Comprehensive Immune Response Profiling

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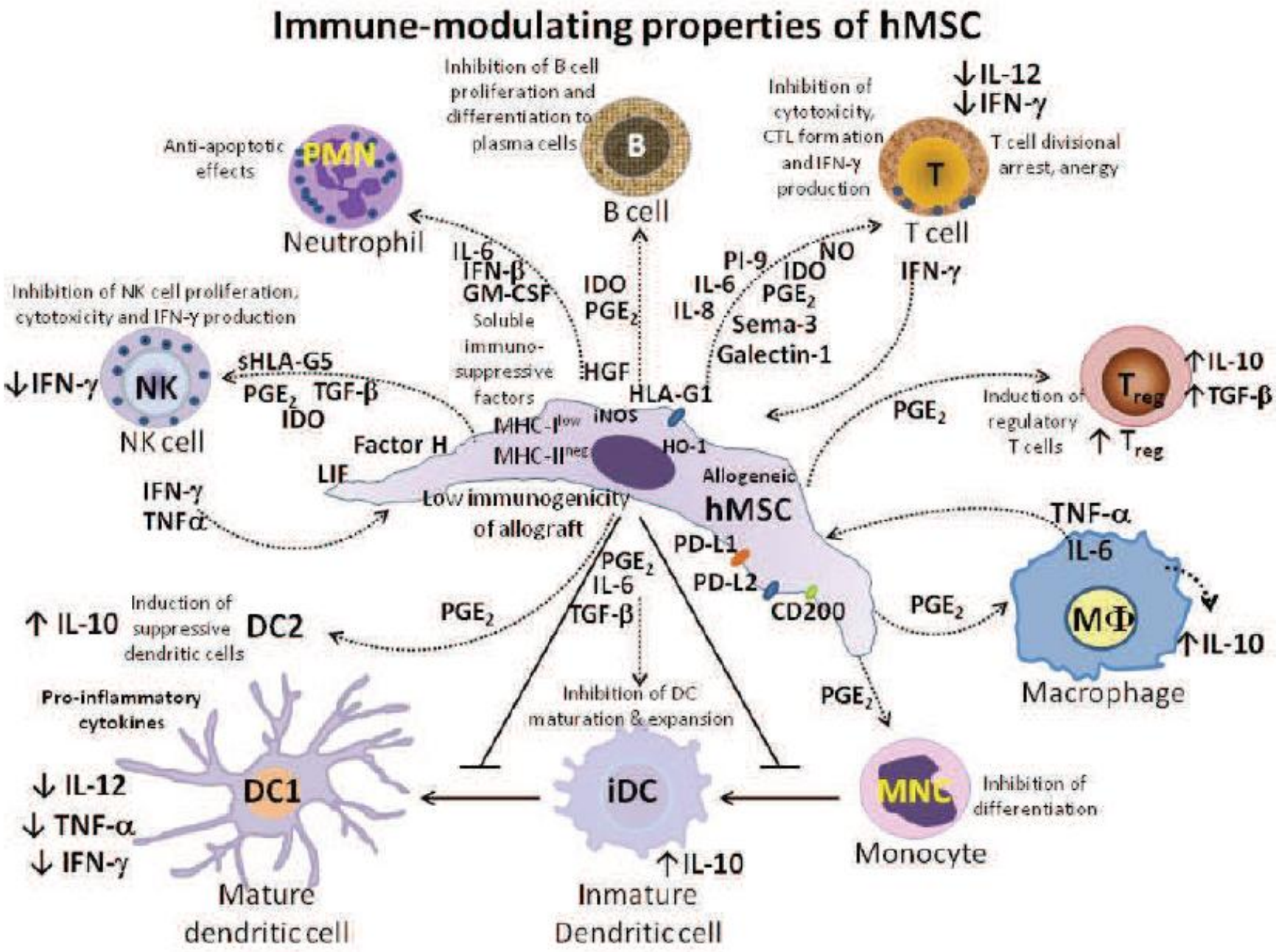
Cell Therapy – Immune Rejection Challenge

