Comprehensive Immune Response Profiling

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Cell Therapy – Immune Rejection Challenge

Success is dependent upon :

- **Prediction** of the immune potential of the cell based therapy
- **Detection** of the immune response specific to the therapeutic cells
- **Amelioration** of immune reaction to the therapeutic cells

Immune Response Monitoring

- **Cellular Response to the cell based therapeutic:**

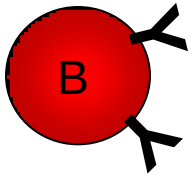
  Intra Cellular Cytokine Response following antigen challenge
  ELISPOT assay following antigen challenge
  CFSE dilution assay for measuring proliferative response
  MHC Tetramers – for estimating frequency of antigen specific T cells

- **Antibody Response : Bead based multiplexed assay platform with ability to detect response to multiple antigens/ epitopes including Ig subclass detection**

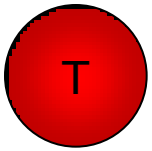
Comprehensive Immune Response Profile

Immunize

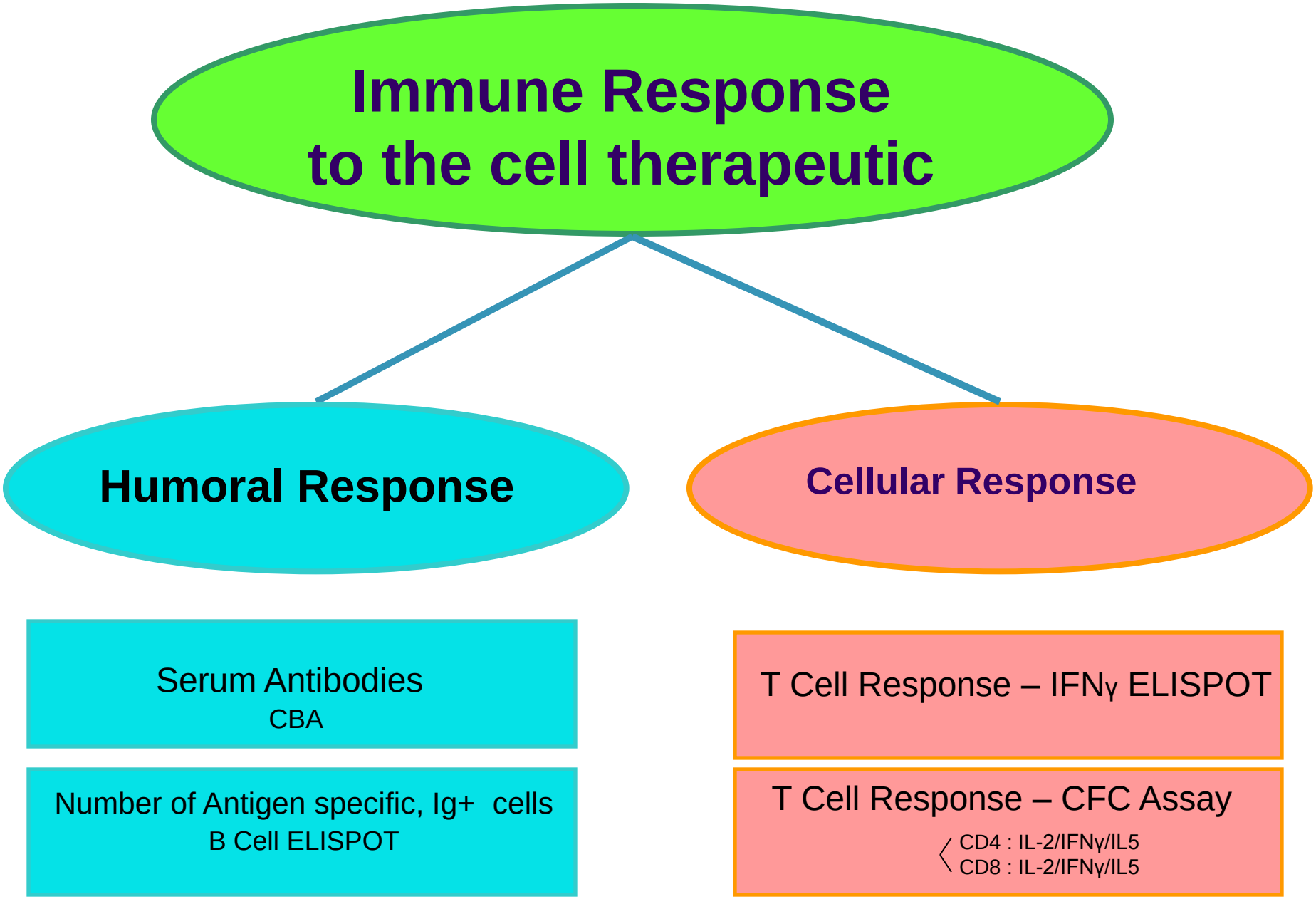
Development and Qualification of Assays



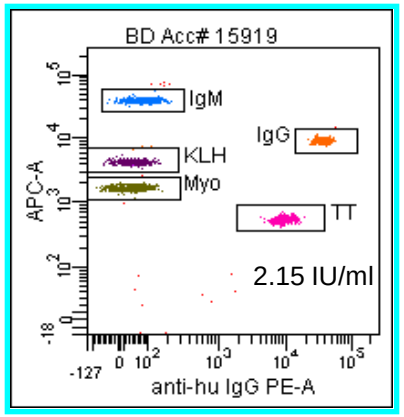
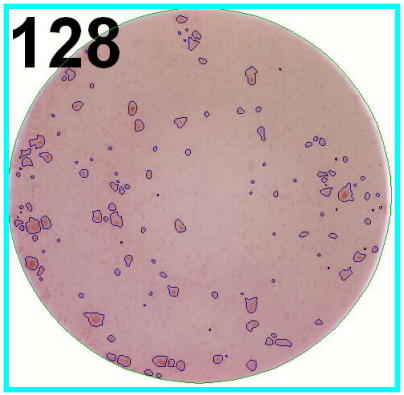
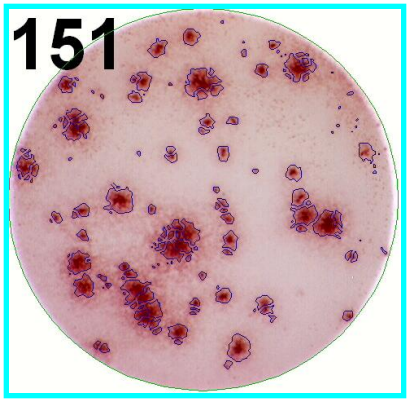
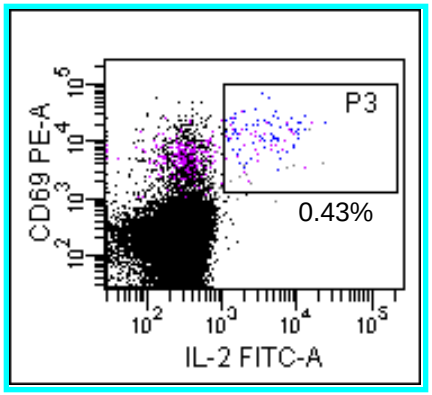
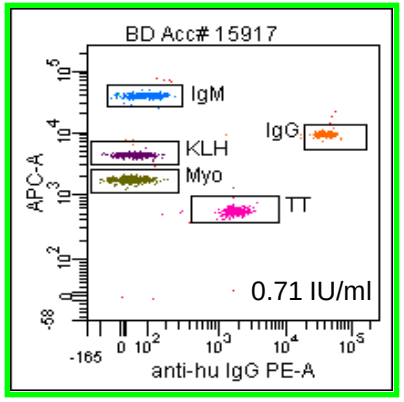
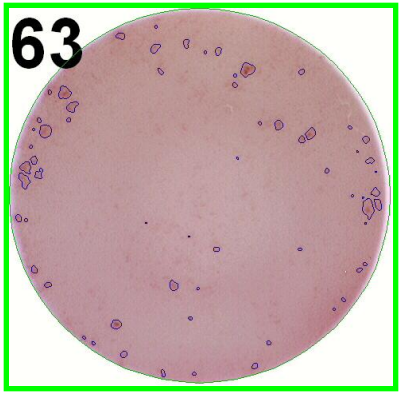
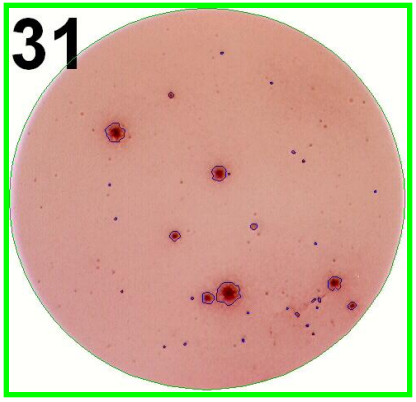
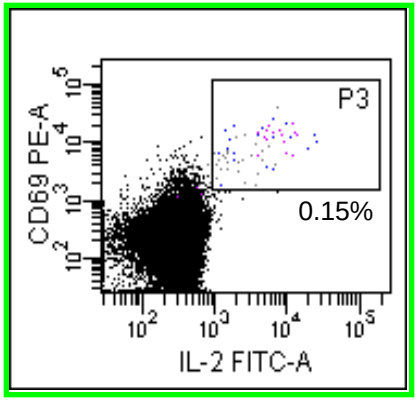
- Frequency of Antigen Specific B cells (slg)
  - Development of Ag specific B cell ELISPOT
- Correlation with Serum Antibodies
  - Using a custom CBA to detect antibodies



