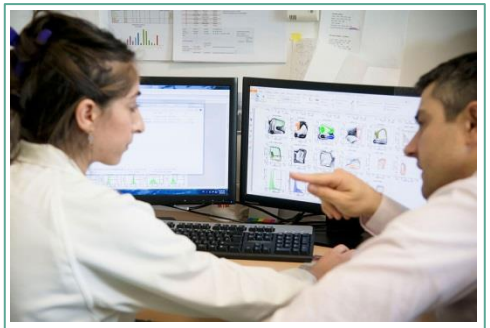
# Immunophenomics

## Immunity profiling and characterization



**CIPHE offers high-throughput solutions for immunity profiling and characterization for academic and industrial projects**

- Advanced multiparametric flow and mass cytometry approaches involving a series of over 200 quantitative parameters to phenotype all the cellular components of the immune system.
- A broad range of instruments and services for the analysis and isolation of cells based on fluorescent and rare metal labeling
- Several organs on a high-throughput mode of steady state and challenged mice via PRR ligand injection or multiples proprietary challenge models (peritonitis, IBD, EAE, tumor immunology, vaccine studies)
- Our experience with inflamation, infection and tumor immunology allows our team to discern the clinical relevance of potential targets identified in mouse models, thereby allowing clients to invest resources in promising therapeutics, increasing their success, and ultimately, improving patient health

# Immunophenomics

## Immunity profiling workflow

CIPHE OR PARTNERS COHORTS

*several experimental groups – up to 24 individuals*

ORGANS and TUMORS

*up to 4 tissues*

AUTOMATED CELL EXTRACTION

CELL SUSPENSION or CELL ENRICHMENT

*up to 24 samples at once*

**EX VIVO EXPERIMENTAL ASSAYS**

- Cell sorting (11 immune cell types) → TRANSCRIPTOMIC ANALYSIS
- High content panel / Metabolic probes → IMMUNOPROFILING → MULTIPARAMETRIC FLOW & SPECTRAL CYTOMETRY
- Surface & Intracellular markers → SINGLE CELL FUNCTIONAL PROFILING → MASS CYTOMETRY

**IN VITRO EXPERIMENTAL ASSAYS**

ACTIVATION & DIFFERENTIATION (B, T, NK cells)

CYTOKINE, CHEMOKINE MULTIPLEX IMMUNO ASSAYS

→ CELL BANKING (BASAL AND ACTIVATED STATES)

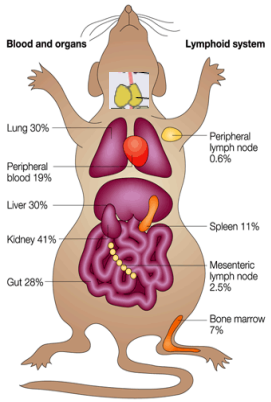
INTEGRATED DATA ANALYSIS

Center for Immunophenomics

# Immunophenomics

## High content immune phenotyping panels

### IMMUNE ORGANS




BLOOD

THYMUS

SPLEEN/LN

BONE MARROW

PERITONEUM

*LUNG*

*INTESTINE*

### CELL TYPES

T cells

B cells

NK cells

LYMPHOCYTES

Monocytes

Neutrophils/Basop

Eosinophils/ Mast

Macrophages

DC, pDC

MYELOID CELLS

### HIGH CONTENT PANELS

**PERIPHERAL BLOOD** 13 populations
Quantitative analysis of hematopoietic cells

**THYMUS**
T cell development 17 populations
Dendritic cells 19 populations

**SPLEEN / LYMPHE NODE**
Orientation panel 34 populations
L-T/NK panel 53 populations
L-B-panel 15 populations
Myeloid panel 19 populations
Dendritic panel 19 populations

**BONE MARROW**
Stem cells/Myeloid cells precursors
B cell development

**LUNG** (broncho-alveolar lavage)
(Steady state/ Inflammation) 17 populations

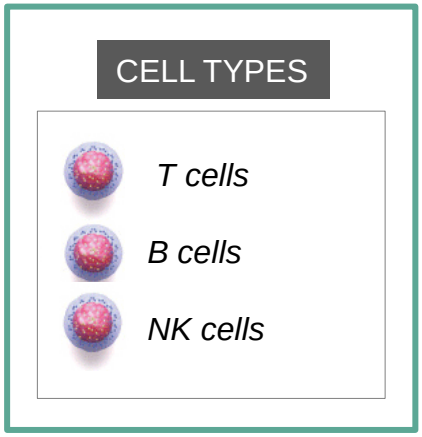
**PERITONEAL CAVITY**
(Steady stade/ Inflammation) 17 populations