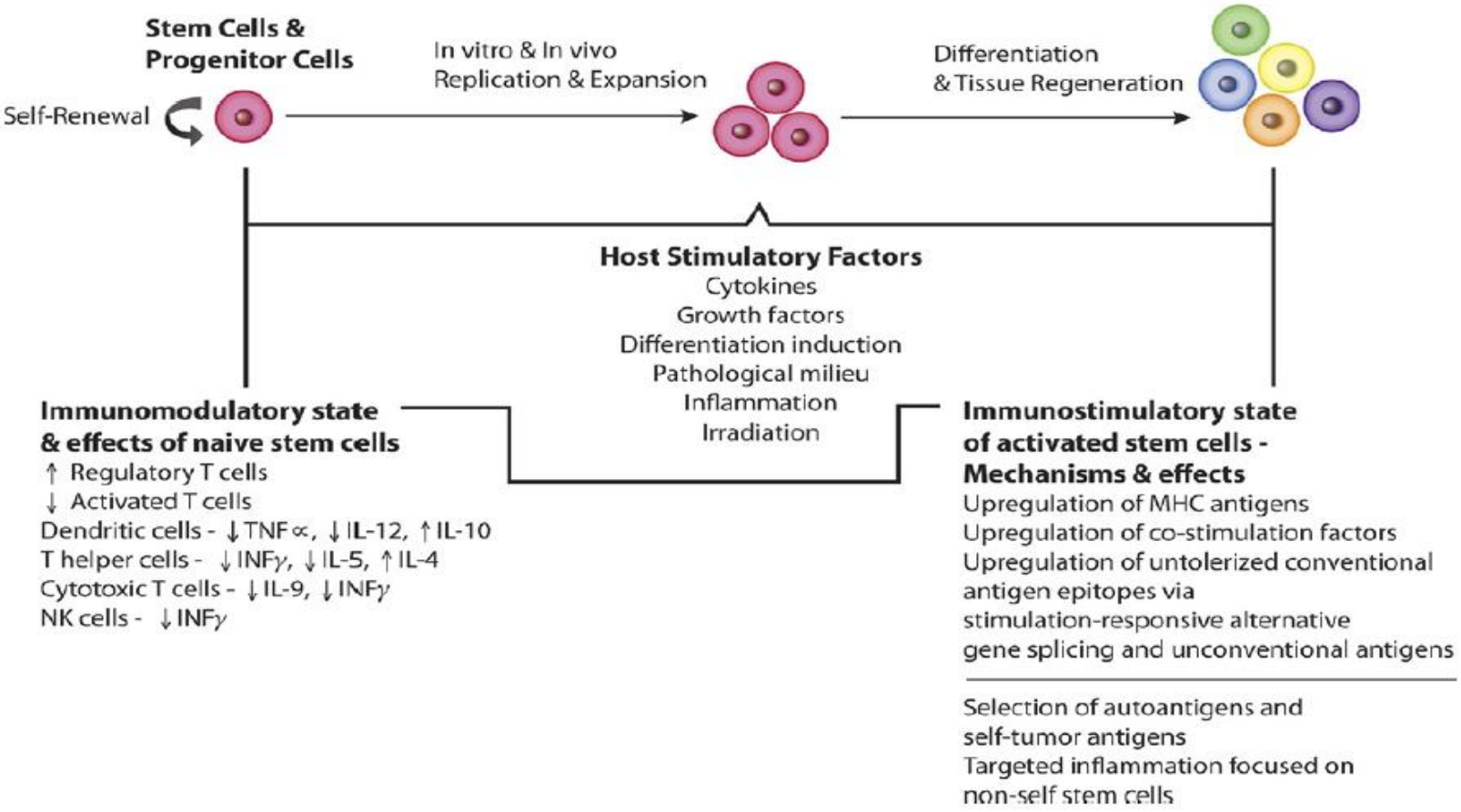
Success is dependent upon :

- **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



Jan Nehlin etal. Immunogenicity and Immune-Modulating Properties of Human Stem Cells. http://www.intechopen.com/

Stem Cells – Immunological Properties



L. Maximilian Buja etal. (2010) Journal of the American College of Cardiology. 56:1693-700.
Yang XF (2007) Cell Mol Immnol 4:161-71

