Les nouveaux outils diagnostiques immunologiques

Divi Cornec

10/10/2020

Centre de Référence des Maladies Auto-Immunes Systémiques Rares Nord et Ouest

Agenda

- Auto-anticorps : quoi de neuf ?

- Etude des cellules : méthodes unicellulaires
  - En suspension : CyTOF
  - Dans le tissu : Cytomètre de masse imageur
  - Transcriptomique

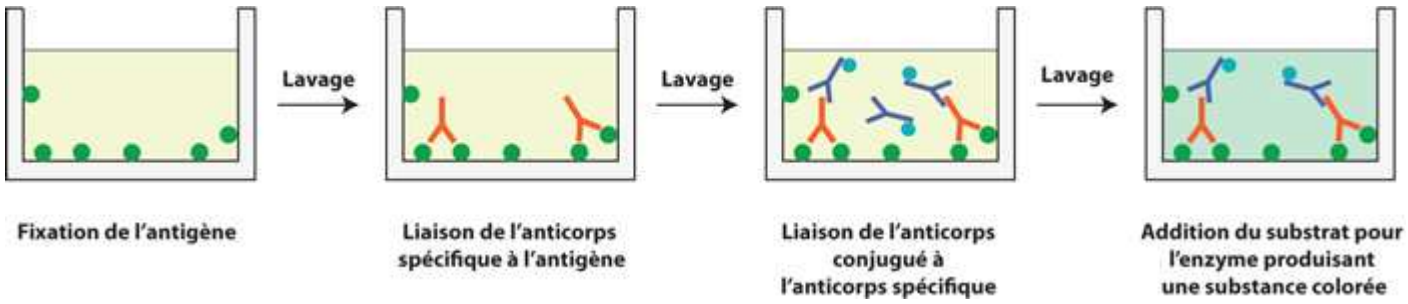
Autoanticorps et maladies autoimmunes systémiques

- Sérologie rhumatoïde
- Anticorps antinucléaires
- Anticorps antiphospholipides
- ANCA

Les auto-anticorps en pratique

Comment les détecter ?

- Par une technique immunologique anticorps-antigène
  - Classiques :
    - En **immunofluorescence indirect** sur des cellules à "gros noyau" (cellule Hep2) ou sur des cellules spécifiques (PNN pour les ANCA)
      → toujours utilisé en dépistage
  - Tests antigènes spécifiques
    - En **ELISA** (immuno-enzymatique)
    - En blot (dot blot)/strip



Les auto-anticorps en pratique

Le dépistage des anticorps anti-nucléaires

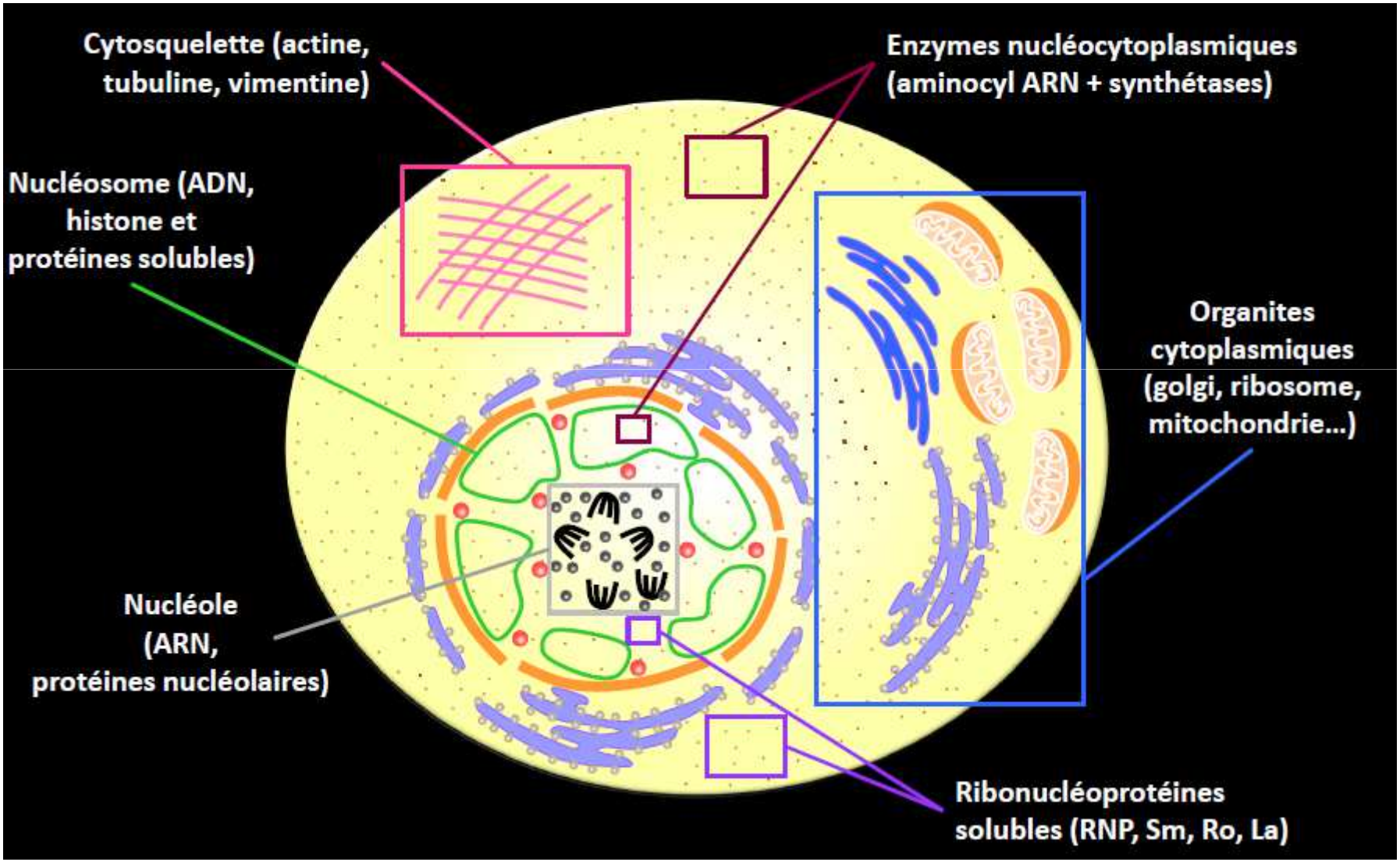
→ par immunofluorescence indirecte (cellule Hep 2)

Avantages : tout auto-antigène présent dans le noyau ou le cytoplasme peut servir de cible à un auto-Ac connu ou inconnu

Limites :

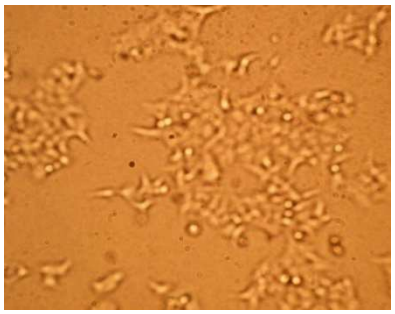
- N'identifie pas formellement (sauf exception) la cible auto-antigénique
- Technique non automatisable très dépendante de "l'œil" du technicien
- Sensibilité limitée et interférence possible (effet de zone)

