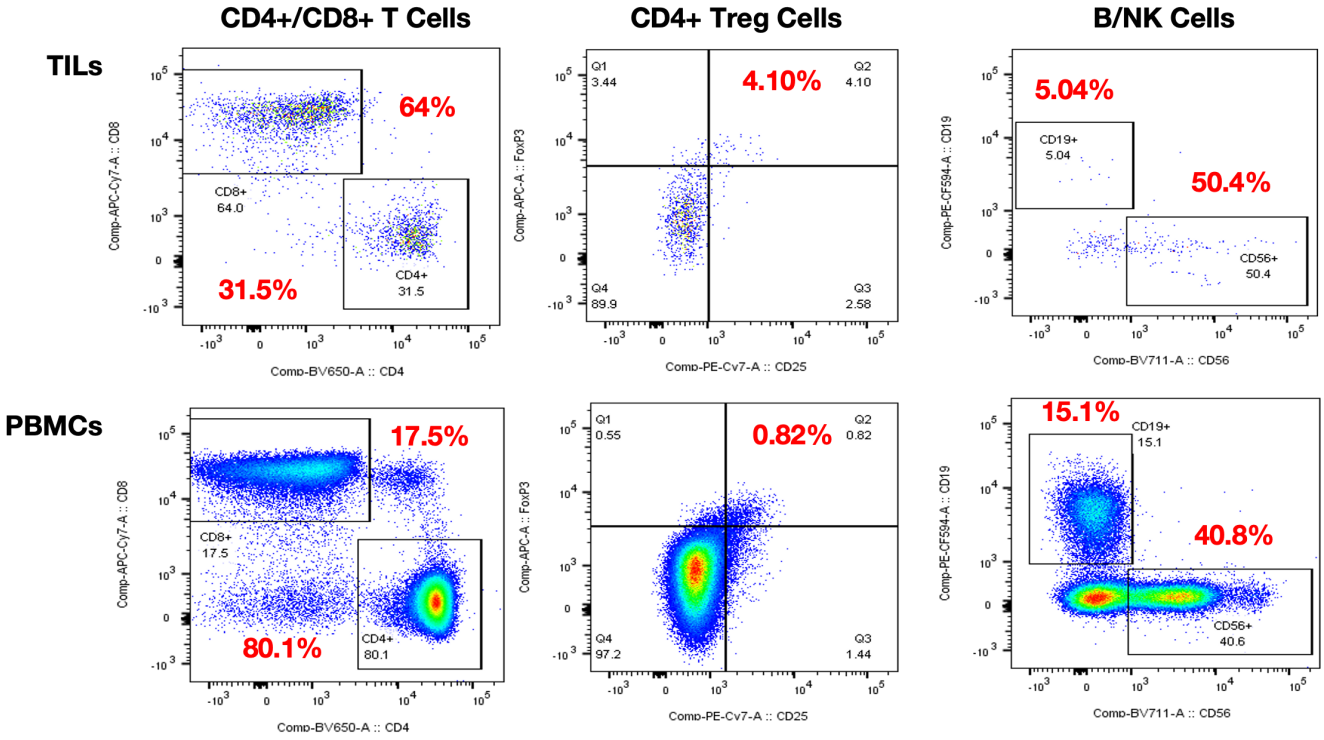


Figure 8. Tumor-infiltrating lymphocytes (TILs) and peripheral blood mononuclear cells (PBMCs) were isolated from a subject with melanoma. The TILs were obtained from fine-needle aspirate (FNA) biopsies, while the PBMCs were isolated separately from whole blood. Flow cytometric analysis revealed phenotypic differences between the TILs infiltrating the tumor microenvironment (TIL) and those in the periphery (PBMC).

Application of Flow Cytometry in Cell Therapy

Cell therapy is an emerging field that utilizes living cells as a therapeutic agent for various diseases, including cancer and autoimmune diseases. Our multiparameter flow cytometry assays can be used to characterize cell therapy products.

Additionally, flow cytometry is a valuable tool to monitor longevity of CAR T-cell populations residing in the periphery alongside molecular assays.

Our team of experts can design custom panels to assess cell viability, phenotype, and function, allowing for comprehensive characterization of cell therapy products.