- Cellular Immune Response
  - Using Ag specific T cell ELISPOT
- Cellular Immune Response
  - IC cytokine assay using multiple cytokine as readouts in subsets of T cells


Immune Response
to the cell therapeutic
Humoral Response
Cellular Response
Serum Antibodies
CBA
Number of Antigen specific, Ig+ cells
B Cell ELISPOT
T Cell Response – IFNγ ELISPOT
T Cell Response – CFC Assay
CD4 : IL-2/IFNγ/IL5
CD8 : IL-2/IFNγ/IL5

Immune Response Analysis Example

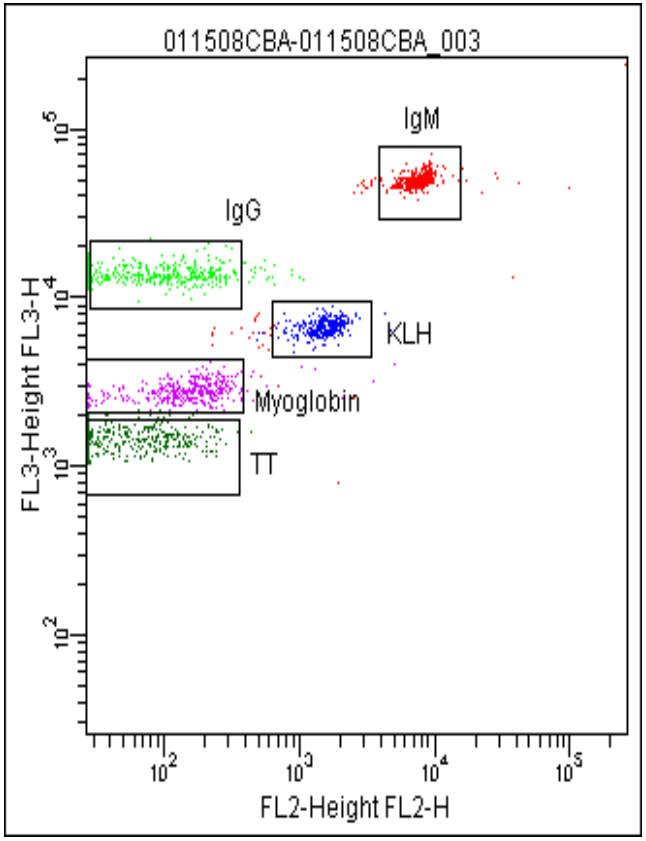
	CBA Data	B-Cell ELISPOT	T-Cell ELISPOT	CD4 - ICC
High				
Low				