Differentiated ESCs are Immunogenic

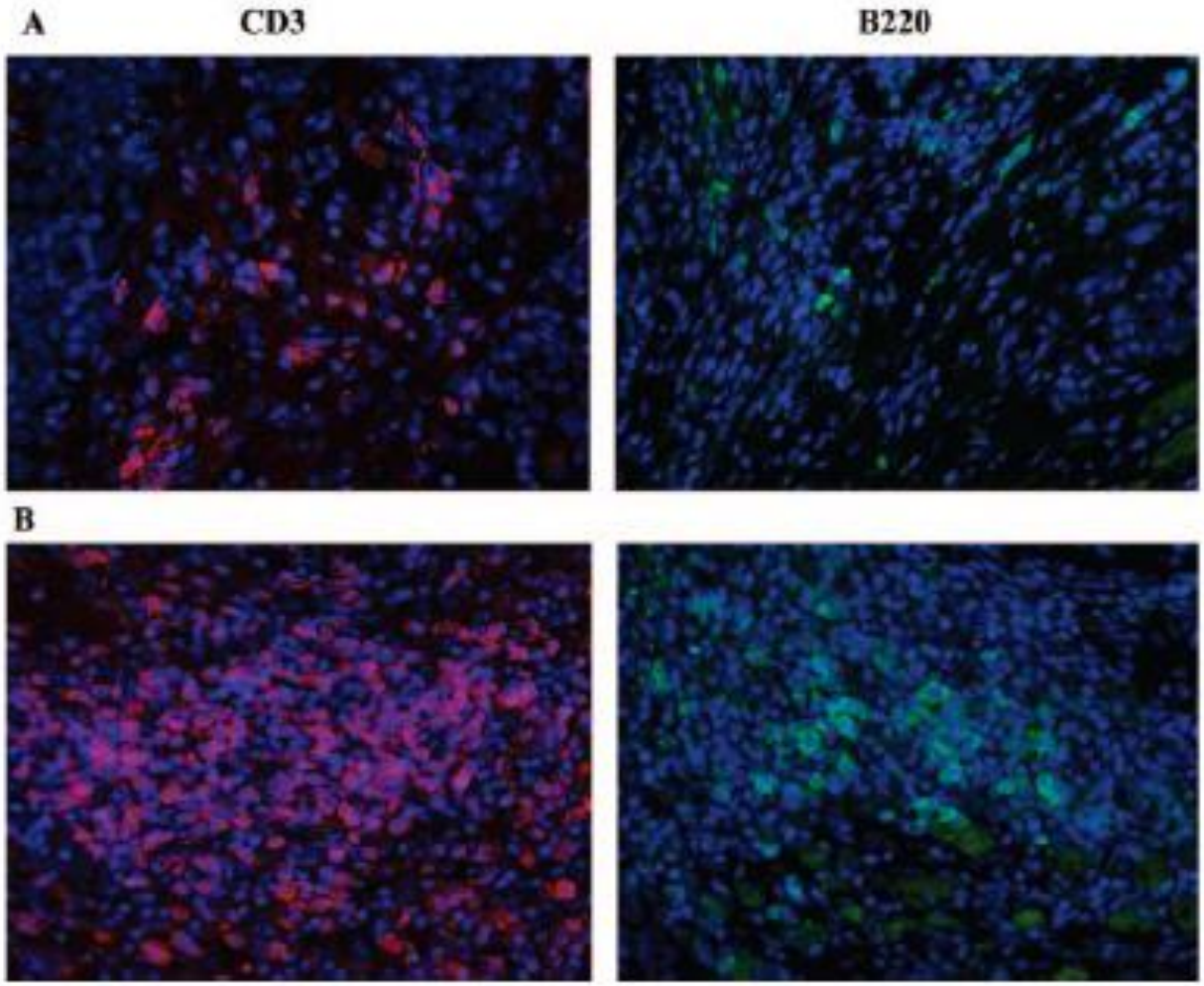


Figure 6. Graft infiltration of immune cells after transplantation of in vivo differentiated ESCs. Representative images of ESC graft infiltration of T (red) and B (green) lymphocytes at 2 weeks after undifferentiated ESC transplantation (A) versus 2 weeks after in vivo differentiated ESC transplantation through heterotopic transplantation of the LAD-ligated and ESC-injected hearts (B). In vivo differentiated ESCs elicited a vigorous and more immediate immune response as compared with undifferentiated ESCs. Counterstaining was performed with DAPI (blue). Original magnification: 400×.