Les antigènes de la cellule



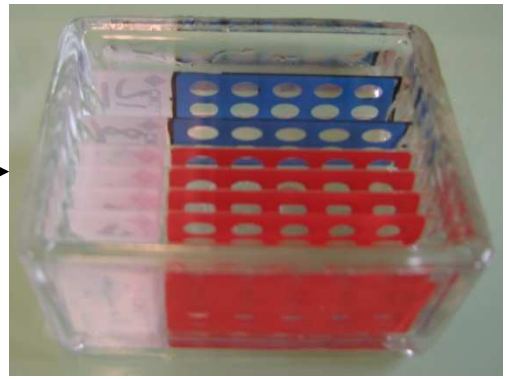
Réalisation des FAN

Culture de cellules HEP-2



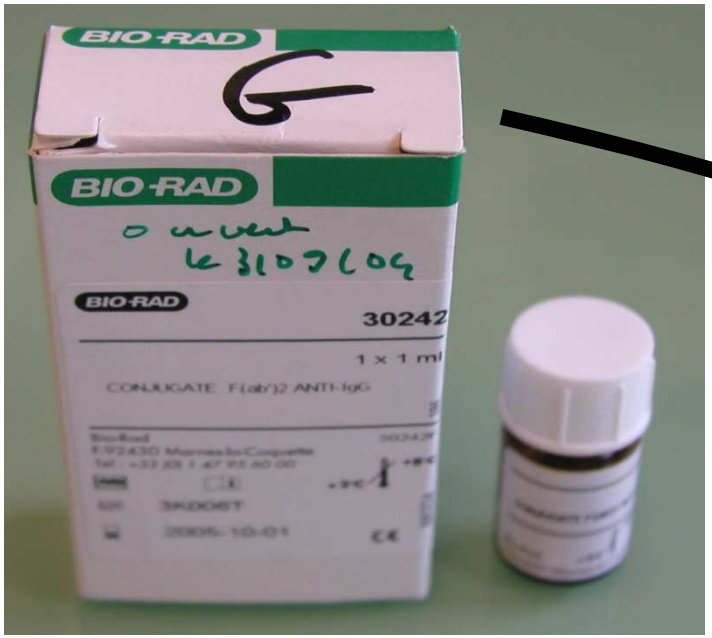
Dépôt des cellules sur lame

Prélèvement du sérum du patient

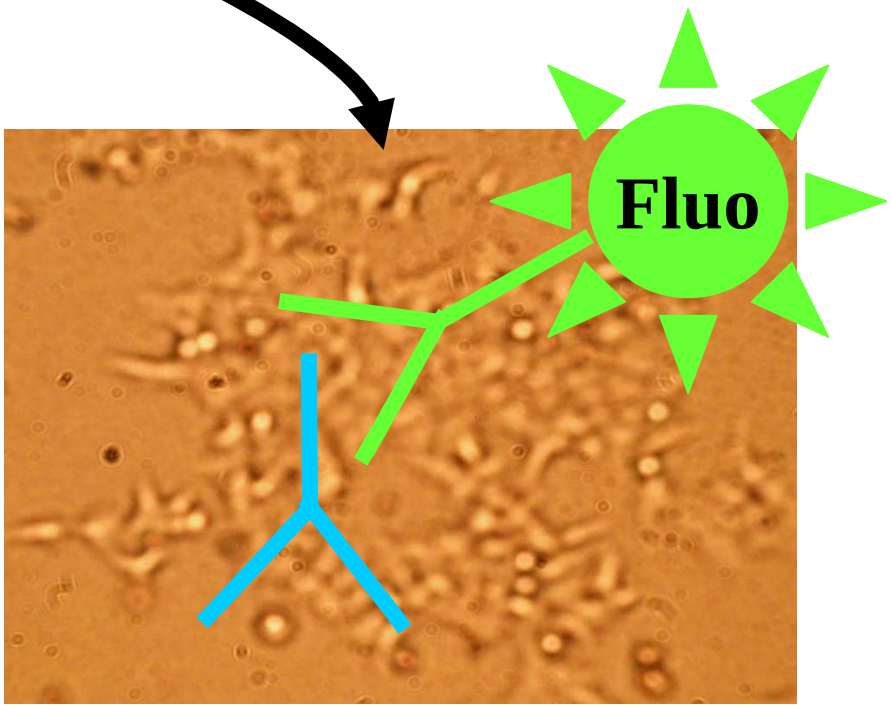


Etuves 5%CO2, 37°C

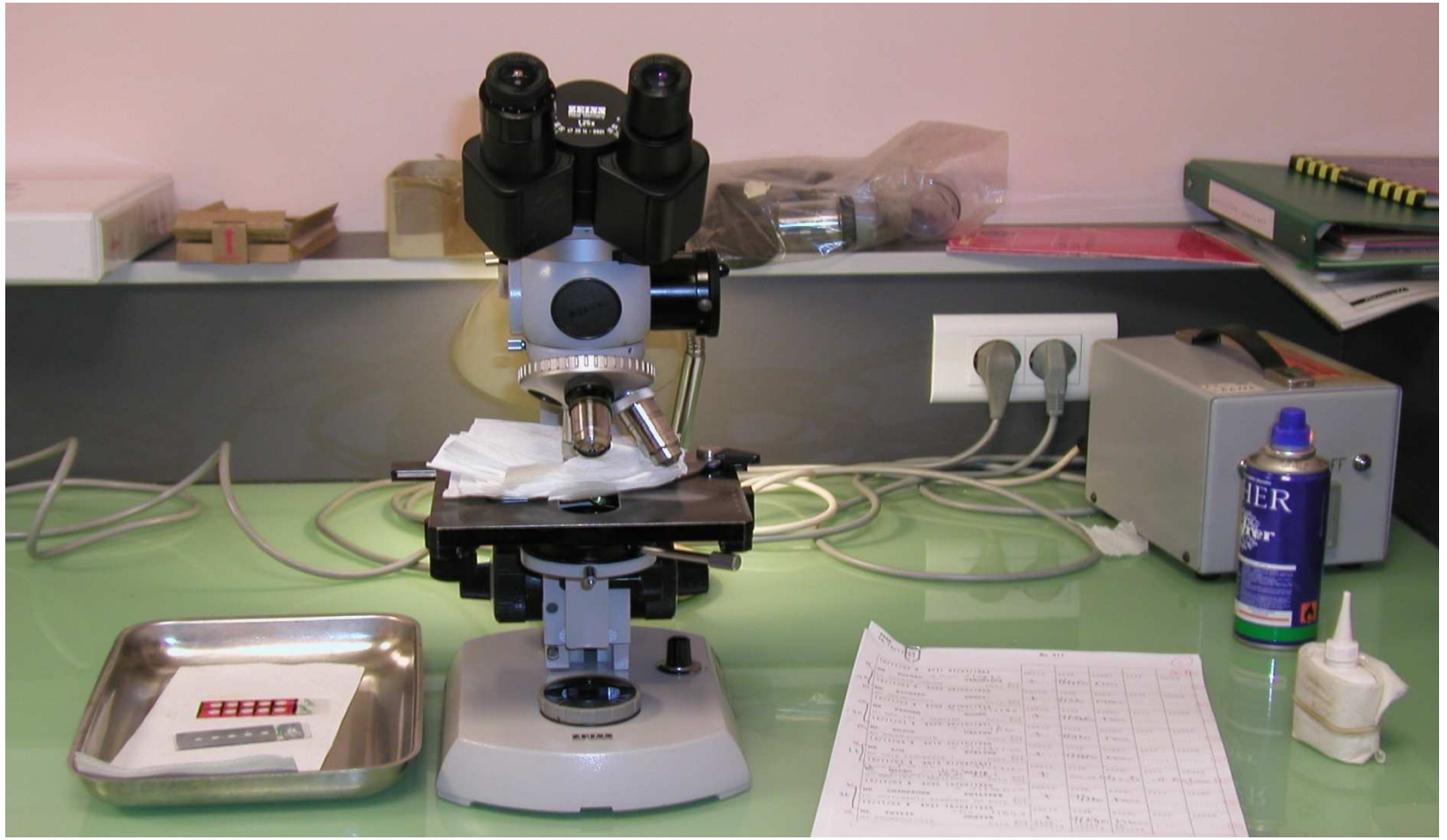
Révélation des Anticorps Anti-nucléaire



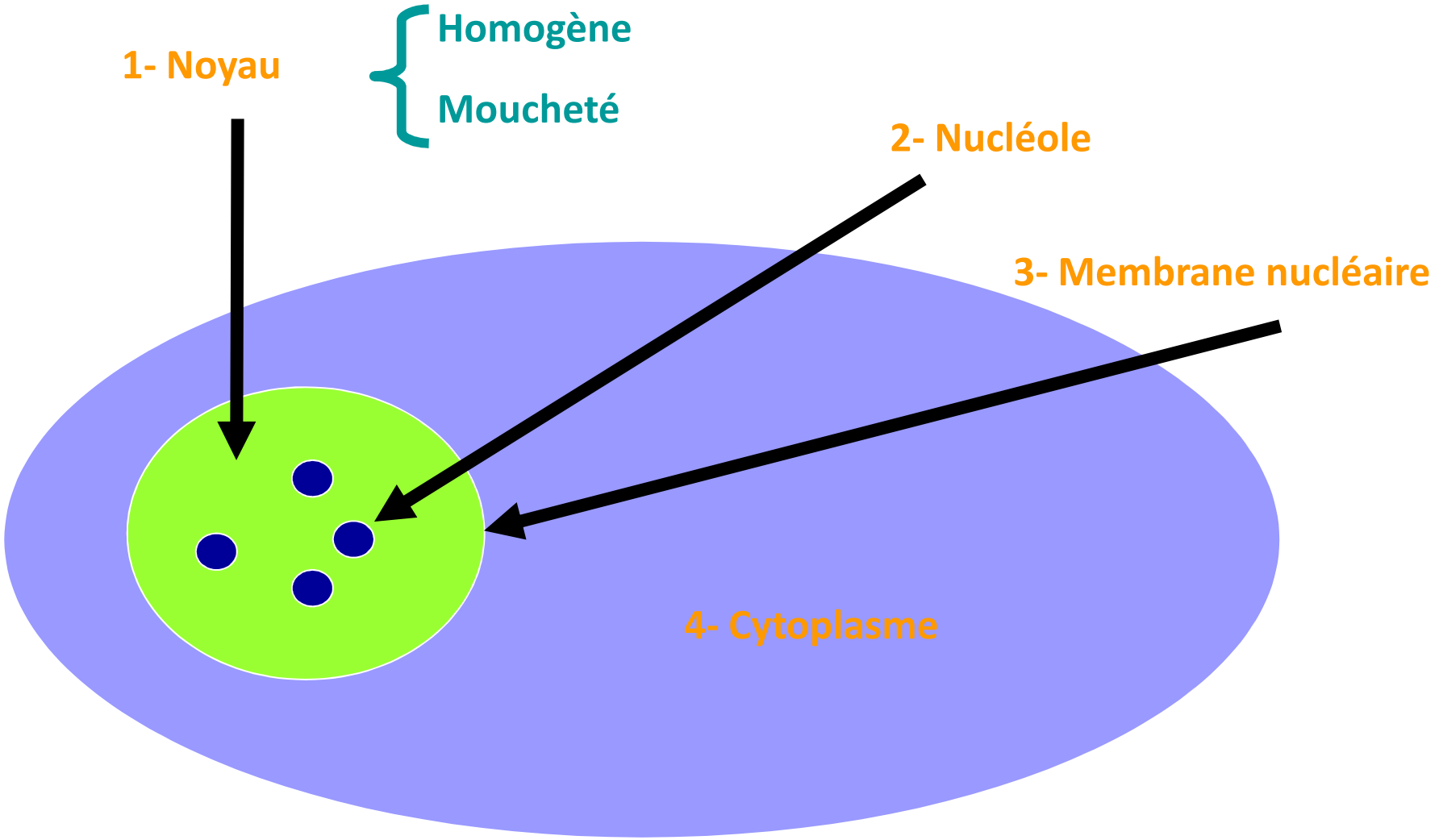
Anti globuline
fluorescente

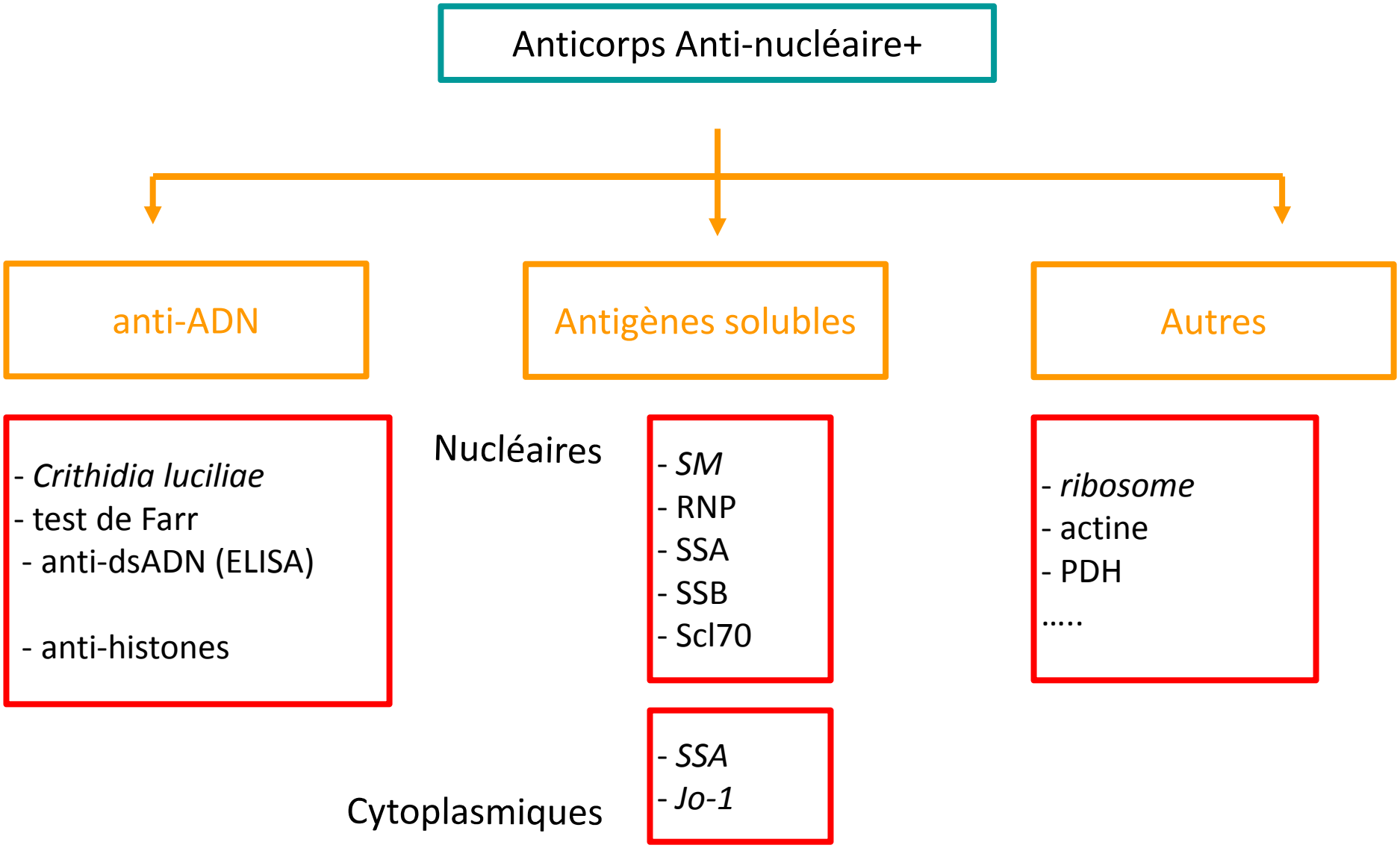


Lecture par microscopie à fluorescence



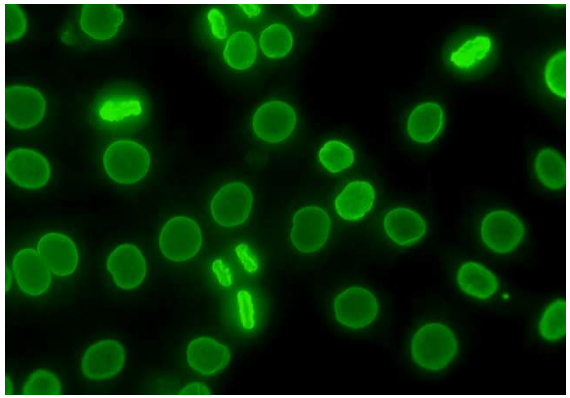
Les aspects des Anticorps Anti-nucléaire



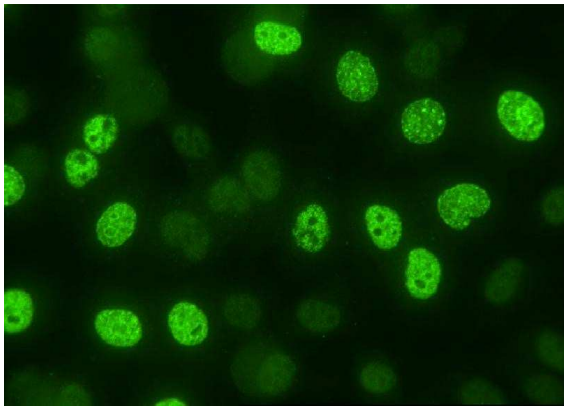

Anticorps Anti-nucléaire+
anti-ADN
Antigènes solubles
Autres
- *Crithidia luciliae*
- test de Farr
- anti-dsADN (ELISA)
- anti-histones
Nucléaires
- *SM*
- RNP
- SSA
- SSB
- Scl70
Cytoplasmiques
- *SSA*
- *Jo-1*
- *ribosome*
- actine
- PDH
…..

Antinucléaires de dépistage

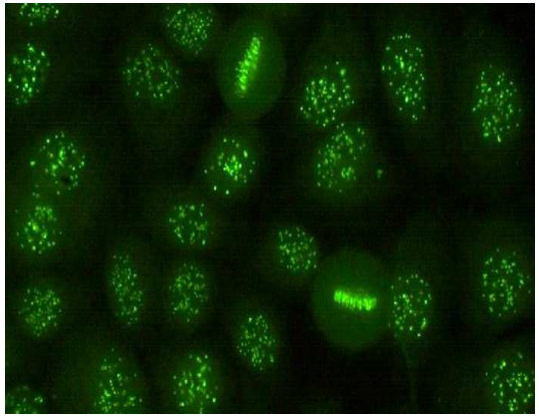
homogène



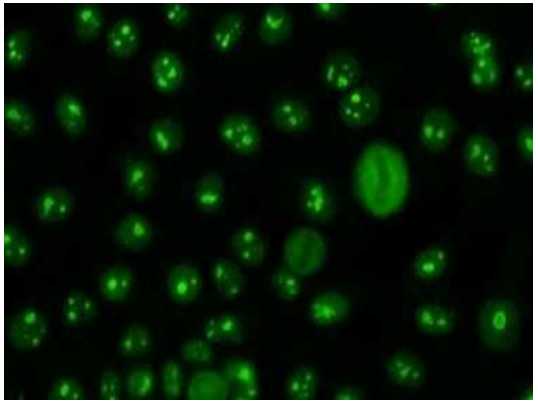
moucheté



centromère



nucléolaire



Les auto-anticorps en pratique

Quelle est leur utilité diagnostique ?

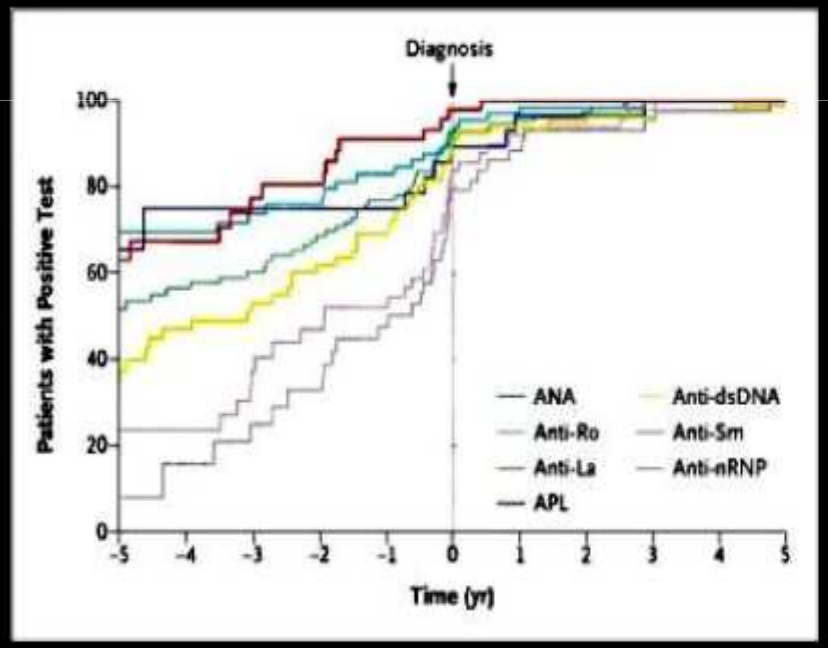
- Les anti-Sm sont quasi spécifiques du lupus
- Les anti-ADN natifs sont très spécifiques du lupus mais parfois induits (ex : anti-TNF)
- Les anti-RNP sont partagés par différentes connectivites (lupus, Sjögren, myosite…)
- Les anti-Ro (SS-A) et La (SS-B) sont partagés par le lupus et le Sjögren
- Les ANCA sont hétérogènes avec certains (anti-PR3, anti-MPO) spécifiques des vascularites mais inductibles par différents phénomènes (infections, toxiques, médicaments…)
- Les anti-phospholipides sont inductibles par de nombreux phénomènes (infections, médicaments, toxiques...)