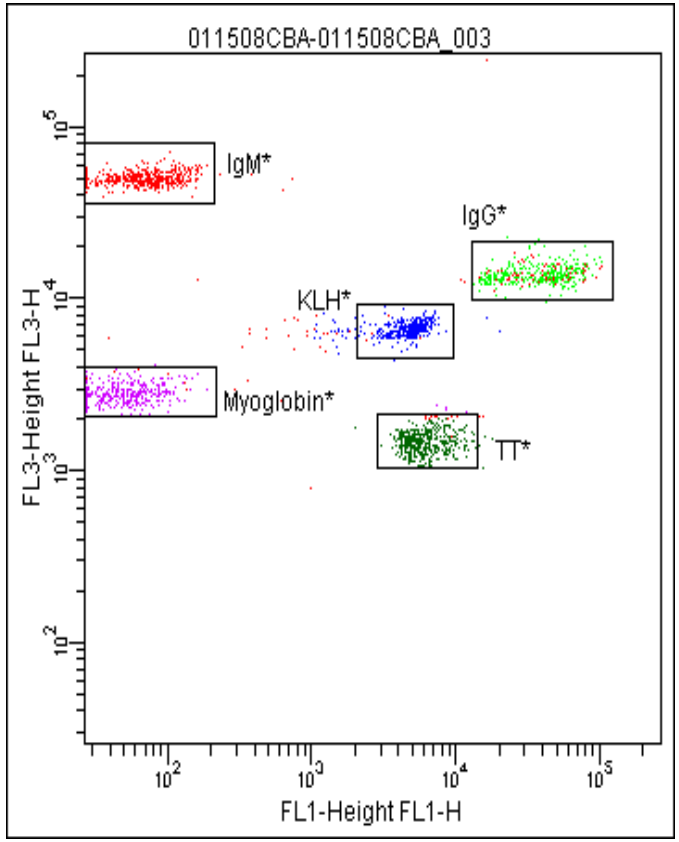
Immune Response Monitoring

- Cellular Response to the cell based therapeutic

- **Antibody Response : Bead based multiplexed assay platform with ability to detect response to multiple antigens/ epitopes including Ig subclass detection**

KLH /TT Antibody Assay :
IgG and IgM Detection from a Single Tube





ICS Quality Assurance Program

(EQAPOL) is a National Institute of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID), Division of AIDS (DAIDS) funded Immunology Quality Assessment Center (IQAC) to support the development, implementation and oversight of external quality assurance programs that monitor laboratories involved in HIV/AIDS research and vaccine trials around the world.

- **ICS (Intracellular Cytokine Survey) : Cellular Immune Response**
  - BD CTT has participated in 9 rounds of ICS surveys
  - They surveys are sent approximately twice (2x) per year
  - 13-15 laboratories around the world participate
  - 3 human peripheral blood lymphocyte (PBMC) samples are assayed in each survey
  - 4 and 7 color immunophenotyping panels are included in the testing
  - A summary report is provided which compares results from all the labs (blinded). The data from each laboratory is also compared to a “Gold Standard” established at the central reference laboratory

❖ EQAPOL ICS (Intracellular Cytokine Survey) RESULTS :

94 points; Laboratory Performance E (Excellent 90-100 points)

ELISPOT Quality Assurance Program

(EQAPOL) is a National Institute of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID), Division of AIDS (DAIDS) Immunology Quality Assessment Center (IQAC) to support the development, implementation and oversight of external quality assurance programs that monitor laboratories involved in HIV/AIDS research and vaccine trials around the world.

- ELISPOT (Enzyme-linked Immunosorbent Spot) : Cellular Immune Response
  - BD has participated in 9 rounds of ICS surveys
  - They surveys are sent approximately twice (2x) per year
  - 13-15 laboratories around the world participate
  - 3 human peripheral blood lymphocyte (PBMC) samples are assayed in each survey
  - The assay tests for **IFNg** producing cells
  - A summary report is provided which compares results from all the labs (blinded). The data from each laboratory is also compared to the “Gold Standard” established at the central laboratory at Duke.

Quality assurance of intracellular cytokine staining assays: analysis of multiple rounds of proficiency testing

J Immunol Methods. 2011 Jan 5;363(2):143-57. Epub 2010 Aug 19.
Jaimes MC, Maecker HT, Yan M, Maino VC, Hanley MB, Greer A, Darden JM, D'Souza MP.

Source

BD Biosciences, 2350 Qume Drive, San Jose, CA 95131, USA.