**QUANTITATIVE MEASUREMENT OF MARKER EXPRESSION** 20 populations

**DESIGN PANEL & CONSULTING**

# Immunophenomics

## *In Vitro & Ex Vivo* Functional Assays




**ACTIVATION / DIFFERENTIATION**

- SURFACE MARKERS
- PROLIFERATION ASSAYS (CTV, Cell Titer Glow)
- CYTOKINES, CHEMOKINES PRODUCTION by ICS, MIA

**DIFFERENTIATION EFFECTOR T CELLS**

- Th1, Th2, Th17, Treg

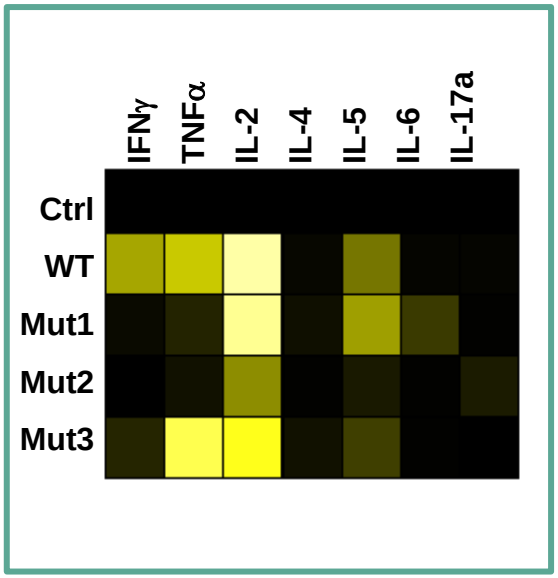
**B CELL RESPONSE IMMUNISATION**

- T-cell dependent
- T-cell independent

- **Th Effector Panel (Th1, Th2, Th17, Th22, Treg) - 8 populations by intra-cellular cytokine**
- **Master Regulator Panels - 8 populations by intra-nuclear staining**

# Immunophenomics

## Single Cell Function - Mass Cytometry



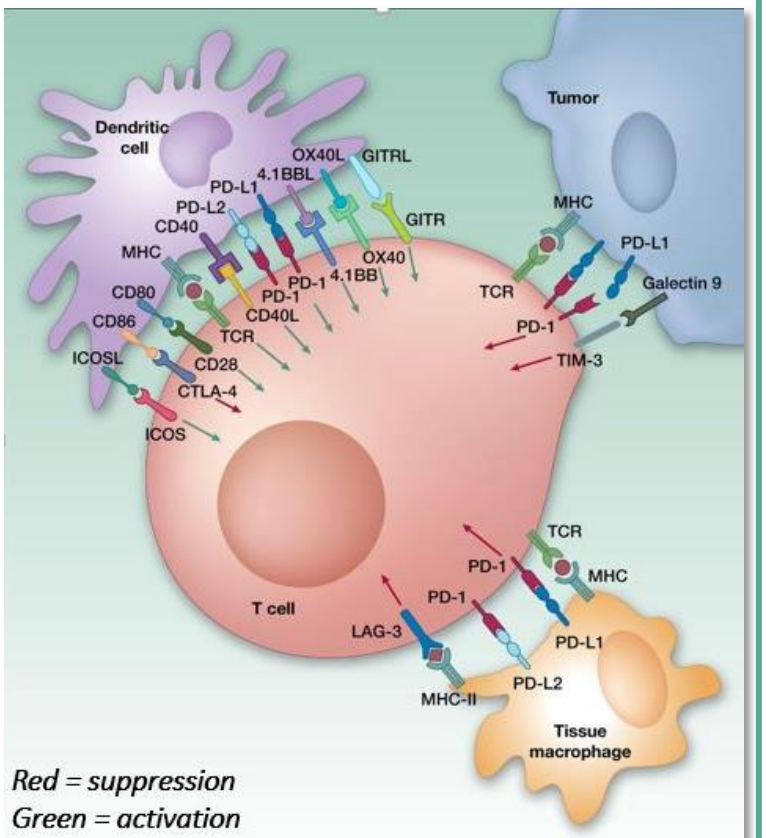

**SINGLE CELL HIGH CONTENT FUNCTIONAL PHENOTYPING (32 specific antibodies per single cell)**

- ANALYSIS OF INTRA-CYTOPLASMIC AND INTRA-NUCLEAR EFFECTOR TC SPECIFIC FACTORS
- ASSAY DIRECTED TOWARDS THE EVALUATION OF EX-VIVO EFFECTORS T CELLS AND MORE SPECIFICALLY TO TH1, TH2, TH17 AND T REG.
- CYTOKINES NETWORK
- TRANSCRIPTION FACTORS
- PHOSPHO-ANTIGENS

- Performed in multiplex conditions
- Possibilities of banking for second line assay