Les auto-anticorps en pratique

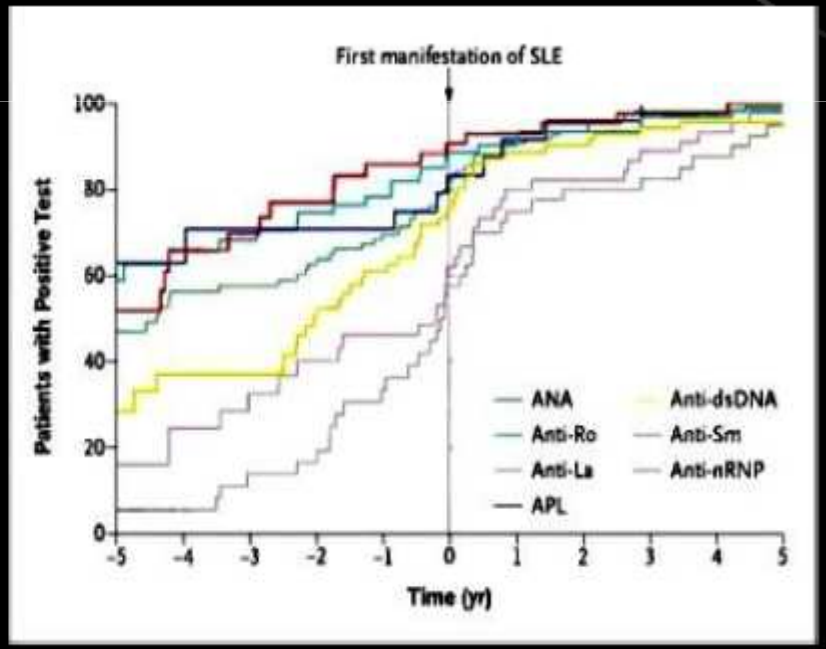
Quelle est leur utilité diagnostique ?

⚠ Les auto-Ac peuvent précéder l'apparition de la maladie auto-immune

Fréquence de chaque Ac avant le diagnostic parmi les malades qui l'ont développé après le diagnostic



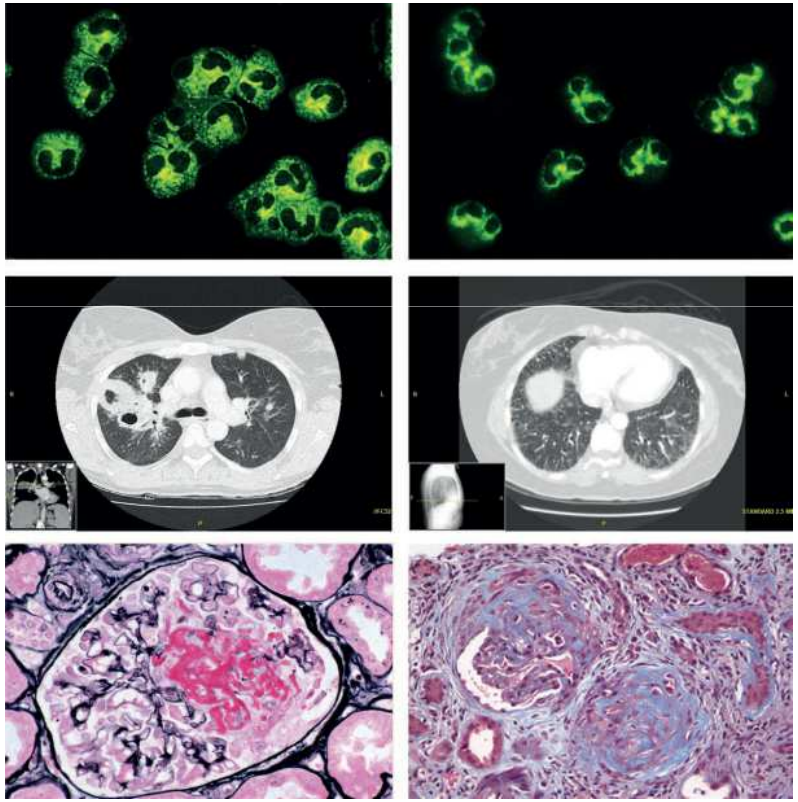
Fréquence de chaque Ac avant le premier signe clinique parmi les malades qui l'ont développé après le diagnostic



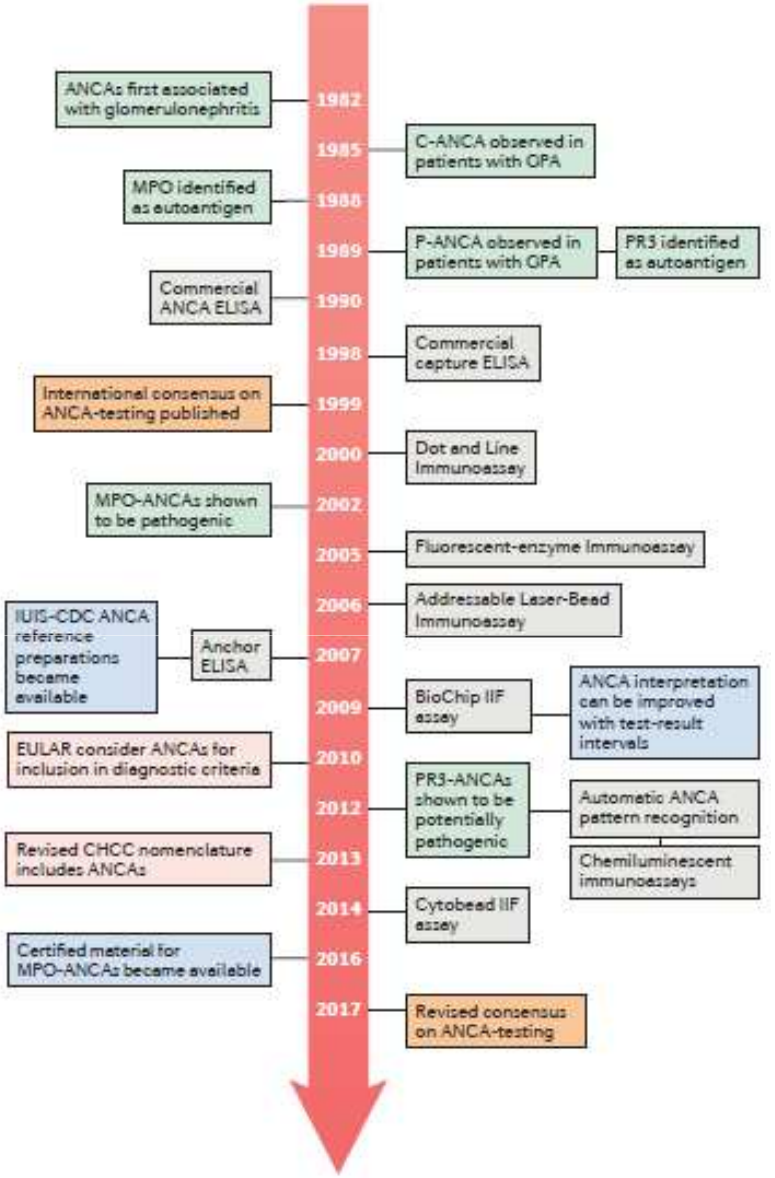
Arbuckle et al. N Engl J Med 2003 ;349:1526-33.

Les ANCA

Anti-PR3 Anti-MPO



Cornec et al, Nat Rev Rheumatol. 2016

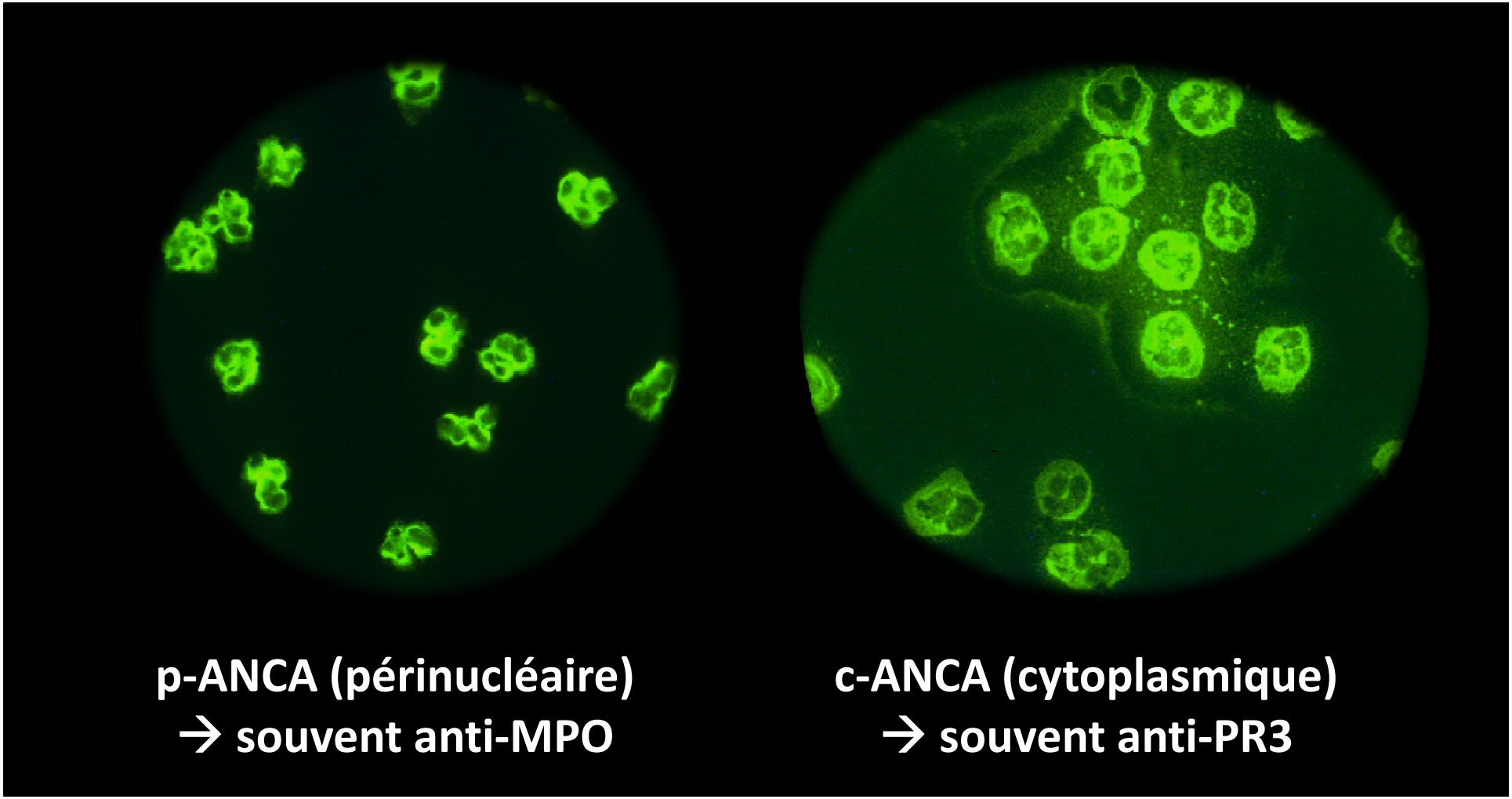


Nat Rev Rheumatol. 2017 Nov;13(11):683-692. doi: 10.1038/nrrheum.2017.140. Epub 2017 Sep 14.

Position paper: Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis.

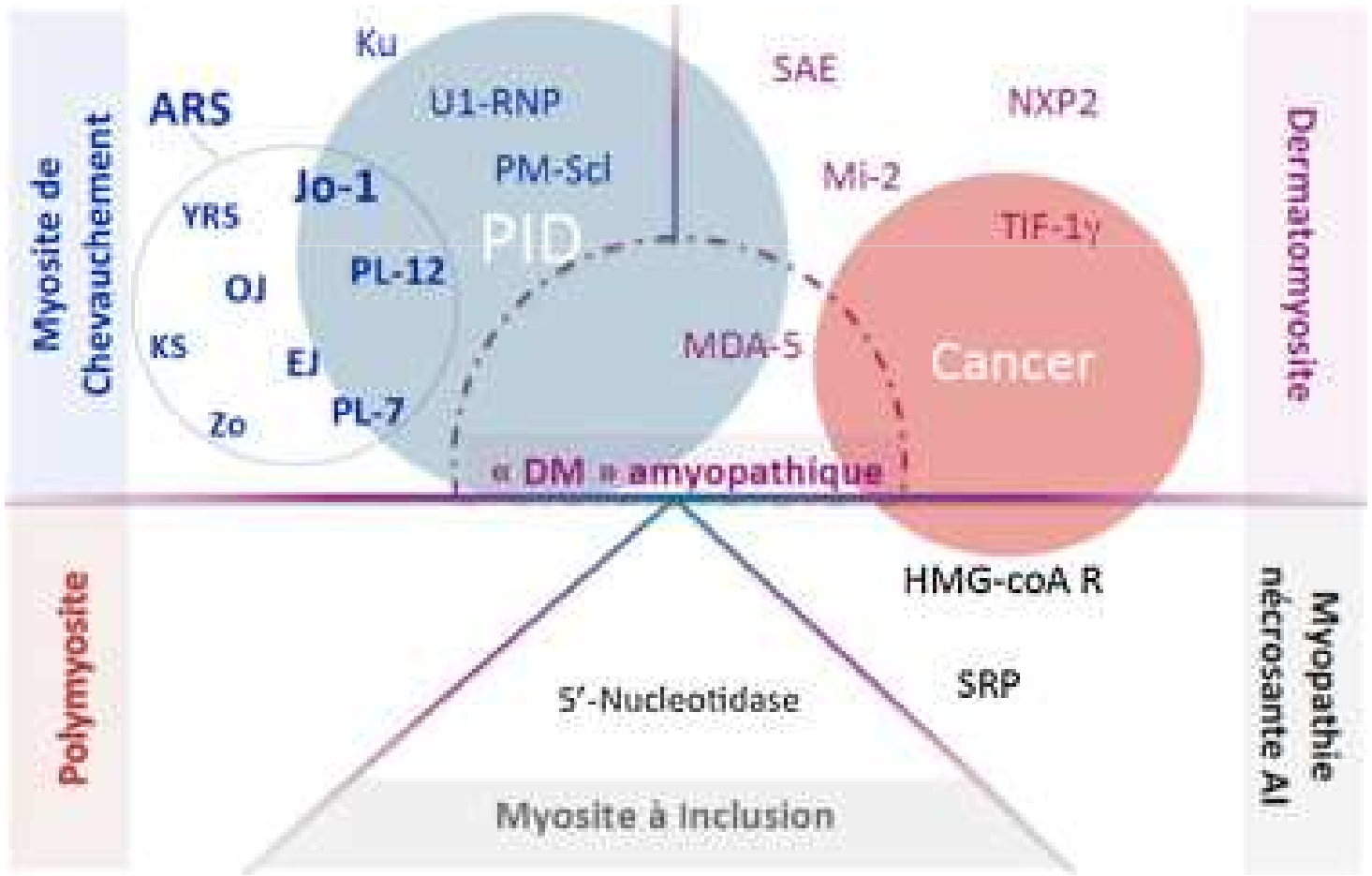
Bossuyt X[1], Cohen Tervaert JW[2], Arimura Y[3], Blockmans D[4], Flores-Suárez LF[5], Guillevin L[6], Hellmich B[7], Jayne D[8], Jennette JC[9], Kallenberg CGM[10], Moiseev S[11], Novikov P[11], Radice A[12], Savige JA[13], Sinico RA[14], Specks U[15], van Paassen P[16], Zhao MH[17], Rasmussen N[18], Damoiseaux J[19], Csernok E[7].