Abstract

When evaluating candidate prophylactic HIV and cancer vaccines, intracellular cytokine staining (ICS) assays that measure the frequency and magnitude of antigen-specific T-cell subsets are one tool to monitor immunogen performance and make product advancement decisions. To assess the inter-laboratory assay variation among multiple laboratories testing vaccine candidates, the NIH/NIAID/DAIDS in collaboration with BD Biosciences implemented an ICS Quality Assurance Program (QAP). Seven rounds of testing have been conducted in which 16 laboratories worldwide participated. In each round, IFN-γ, IL-2 and/or TNF-α responses in CD4+ and CD8+ T-cells to CEF or CMV pp65 peptide mixes were tested using cryopreserved peripheral blood mononuclear cells (PBMC) from CMV seropositive donors. We found that for responses measured above 0.2%, inter-laboratory %CVs were, on average, 35%. No differences in inter-laboratory variation were observed if a 4-color antibody cocktail or a 7-color combination was used. Moreover, the data allowed identification of important sources of variability for flow cytometry-based assays, including: number of collected events, gating strategy and instrument setup and performance. As a consequence, in this multi-site study we were able to define pass and fail criteria for ICS assays, which will be adopted in the subsequent rounds of testing and could be easily extrapolated to QAP for other flow cytometry-based assays.

Immune Response Prediction

- **Simultaneous measurement of multiple soluble mediators e.g., cytokines, chemokines (SARS example)**
- **Cellular Expression Profiling : Using over 220 Ab library against cell surface markers**
- **Kinase Pathway Probe : e.g., Phosflow**

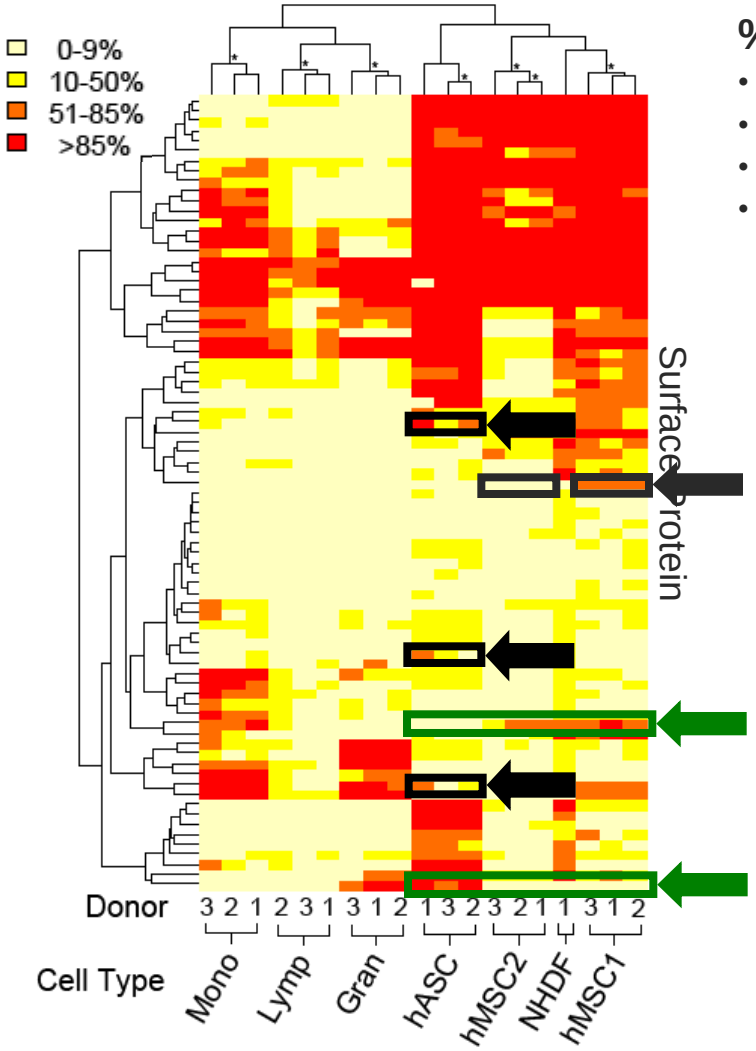
BD FACS CAP : Combinatorial Antibody Profiling

- FACS CAP is a powerful high throughput flow cytometry screening technology which enables the rapid characterization of human cell surface protein expression profiles

- This technology uses over 220 directly conjugated antibodies to profile the cell surface. Current format uses 229 antibodies formulated as three-color cocktails (FITC/PE/APC) arrayed in 96-well plates

- The majority of antibodies are directed against cell surface receptors. Of the 229 surface antigens, 208 are specific to a single protein, 11 bind small sets of related proteins, and 10 bind to uncharacterized proteins or carbohydrate antigens.