R. Swijnenburg et al. (2005) Circulation112:166-172.

iPSCs – Immunogenic Potential

Table. Summary of Teratoma Formation on Day 30

Type of Model	Donor Cell	Recipient	Average Teratoma Formation Rate (%)	Lymphocytic Infiltration
iPSC autograft	ViPSC (B6)	Mouse (B6)	64.7% (22/34)	+ (10/10)
iPSC autograft	EiPSC (B6)	Mouse (B6)	83.9% (26/31)	+ (8/13), – (5/13)
ESC autograft	ESC (B6)	Mouse (B6)	96.8% (30/31)	–
ESC allograft	ESC (129/SvJ)	Mouse (B6)	3.2% (1*/31)	+ (1/1)

The average tumor formation rates for iPSC autograft and ESC allograft/autograft models. This table was calculated based on Figures 1B and 3D, and Supplemental Figure 3E in the article.[2] The authors noted that only small teratoma formation was observed in the ESC allograft model (asterisk).

Keisuke Okita etal. (2011) Immunogenicity of Induced Pluripotent Stem Cells. Nature 109:720-721.

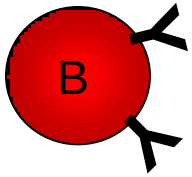
Immune Response Monitoring

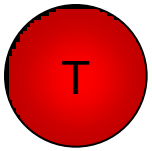
- **Cellular Response to the cell based therapeutic:**
  ICC, ELISPOT, CFSE, MHC Tetramers, MLR