Les ANCA : le dépistage en immunofluorescence sur des frottis de polynucléaires humains fixés à l'éthanol



Radice et al. Autoimmun Rev 2013; 42 : 487-95.

Auto-anticorps des myosites

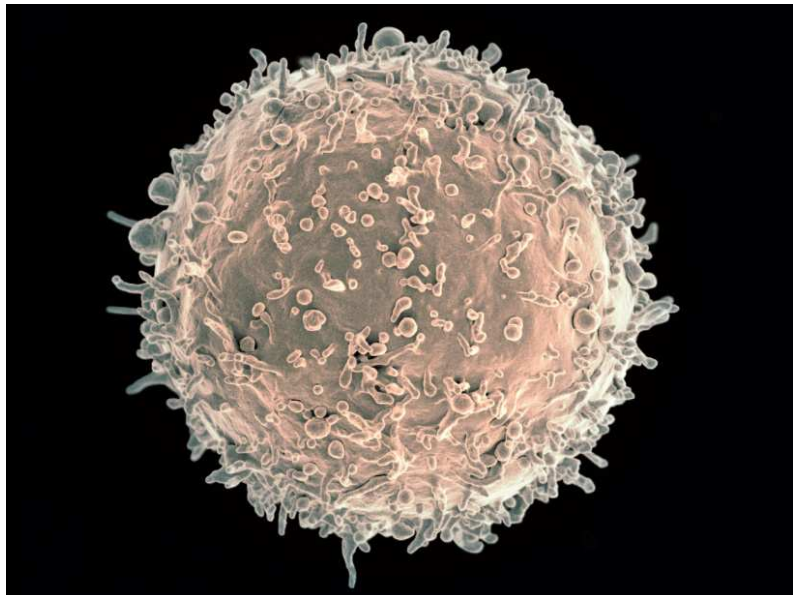


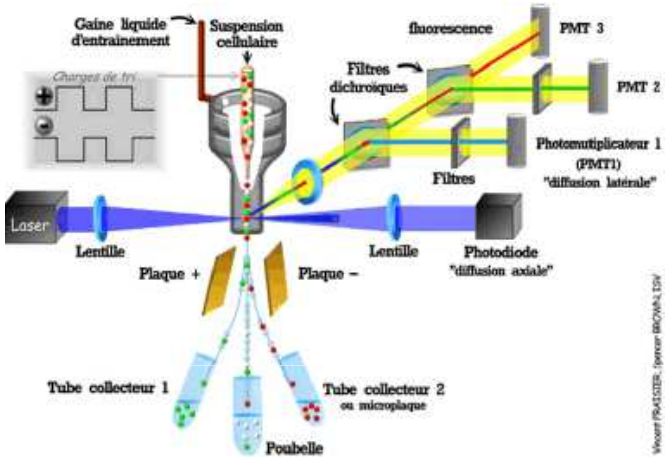
Méthodes d'étude des cellules immunitaires

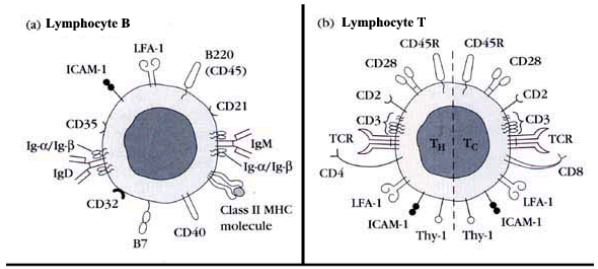
- Phénotype membranaire
  - Cellules en suspension
  - Dans le tissu

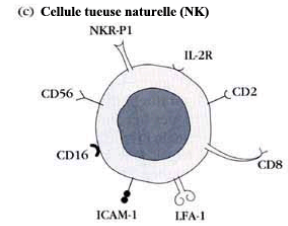
- Etude du transcriptome
  - Arrays
  - RNA Seq
  - Single-cell RNA Seq

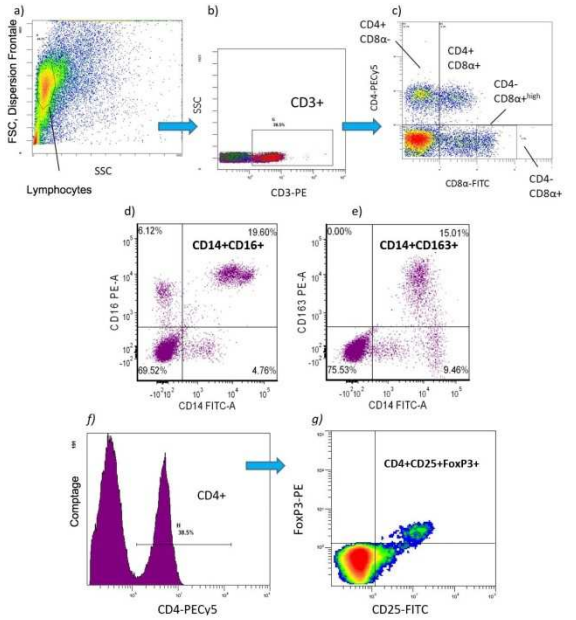
Phénotype cellulaire





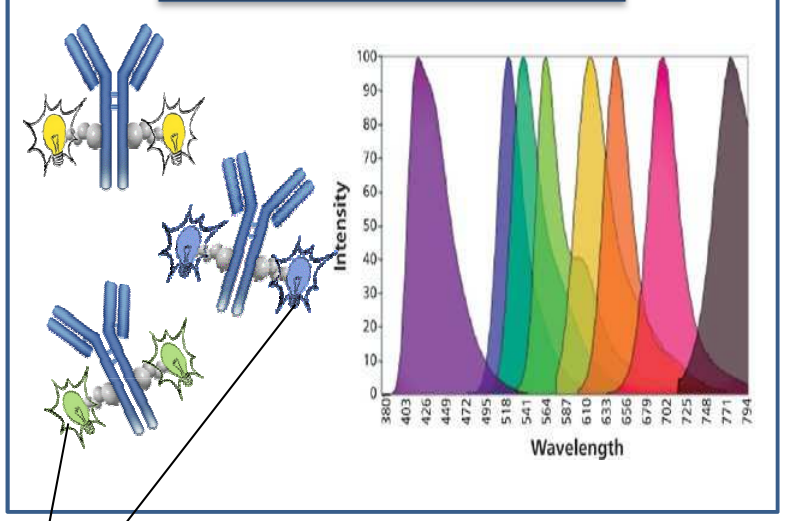






AVANCEES DE LA CYTOMETRIE DE MASSE

Cytométrie en flux



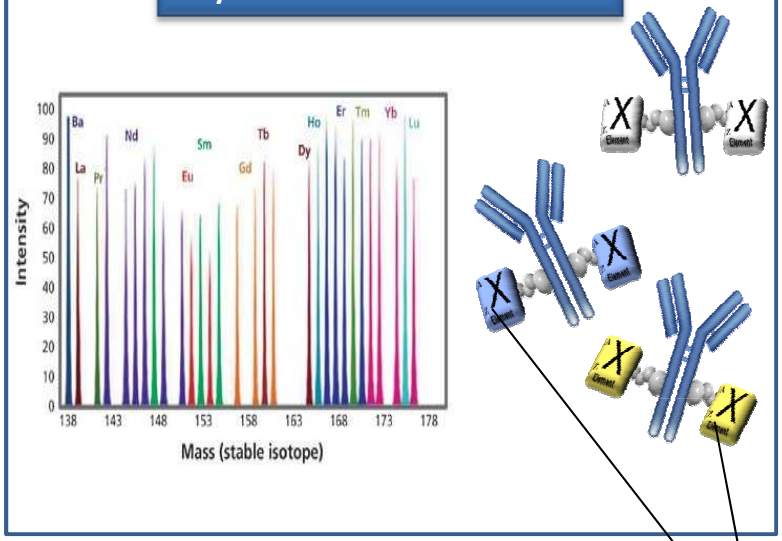
Fluorochromes variés

Recouvrement des bandes à différentes longueurs d'ondes

Perte de sensibilité

(15-20 paramètres / cellules possibles)

Cytométrie de masse



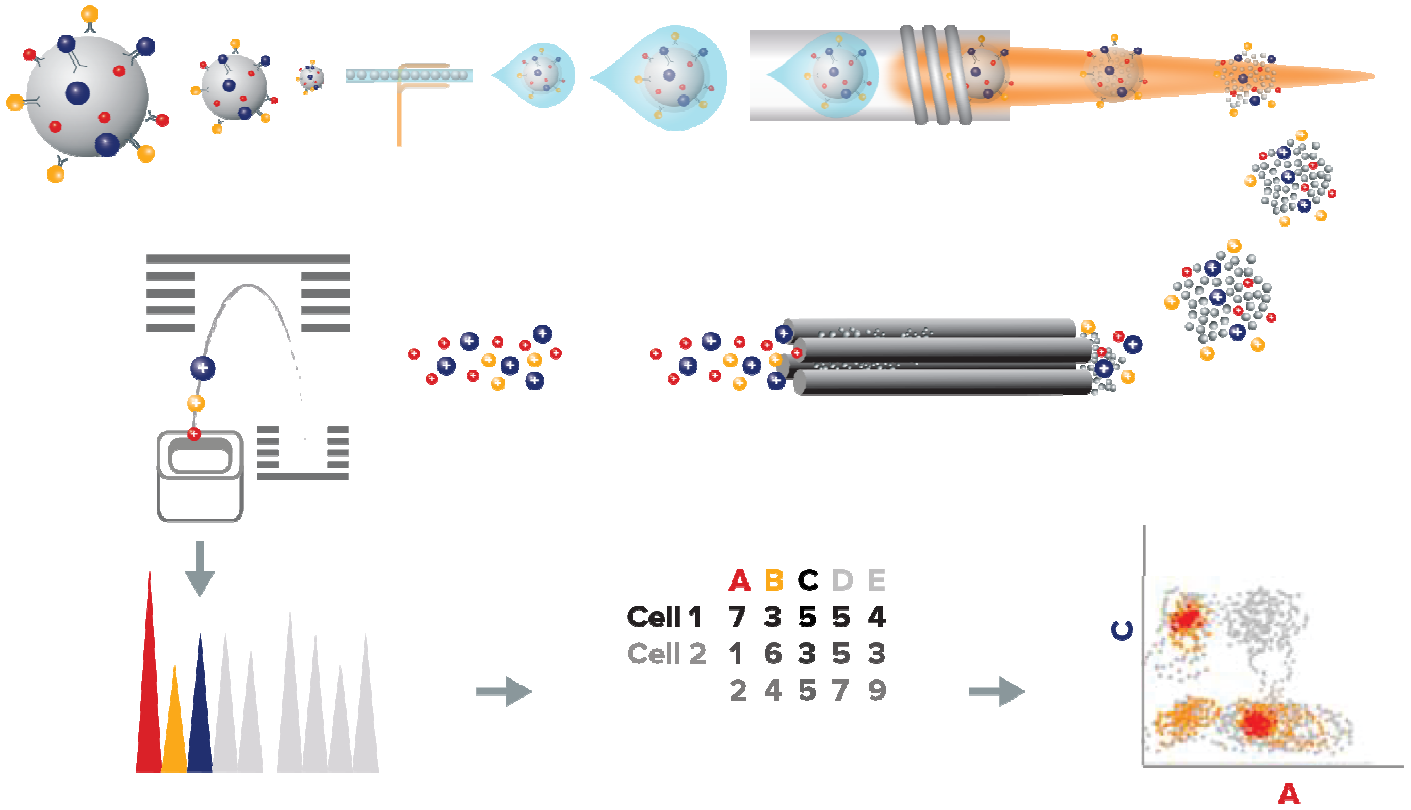
Cations multiples isotopes

Aucun recouvrement car un isotope correspond à une masse unique

Grande sensibilité

(50 paramètres / cellules possibles)