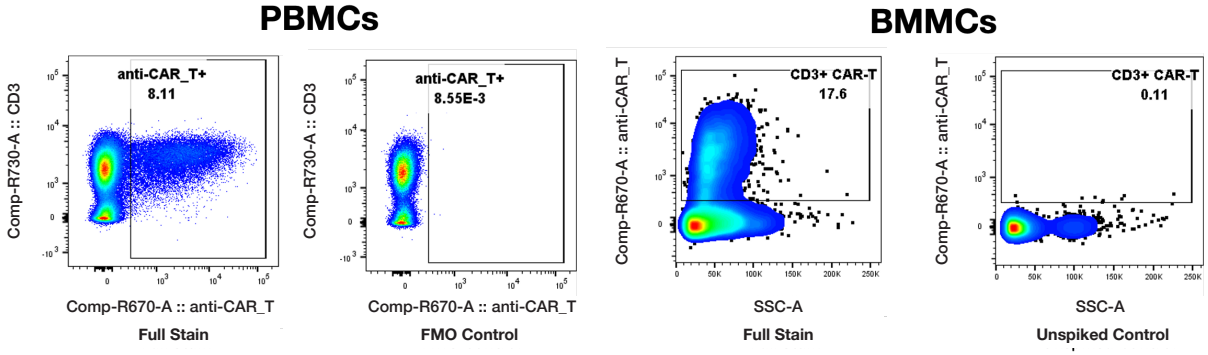


Figure 9. CAR T cells were spiked into healthy donor peripheral blood mononuclear cells (PBMCs) and bone marrow mononuclear cells (BMMCs) prior to detection with a 10-color flow cytometry panel. Fluorescence minus one (FMO) control staining or unspiked samples can be used as gating controls to identify CAR T cells.

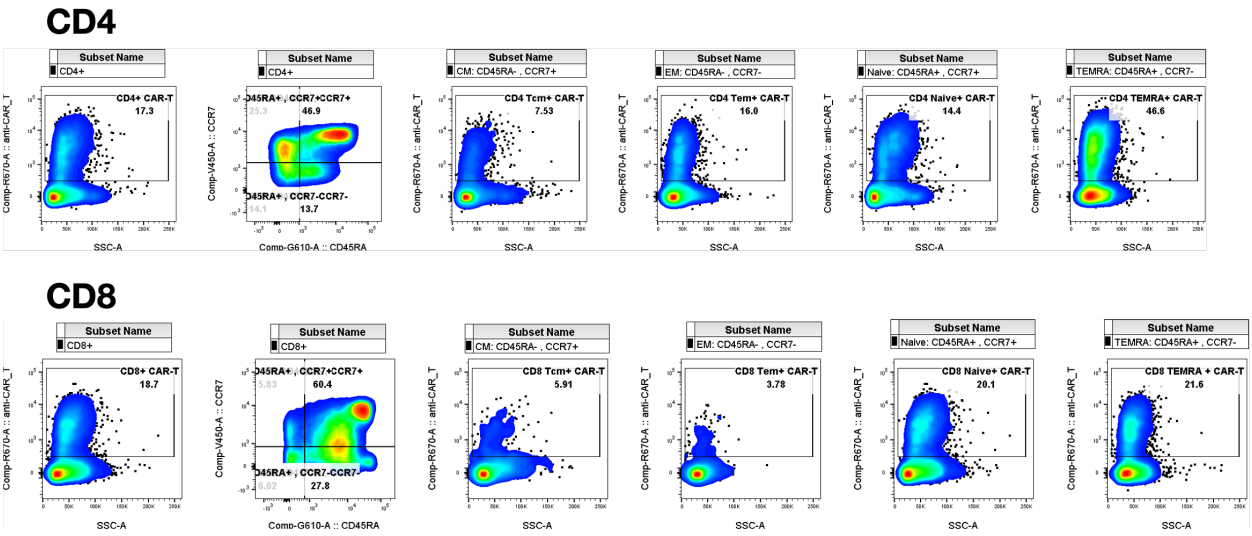


Figure 10. A 10-color flow cytometry panel was used to perform phenotypical characterization of the CAR T cells.

Fluorescence-Activated Cell Sorting (FACS): A Tool for Downstream Applications

Fluorescence-activated cell sorting (FACS) is a powerful technique used to isolate specific cell populations based on their phenotype. Precision utilizes FACS instruments that can perform up to 18-color sorts, allowing for efficient and accurate cell sorting.