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

Comprehensive Immune Response Profile

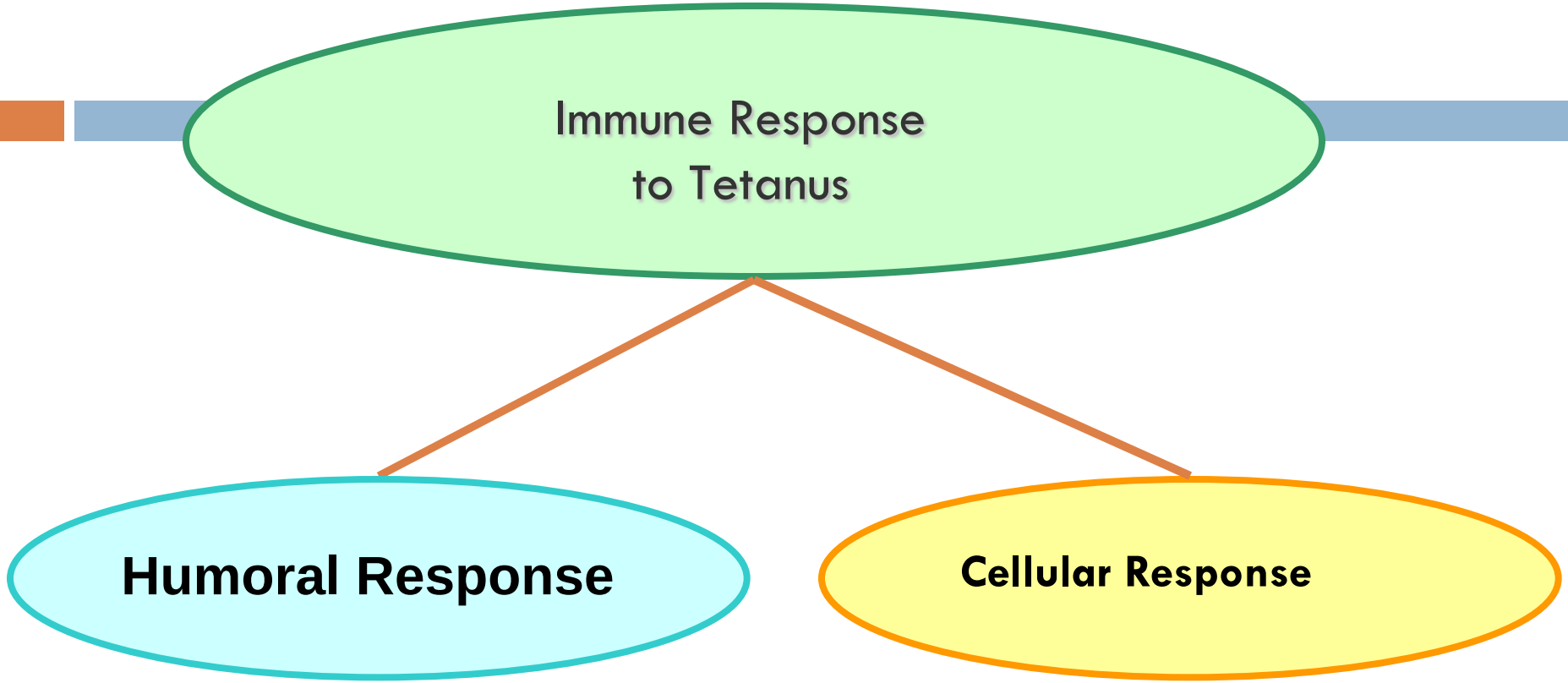
Immunize



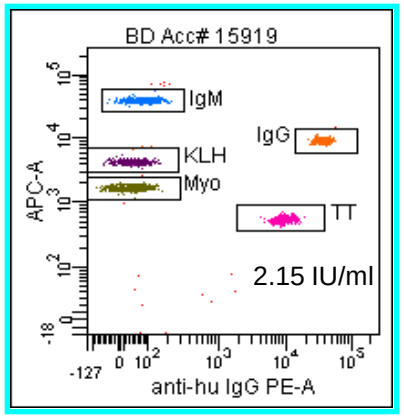
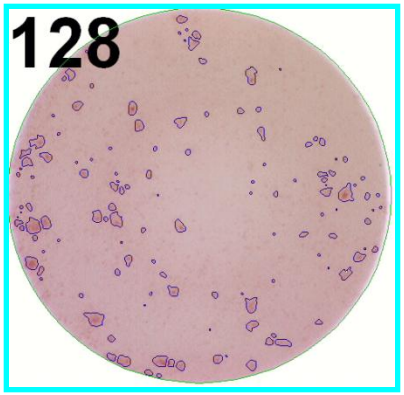
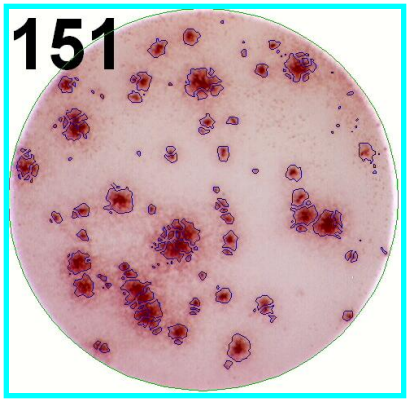
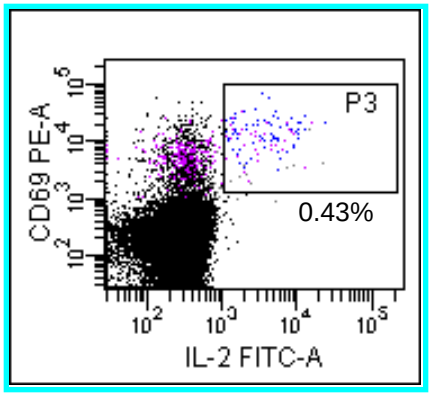
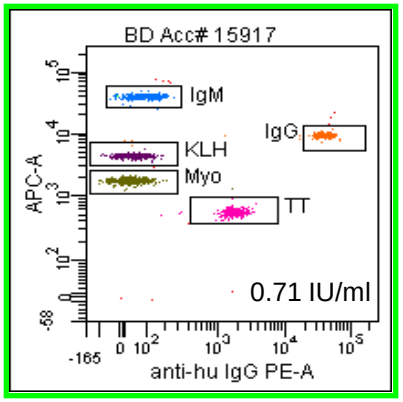
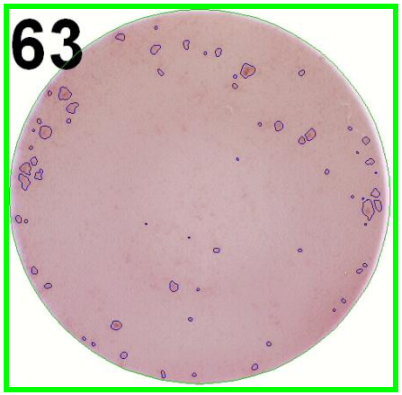
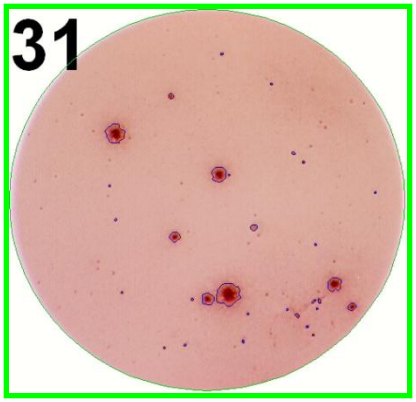
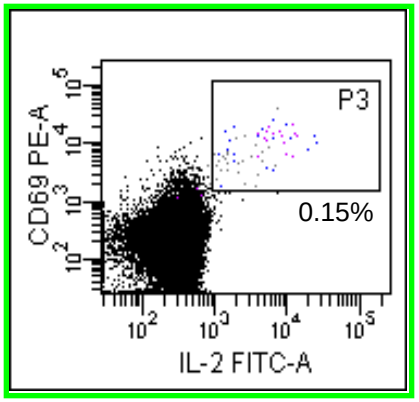