SUR LA BASE DE LA TECHNOLOGIE CyTOF



Evolution exponentielle de la quantité de données en cytométrie de masse

Cytométrie en flux

15-20 paramètres

> 200 Dots possibles

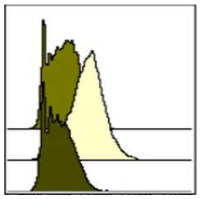
Cytométrie de masse

50 paramètres

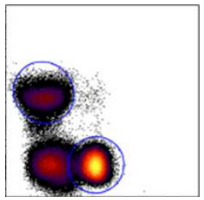
> 1200 Dots possibles

ANALYSES : Cytobank

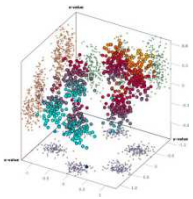
Plot raw data



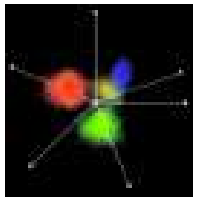
Histogram



Biaxial plot

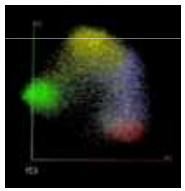


3D plot

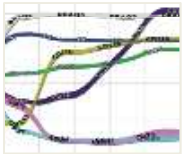


Radar

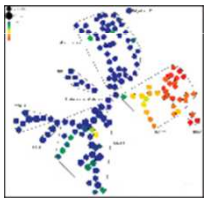
Reduce dimensionality



PCA

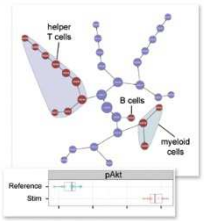


GemStone™



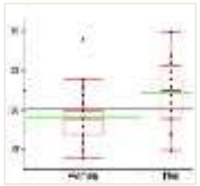
SPADE

viSNE

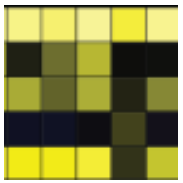


Citrus

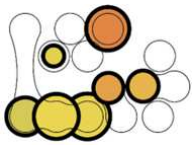
Summarize statistics



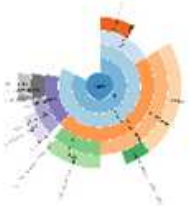
Box plot



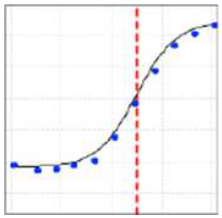
Heat map



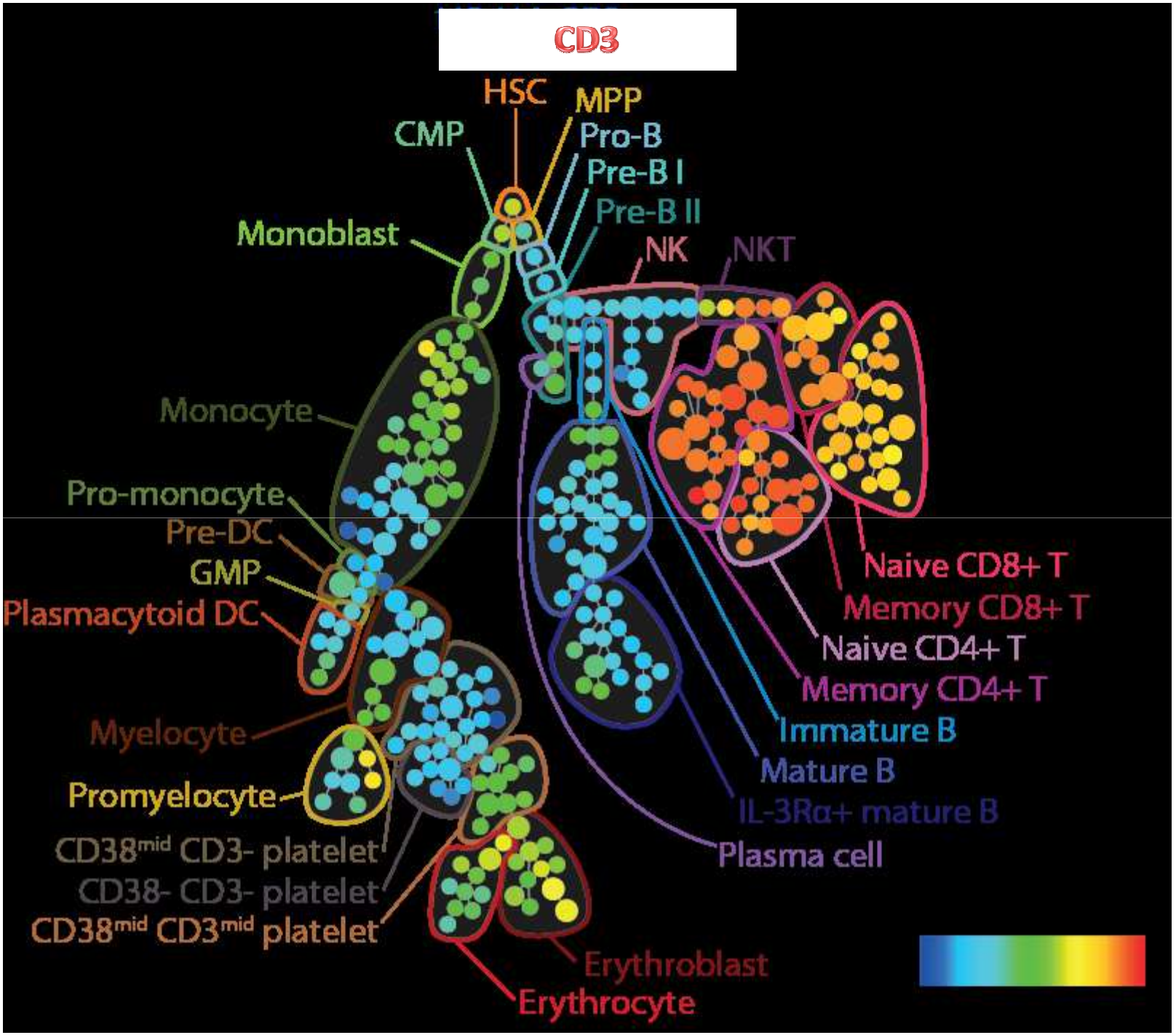
Network

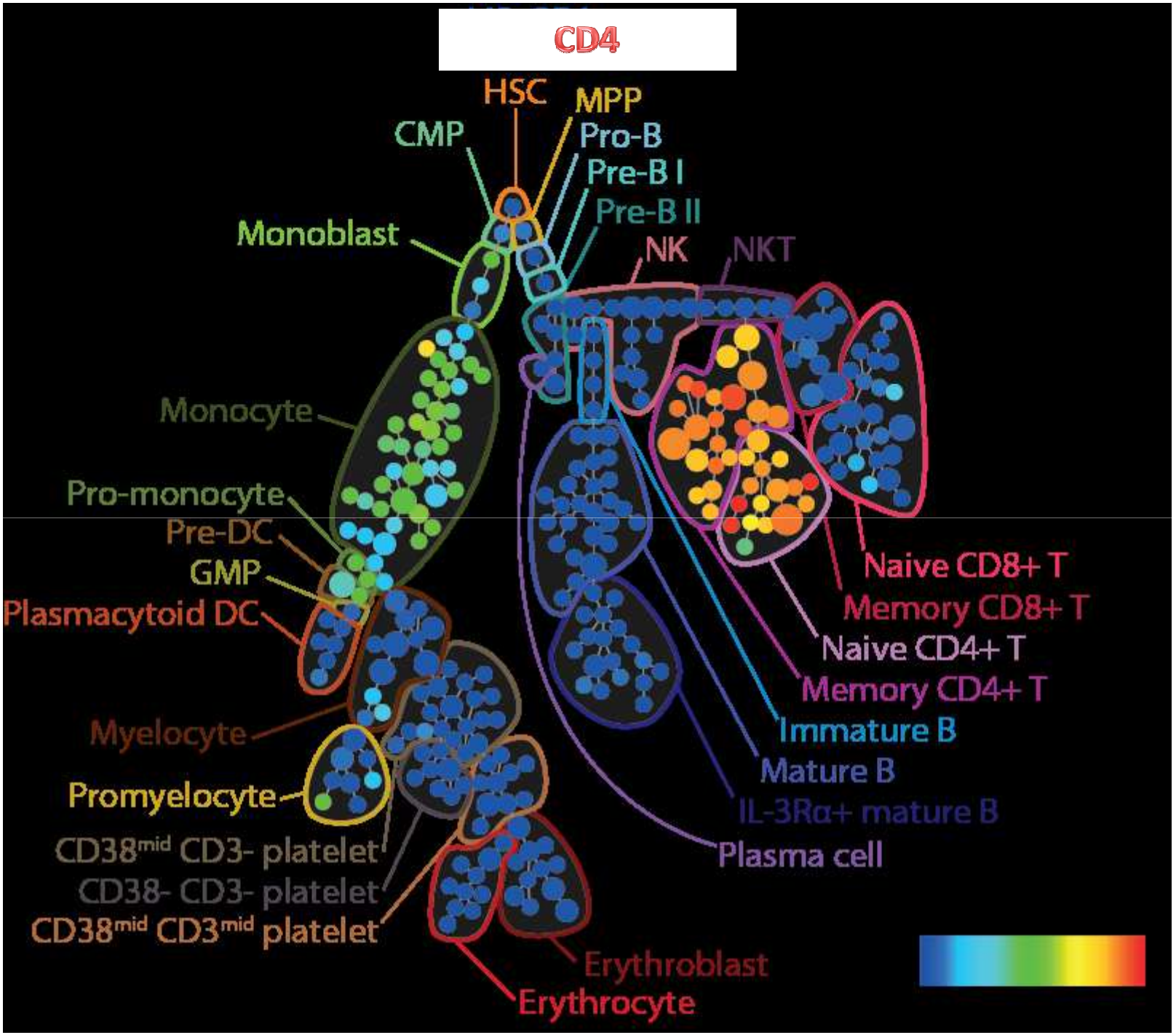


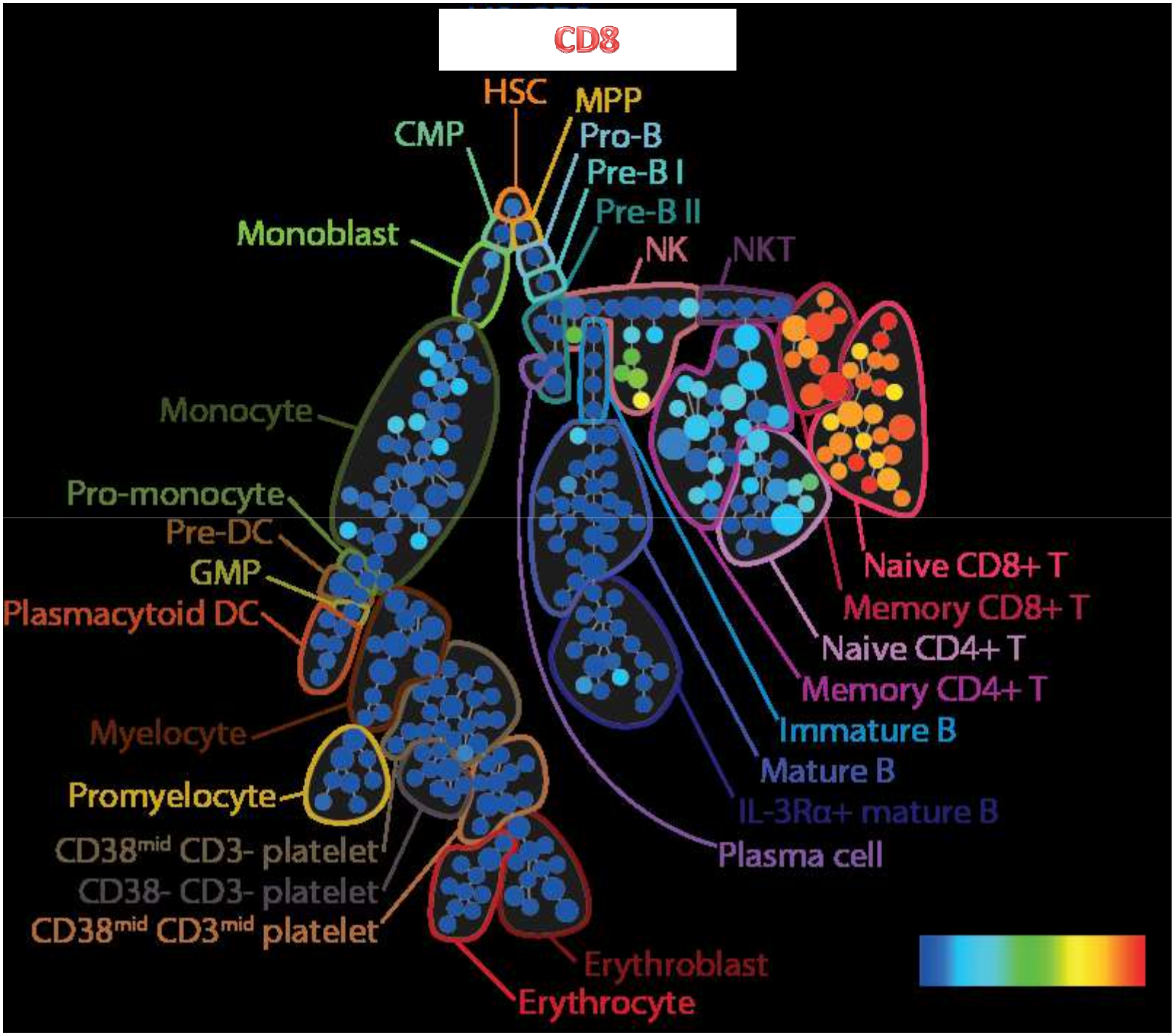
Sunburst

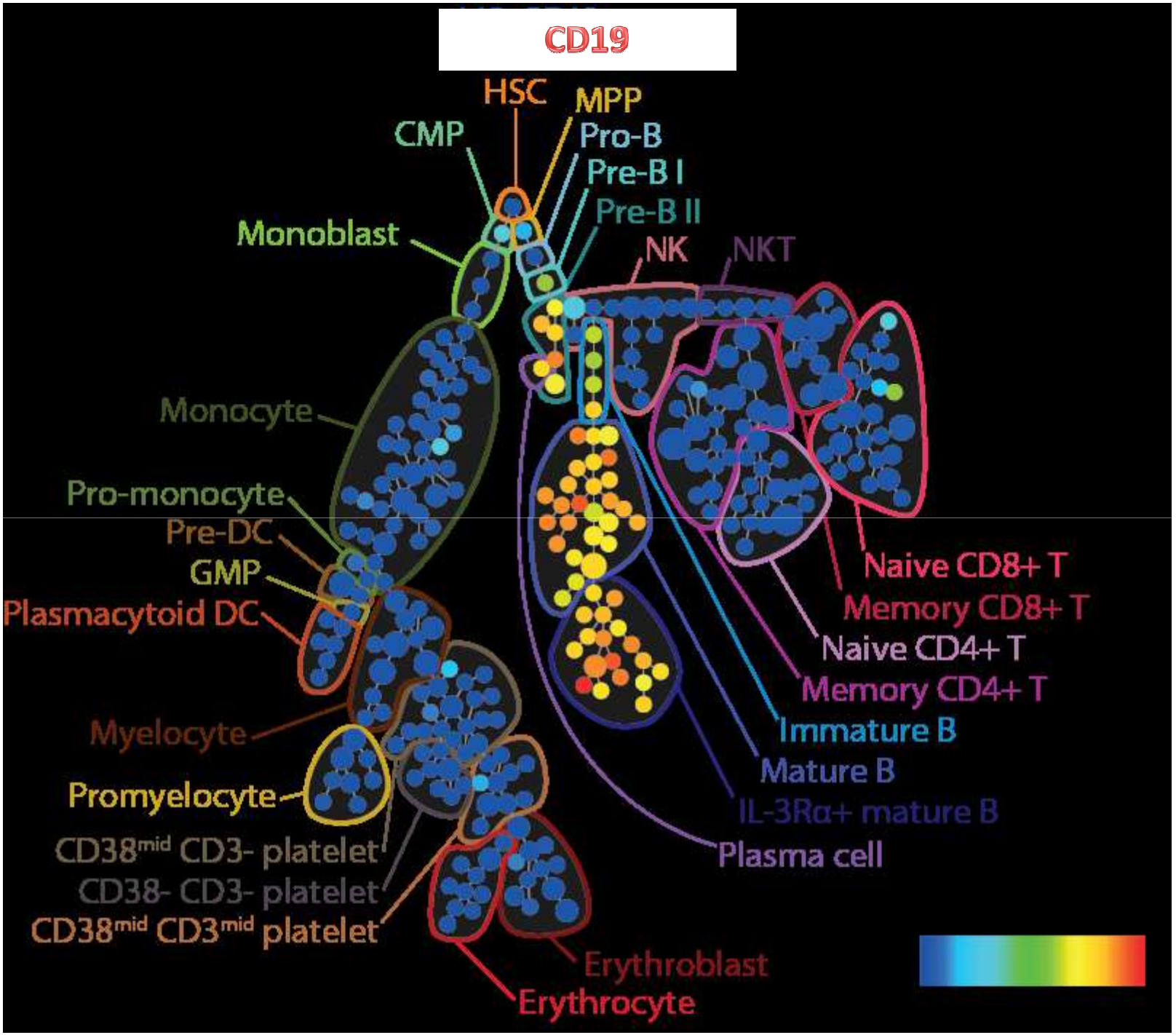


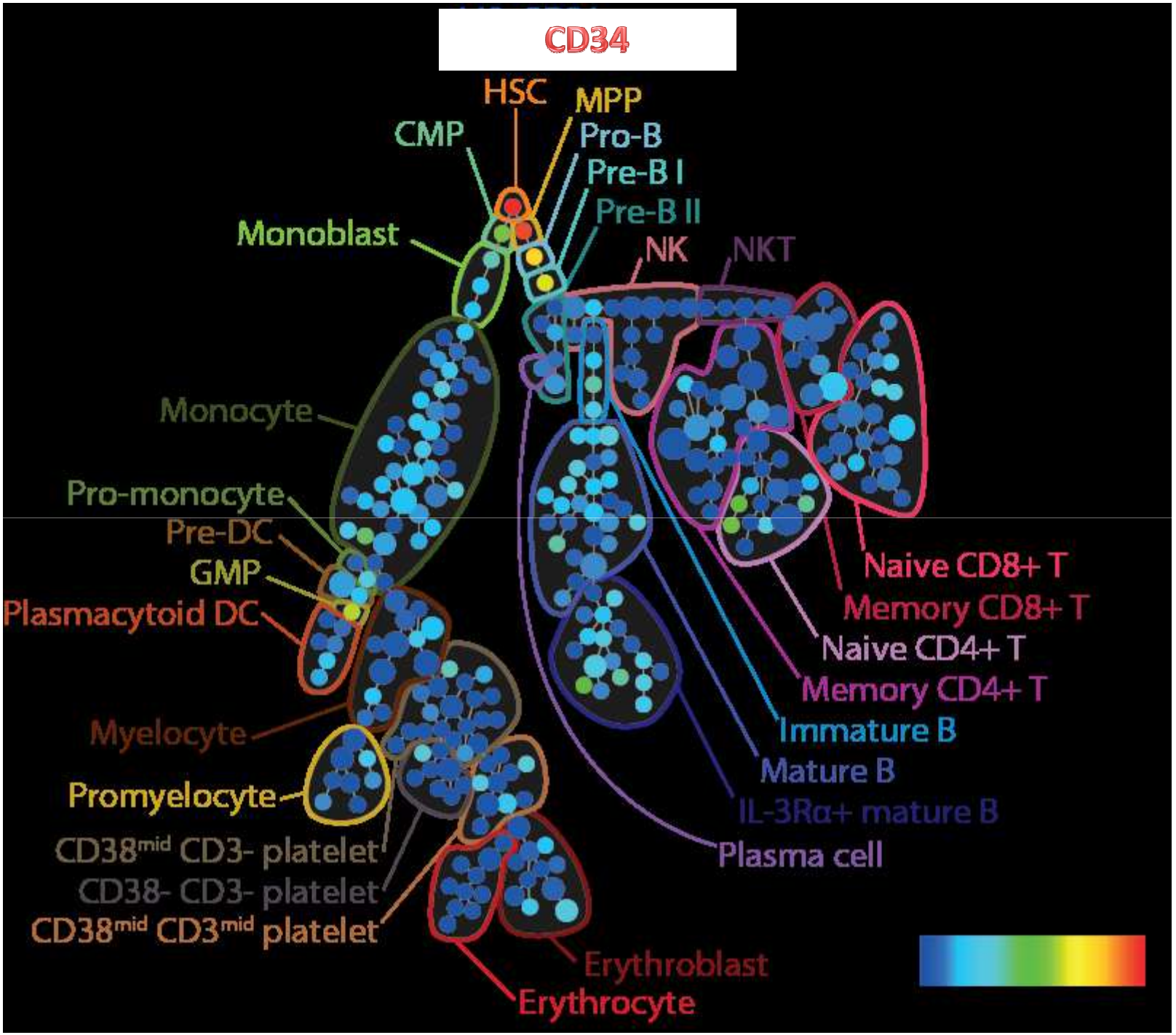
Dose curve

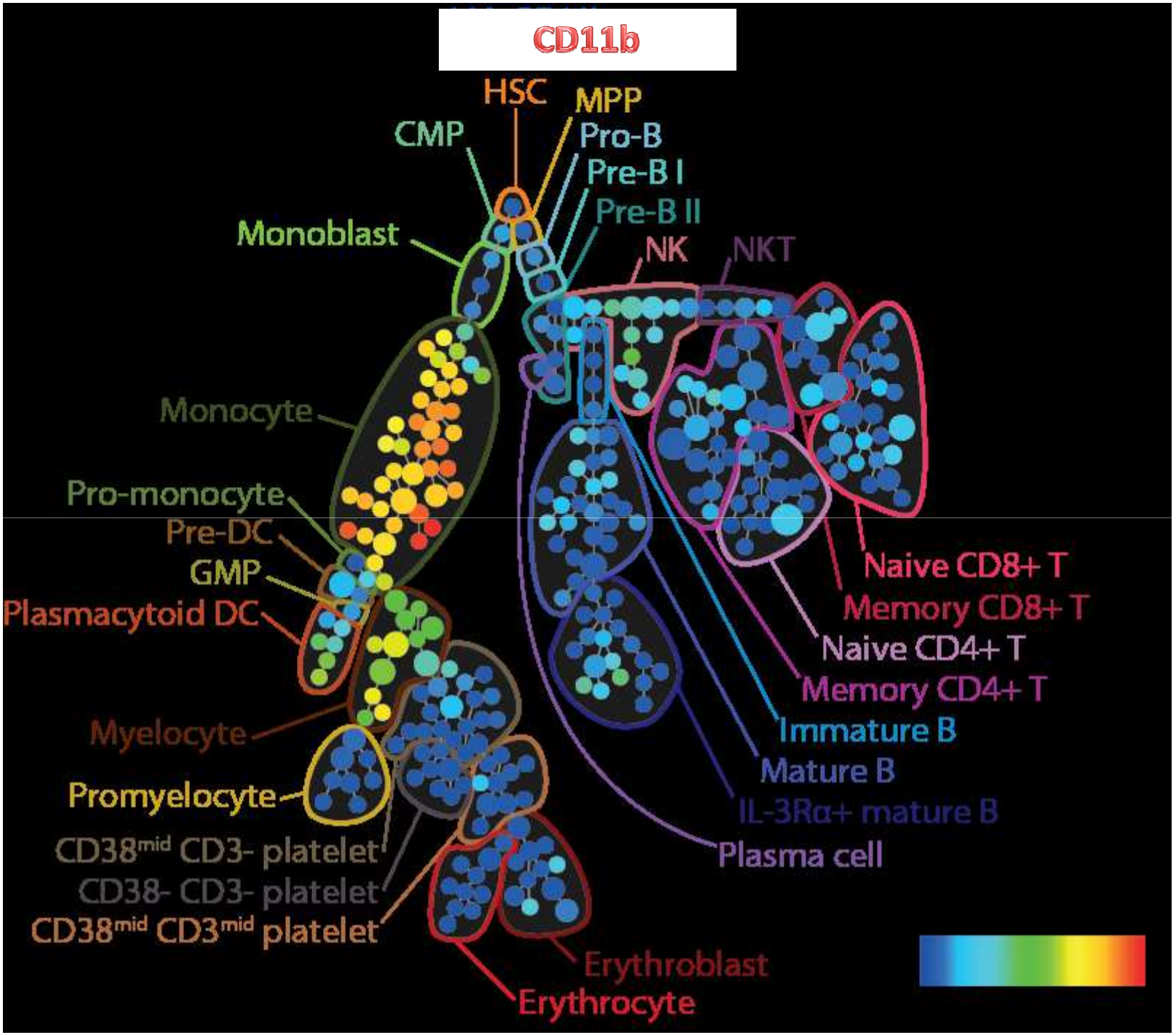

CD3
HSC
MPP
CMP
Pro-B
Pre-B I
Pre-B II
Monoblast
NK
NKT
Monocyte
Pro-monocyte
Pre-DC
GMP
Plasmacytoid DC
Naive CD8+ T
Memory CD8+ T
Naive CD4+ T
Memory CD4+ T
Immature B
Mature B
IL-3Rα+ mature B
Plasma cell
Myelocyte
Promyelocyte
CD38mid CD3- platelet
CD38- CD3- platelet
CD38mid CD3mid platelet
Erythroblast
Erythrocyte


CD4
HSC
MPP
CMP
Pro-B
Pre-B I
Pre-B II
Monoblast