CTT: BD FACS – Combinatorial Antibody Profile



% positive cells for 79 surface proteins in human:

- Monocytes *(Mono)*, Lymphocytes *(Lymp)*, Granulocytes *(Gran)*
- Adipose-derived stem cells *(hASC)*
- Dermal fibroblasts *(NHDF)*
- Bone marrow-derived mesenchymal stem cells *(two preparations)*

Inventory of Receptors and Ligands

Donor-to-Donor Variations, for:

- Donor Qualification
- QA
- Expression/Function Correlations

Cell Type-Specific Markers, for:

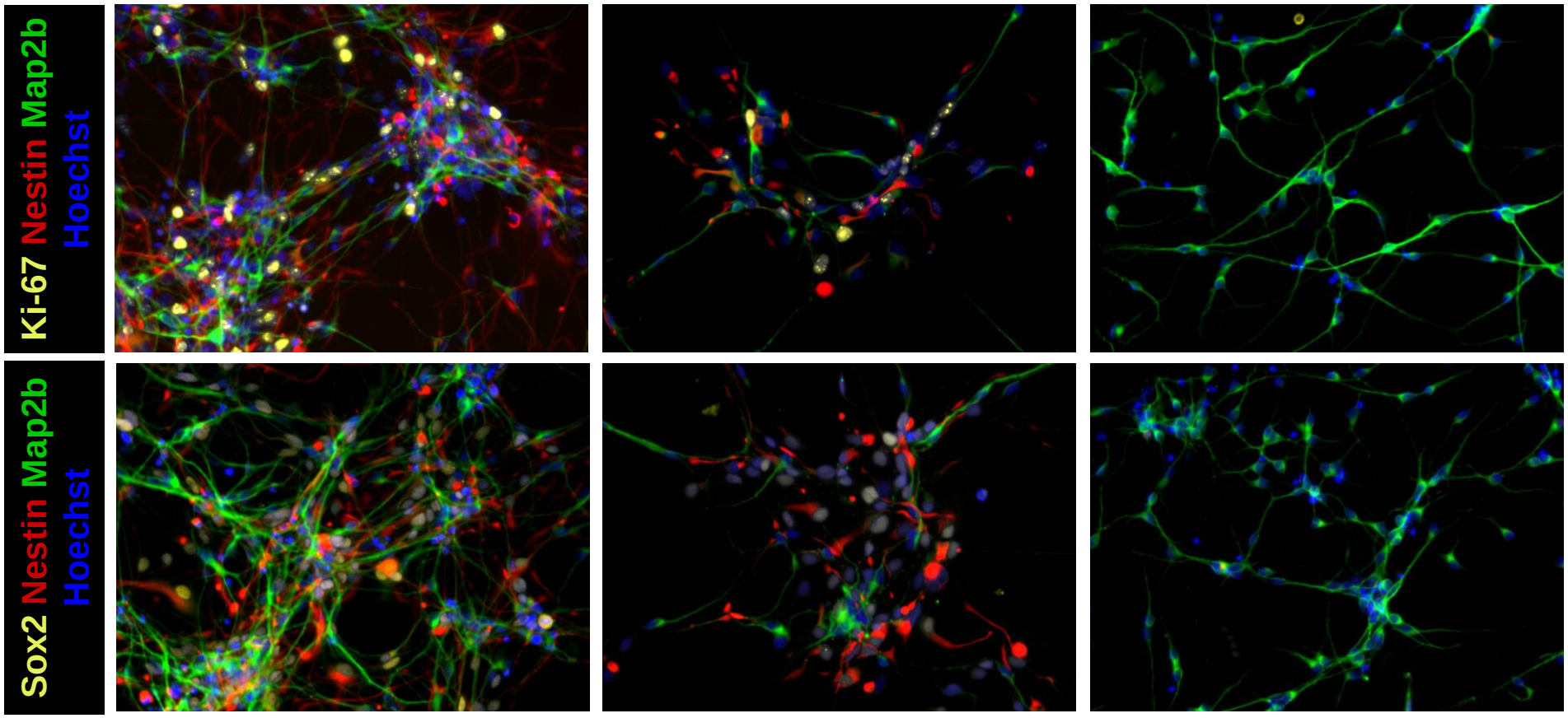
- Cell Sorting
- Cell Purification
- Analyzing sub-populations

Treatment-Specific Changes, for:

- Process Development
- Media Optimization
- Discovery Biology

Flow Cytometric Separation of Neurons and Undifferentiated NSC

2 days post FACS



Sox2: NSC	Map2: Neuron
Ki-67: proliferation	Nestin: NSC

BD