Development and Qualification of Assays

- Frequency of Antigen Specific B cells (sIg)
  - 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 Tetanus
Humoral Response
Cellular Response
Serum Antibodies
CBA
Number of TT 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 to Tetanus

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

Immune Response to CMV

Cellular Response:

Fresh vs Frozen

T Cell Response – IFNγ ELISPOT

T Cell Response – CFC Assay

CD4 : IL-2/IFNγ/IL5
CD8 : IL-2/IFNγ/IL5

T Cell Response – CFSE Assay

Immune Response Monitoring – Lyophilized Reagents

Lyophilized pre-optimized Antibody Cocktails :

4, 6 or 8+ color antibody cocktails, include surface and intracellular cytokine specific antibodies; single tests per well or tube; stable for at least 18 months at room temperature

Lyophilized Stimulation Reagents :

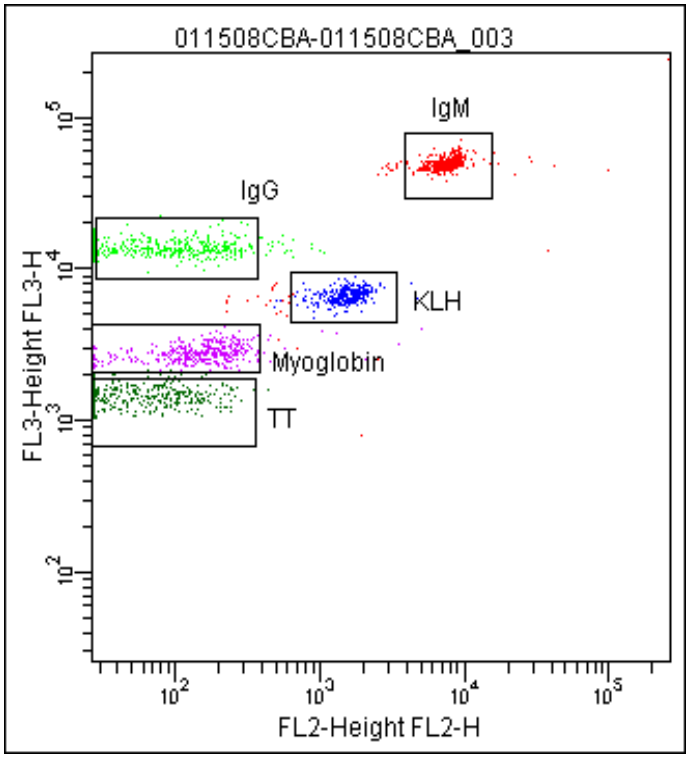
Peptide antigens, protein antigens, cells, super antigens etc., in 96 well plates, 384 well plates, tubes or vials