NK
NKT
Monocyte
Pro-monocyte
Pre-DC
GMP
Plasmacytoid DC
Naive CD8+ T
Memory CD8+ T
Naive CD4+ T
Memory CD4+ T
Immature B
Mature B
IL-3Rα+ mature B
Plasma cell
Myelocyte
Promyelocyte
CD38mid CD3- platelet
CD38- CD3- platelet
CD38mid CD3mid platelet
Erythroblast
Erythrocyte


CD8
HSC
MPP
CMP
Pro-B
Pre-B I
Pre-B II
Monoblast
NK
NKT
Monocyte
Pro-monocyte
Pre-DC
GMP
Plasmacytoid DC
Naive CD8+ T
Memory CD8+ T
Naive CD4+ T
Memory CD4+ T
Immature B
Mature B
IL-3Rα+ mature B
Plasma cell
Myelocyte
Promyelocyte
CD38mid CD3- platelet
CD38- CD3- platelet
CD38mid CD3mid platelet
Erythroblast
Erythrocyte


CD19
HSC
MPP
CMP
Pro-B
Pre-B I
Pre-B II
Monoblast
NK
NKT
Monocyte
Pro-monocyte
Pre-DC
GMP
Plasmacytoid DC
Naive CD8+ T
Memory CD8+ T
Naive CD4+ T
Memory CD4+ T
Immature B
Mature B
IL-3Rα+ mature B
Plasma cell
Myelocyte
Promyelocyte
CD38mid CD3- platelet
CD38- CD3- platelet
CD38mid CD3mid platelet
Erythroblast
Erythrocyte

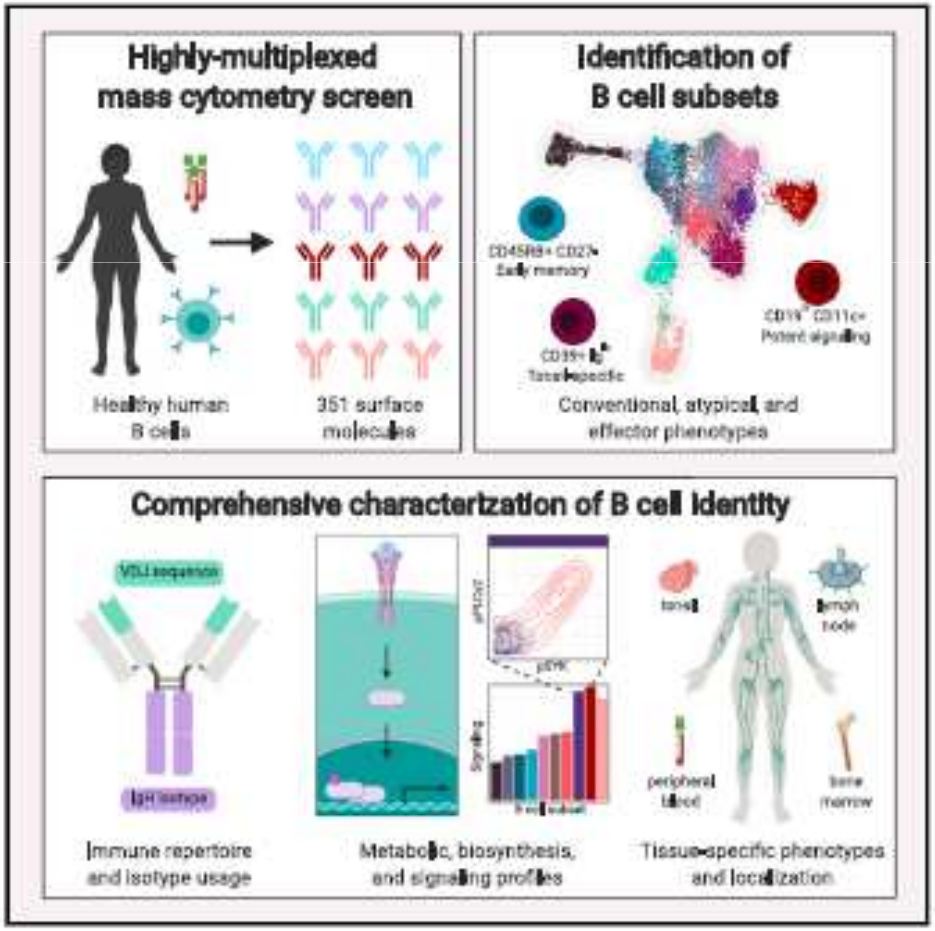

CD34
HSC
MPP
CMP
Pro-B
Pre-B I
Pre-B II
Monoblast
NK
NKT
Monocyte
Pro-monocyte
Pre-DC
GMP
Plasmacytoid DC
Naive CD8+ T
Memory CD8+ T
Naive CD4+ T
Memory CD4+ T
Immature B
Mature B
IL-3Rα+ mature B
Plasma cell
Myelocyte
Promyelocyte
CD38mid CD3- platelet
CD38- CD3- platelet
CD38mid CD3mid platelet
Erythroblast
Erythrocyte


CD11b
HSC
MPP
CMP
Pro-B
Pre-B I
Pre-B II
Monoblast
NK
NKT
Monocyte
Pro-monocyte
Pre-DC
GMP
Plasmacytoid DC
Naive CD8+ T
Memory CD8+ T
Naive CD4+ T
Memory CD4+ T
Immature B
Mature B
IL-3Rα+ mature B
Plasma cell
Myelocyte
Promyelocyte
CD38mid CD3- platelet
CD38- CD3- platelet
CD38mid CD3mid platelet
Erythroblast
Erythrocyte