Sorted immune cell populations can be assessed by further downstream applications such as functional assays, cell differentiation, RNA-seq, and epigenetic characterization.

BD FACSAria™ Fusion Cell Sorter — 16- to 18-color sorts: 4-way sorting capabilities, 384 well/96 well/single tubes

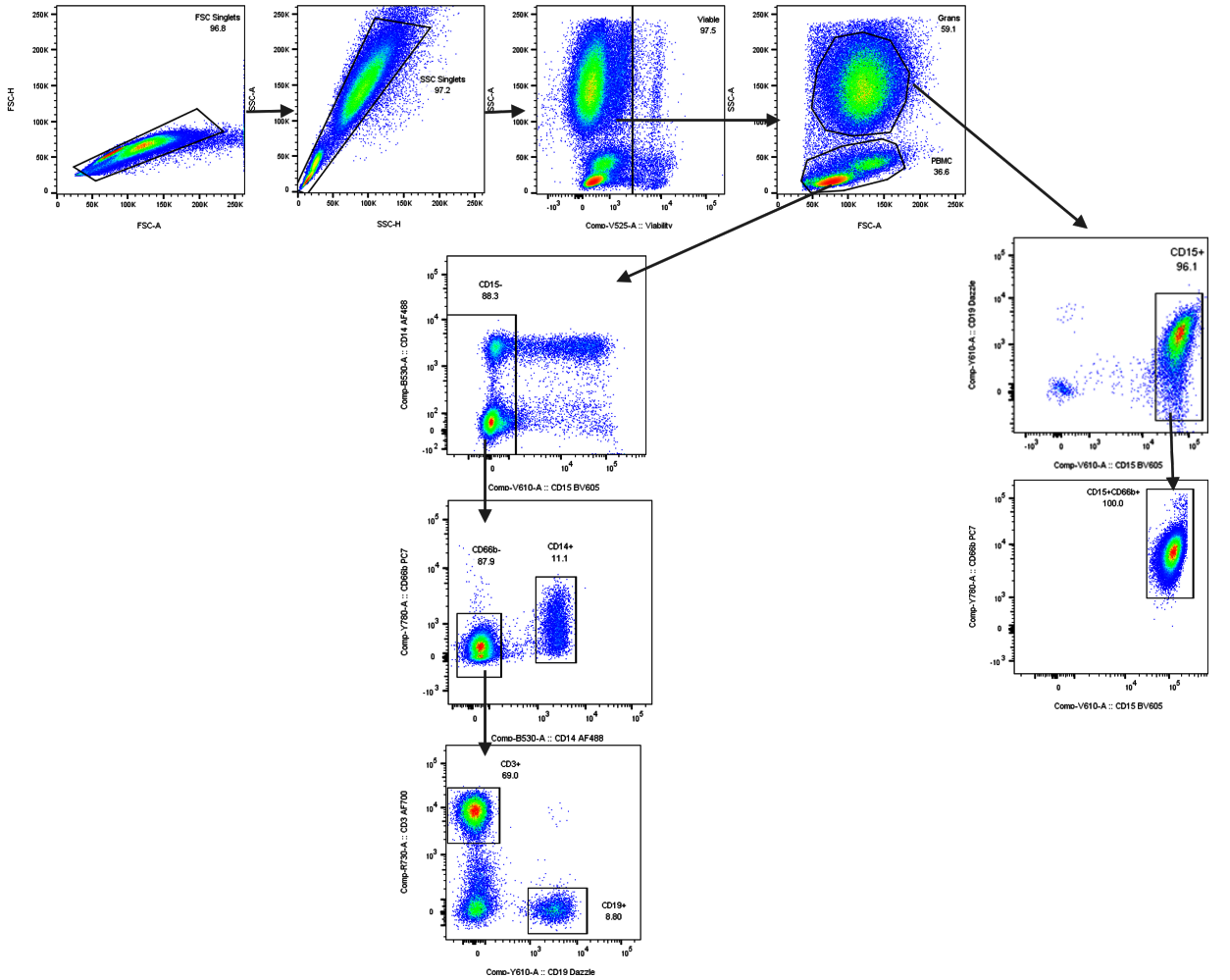


Figure 11. Cell sorting of PBMCs was performed to isolate T-cell, B-cell, monocyte, and granulocyte cell populations for further downstream applications.

Solving the most complex challenges in biomarker-driven and precision therapeutic development

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Rev. 01

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