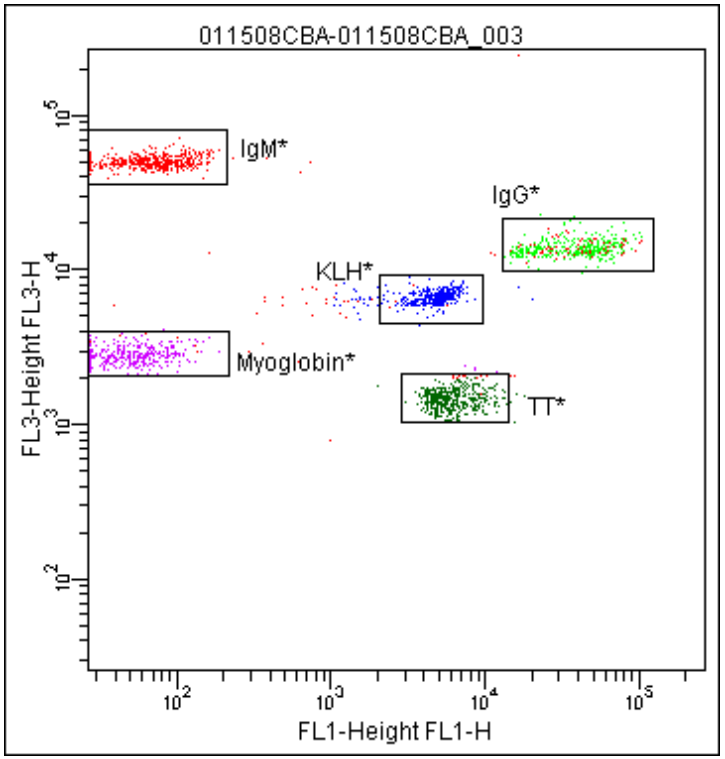
Immune Response Monitoring

- Cellular Response to the cell based therapeutic:
  ICC, ELISPOT, CFSE, MHC Tetramers, MLR

- **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.

EQAPOL ELISPOT Survey RESULTS :

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

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

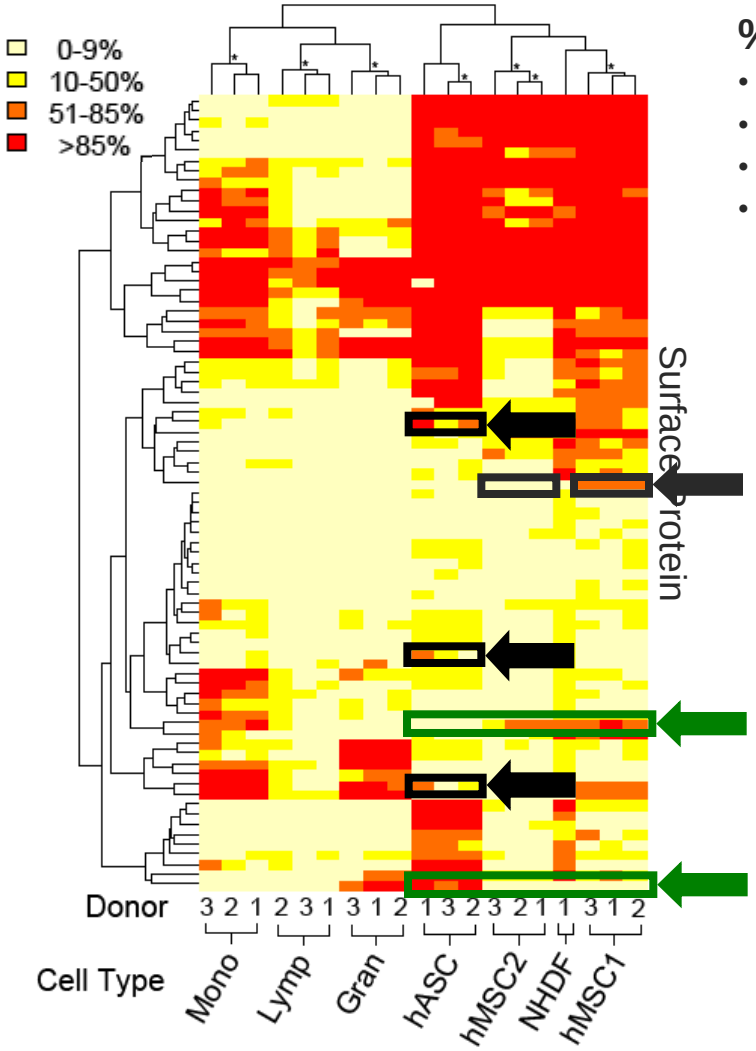
BD FACS CAP : Combinatorial Antibody Profiling

- 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

Thanks !

Acknowledgement

Christian Carson

John Hodgson

Joshua Kotner

Ray Chan