Immunity

An Integrated Multi-omic Single-Cell Atlas of Human B Cell Identity

Graphical Abstract



Authors

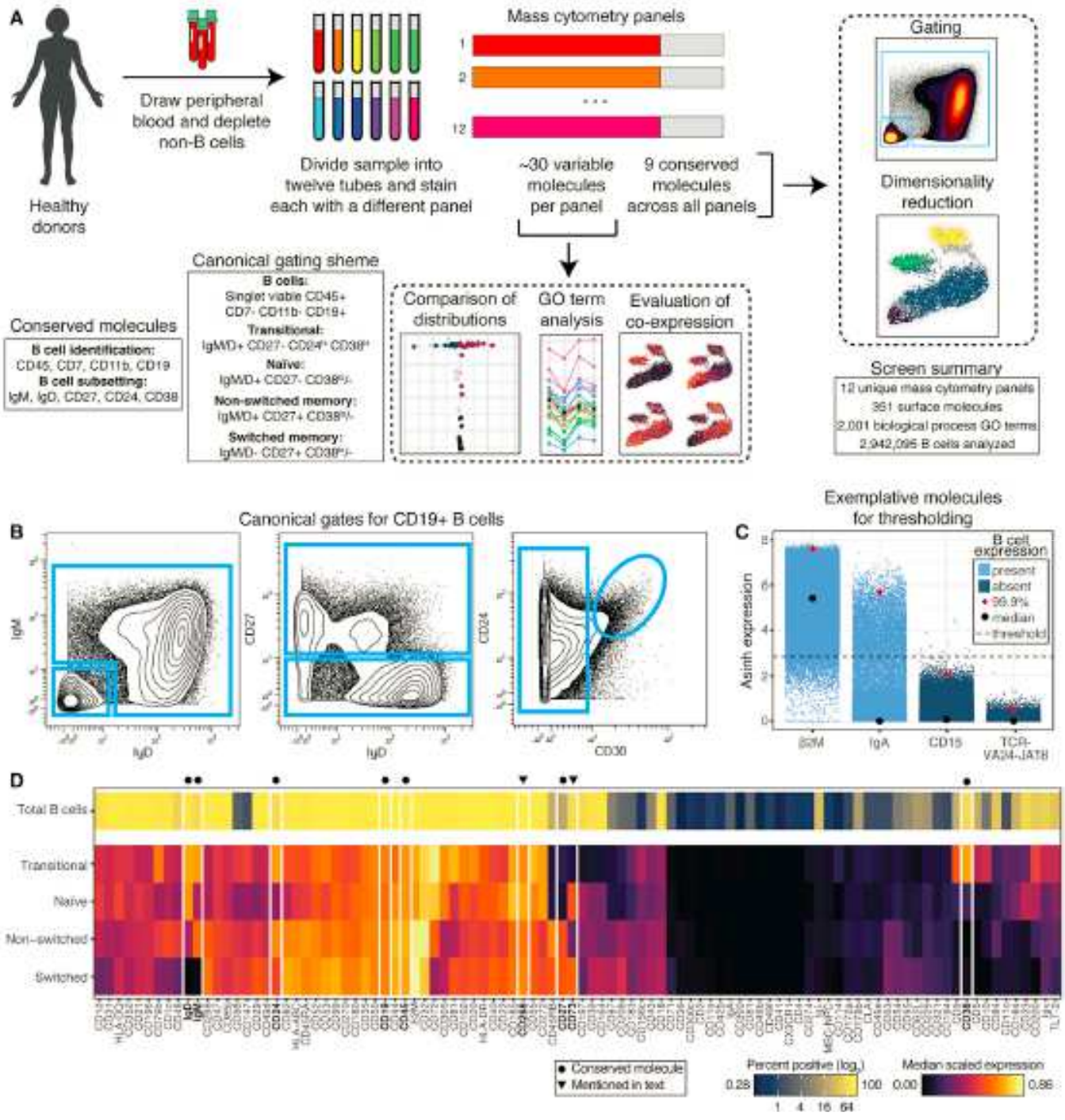
David R. Glass, Albert G. Tsai, John Paul Oliveria, ..., Lisa E. Wagar, Mark M. Davis, Sean C. Bendall

Correspondence

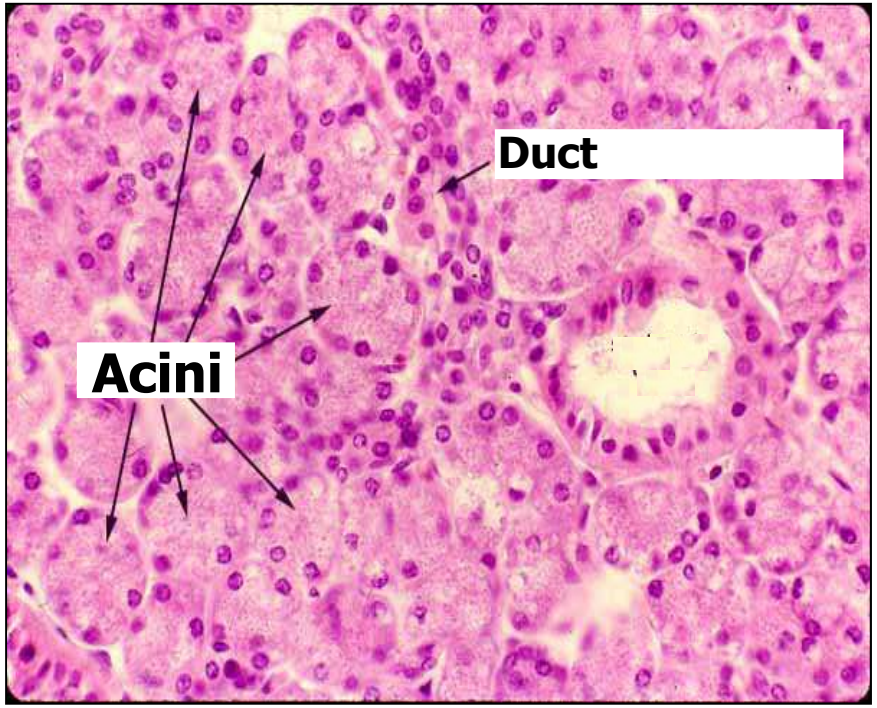
bendall@stanford.edu

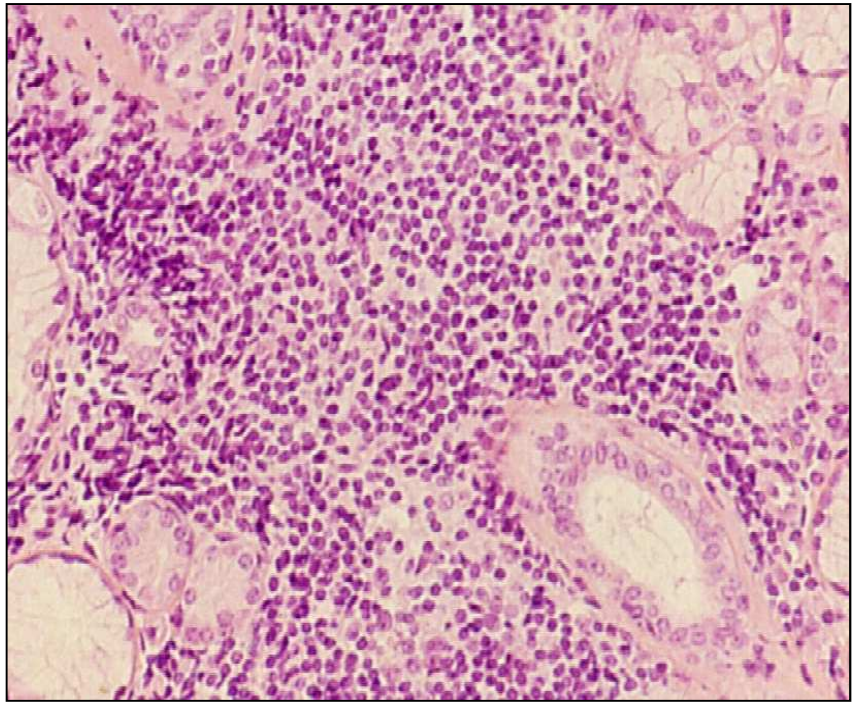
In Brief

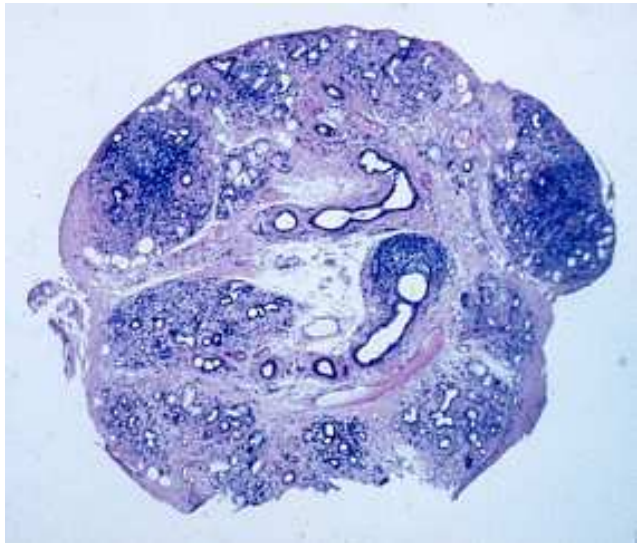
To identify molecules that distinguish functionally distinct subsets of human B cells, Glass et al. screened the expression of 351 surface molecules by mass cytometry. By combining identified molecules with functional readouts, they propose a new classification scheme to segregate B cells from four lymphoid tissues into twelve unique subsets.

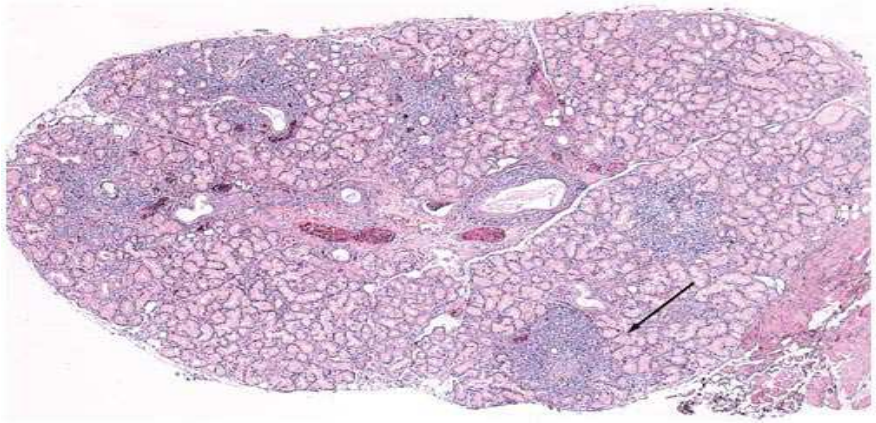

A
Healthy donors
Draw peripheral blood and deplete non-B cells
Divide sample into twelve tubes and stain each with a different panel
Mass cytometry panels
1
2
12
~30 variable molecules per panel
9 conserved molecules across all panels
Gating
Dimensionality reduction
Screen summary
12 unique mass cytometry panels
351 surface molecules
2,001 biological process GO terms
2,942,095 B cells analyzed
Canonical gating sheme
B cells:
Singlet viable CD45+ CD7- CD11b- CD19+
Transitional:
IgMD+ CD27- CD24+ CD38+
Naive:
IgMD+ CD27- CD38+/-
Non-switched memory:
IgMD+ CD27+ CD38+/-
Switched memory:
IgMD- CD27+ CD38+/-
Conserved molecules
B cell identification:
CD45, CD7, CD11b, CD19
B cell subsetting:
IgM, IgD, CD27, CD24, CD38
Comparison of distributions
GO term analysis
Evaluation of co-expression
B
Canonical gates for CD19+ B cells
IgM
IgD
CD27
IgD
CD24
CD38
C
Exemplative molecules for thresholding
B cell expression
present
absent
99.9%
median
threshold
Asinh expression
IgM
IgA
CD15
TCR-VA24-JATB
D
Total B cells
Transitional
Naive
Non-switched
Switched
Conserved molecule
Mentioned in text
Percent positive (log4)
0.25
100
1
4
16
64
Median scaled expression
0.00
0.86

Et dans le tissu ?





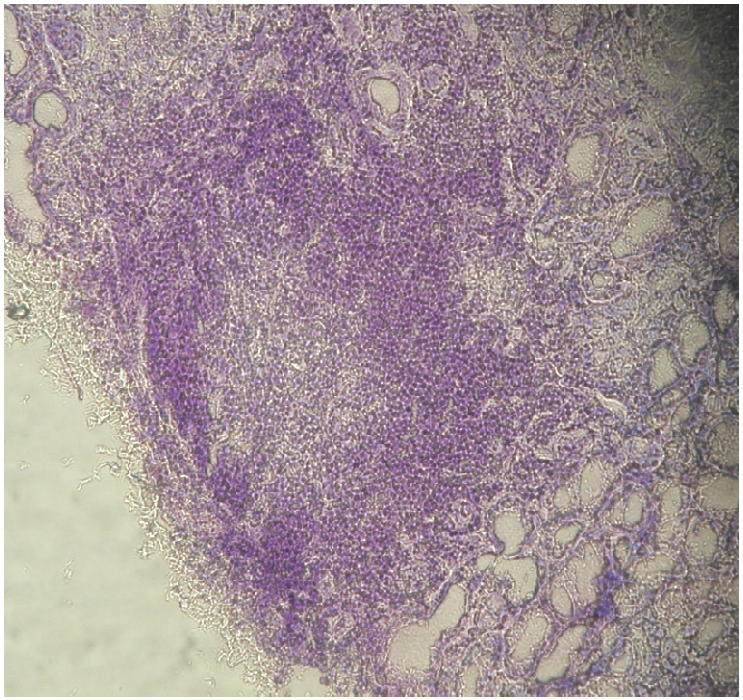


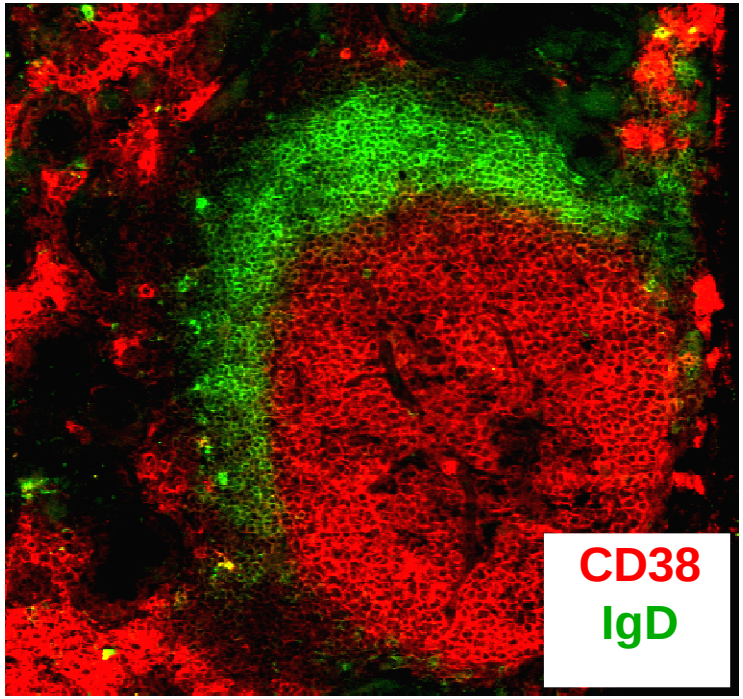


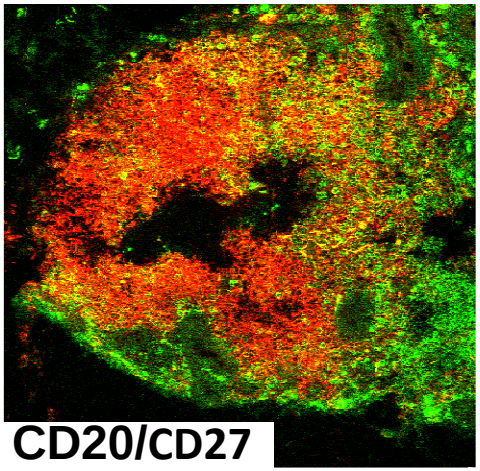
Focus score
= nb of foci / 4 mm²

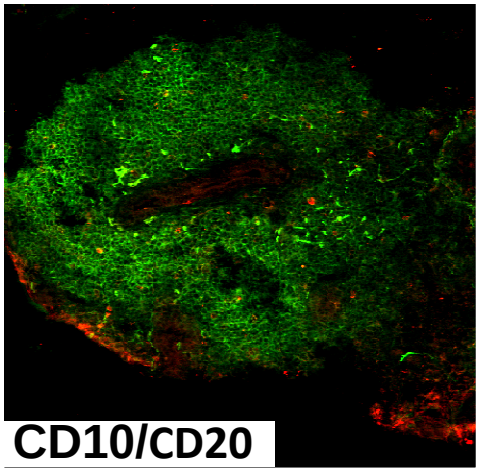
A role for B-cells in Sjögren's syndrome?