# Tumor Immunophenomics

## Tumor Specific Multiparametric Panels




*Picture Source : https://blogs.shu.edu/*

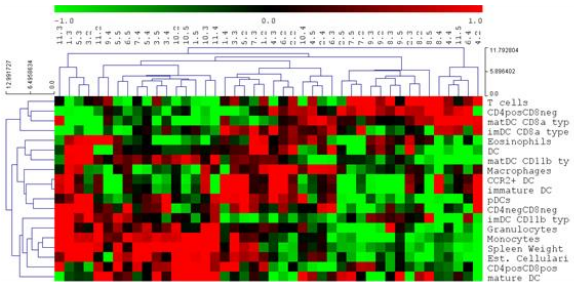
- CHARACTERIZATION OF **ALL IMMUNE CELLS INFILTRATING THE TUMOR** AND THEIR ACTIVATION STATUS.
- EXPRESSION OF IDENTIFIED **CHECKPOINT, INHIBITORY RECEPTORS** OR OTHER **IMMUNO-REGULATORS** ON T CELLS.
- EXPRESSION OF **CHECKPOINT INHIBITORS LIGANDS** AT THE SURFACE OF **TUMOR CELLS AND MYELOID CELLS**
- CHARACTERIZATION OF IMMUNE CELL AND TUMOR **METABOLISM**

  Metabolic probes:
  - ROS production
  - Glu transporter
  - Amino-acid transporter
  - B-galactosidase activity

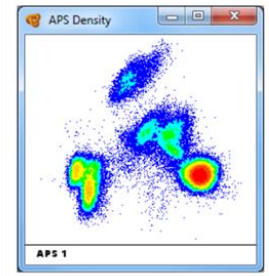
# Immunophenomics

## Multiple Bioinformatic Analysis Toolbox

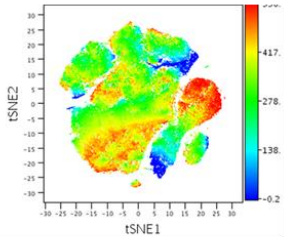
### Cluster analysis and statistical tests



### Data Dimension reduction

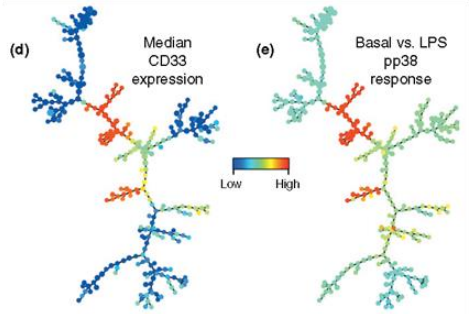



PCA




ViSNE

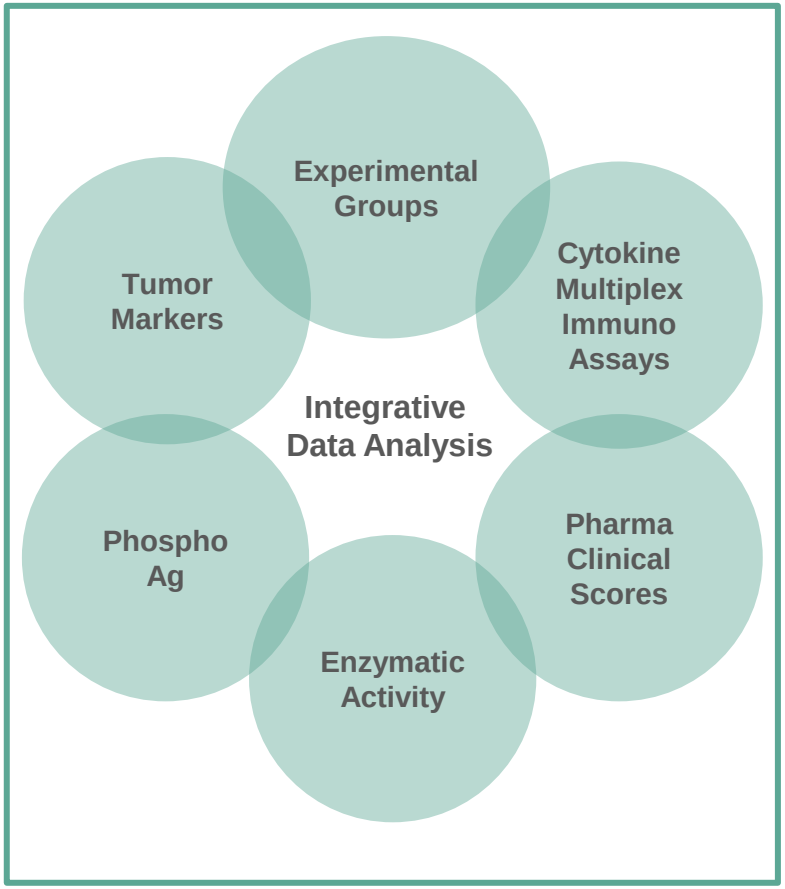
### Unsupervised clustering analysis




- AUTOMATED ANALYSIS PIPELINE
- INFORMATION MANAGEMENT SYSTEM
- BATLAB LIMS

# Immunophenomics

## Integrative Data Analysis




- By looking at more than 200 quantitative parameters at once, the Immunophenomics platform performs integrative data analysis of unprecedented resolution on all subsets of leukocytes in wild-type and mutant mouse at steady state, inflammatory or infectious conditions.
- All type of measurements can be combined in one single illustration as values are expressed in variation to the mean for every given parameter
- Variations of immune signature is a more robust way to investigate a phenotype (consideration of many parameters at once and not parameter by parameter)