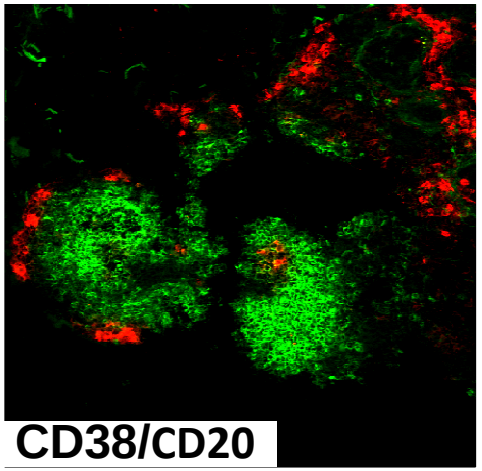
ECTOPIC GERMINAL CENTERS





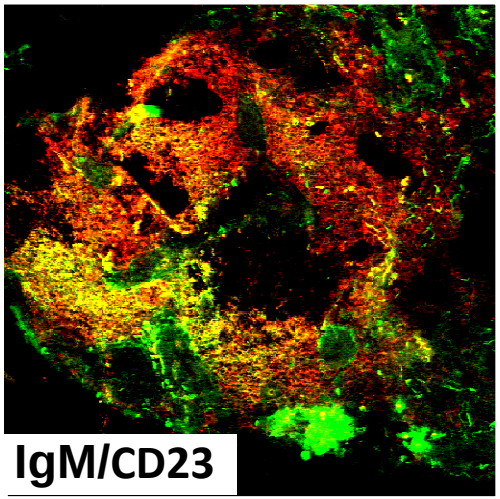


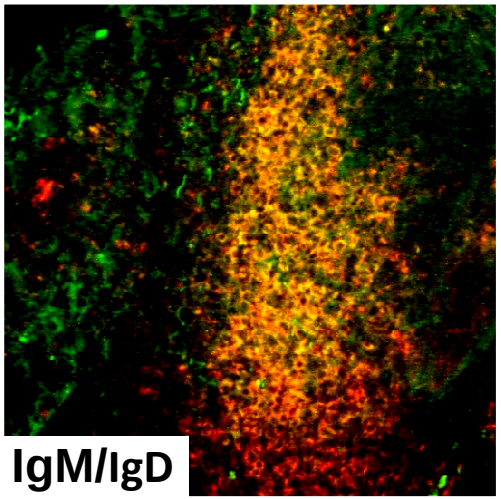


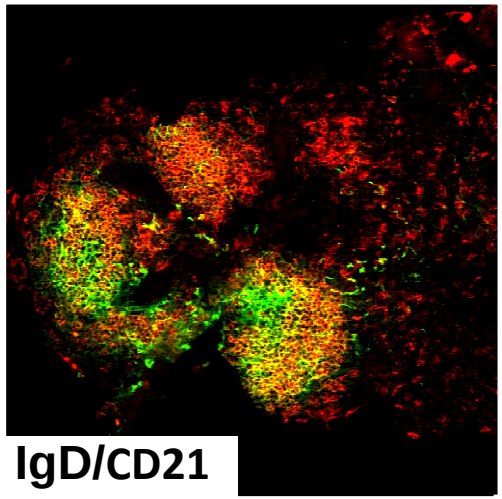


Memory B cells

Non CG B cells





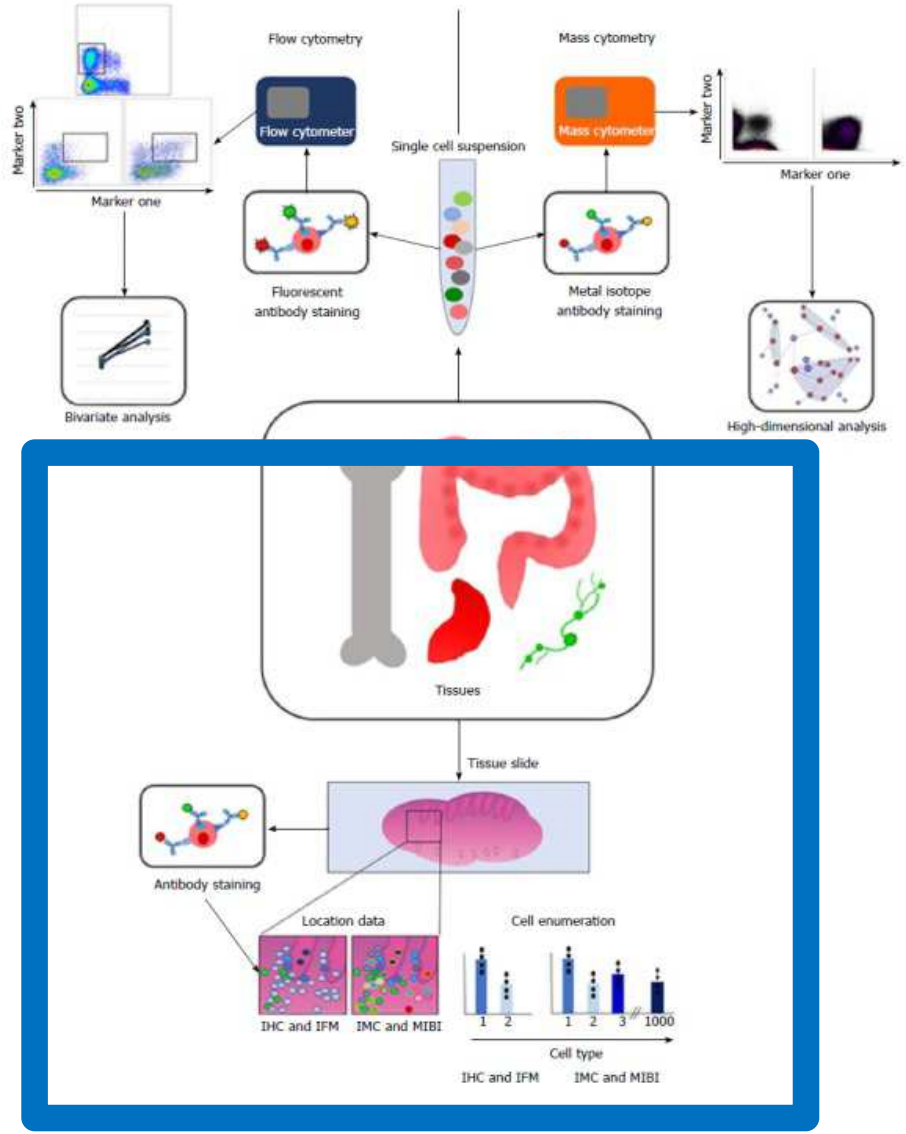


Transitionnal Type 2 B cells

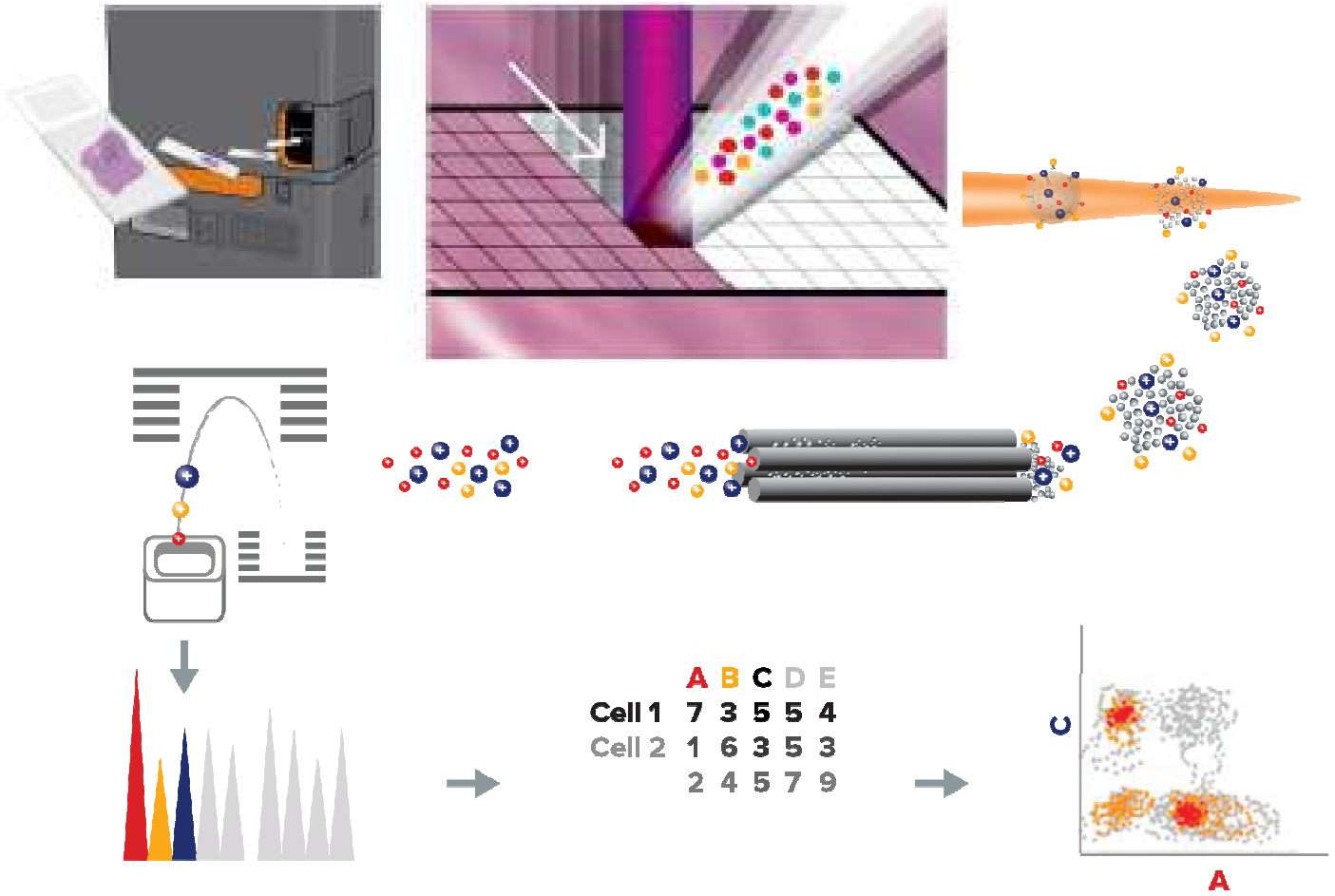
Daridon *et coll., Arthritis Rheum, 2006*

HELIOS / HYPERION

HYPERION



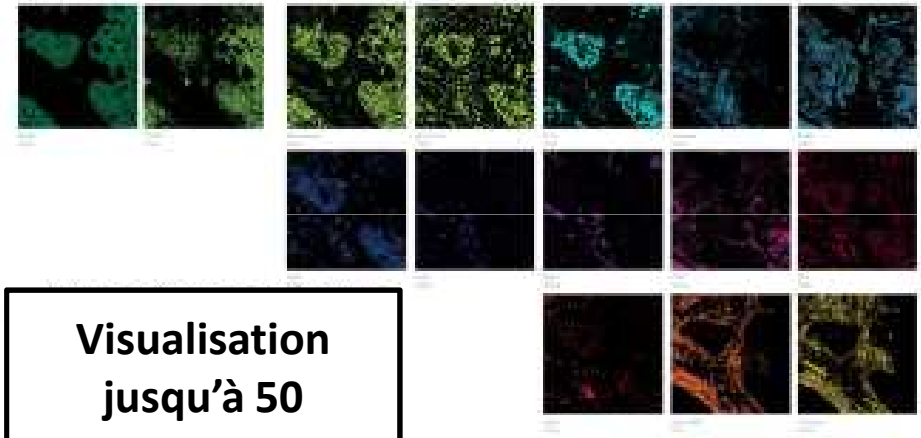
SUR LA BASE DE LA TECHNOLOGIE CyTOF



HYPERION

<u>Acquisition</u> :
Imageur Hyperion

<u>Détection</u>:
Technologie CyTOF



Visualisation jusqu'à 50 marqueurs

Sur tissus congelées, parafines ou cellules adhérentes sur lames avec une résolution de 1µm

HYPERION

1

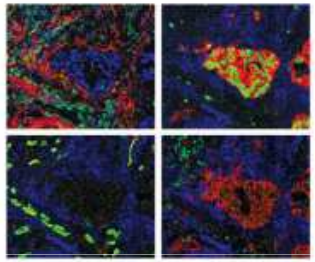
Design de panel en utilisant des anticorps fluidigm validés ou anticorps couplé à façon

2

Marquage sur lame FFPE or congelées avec des protocole d'IHC classique

3

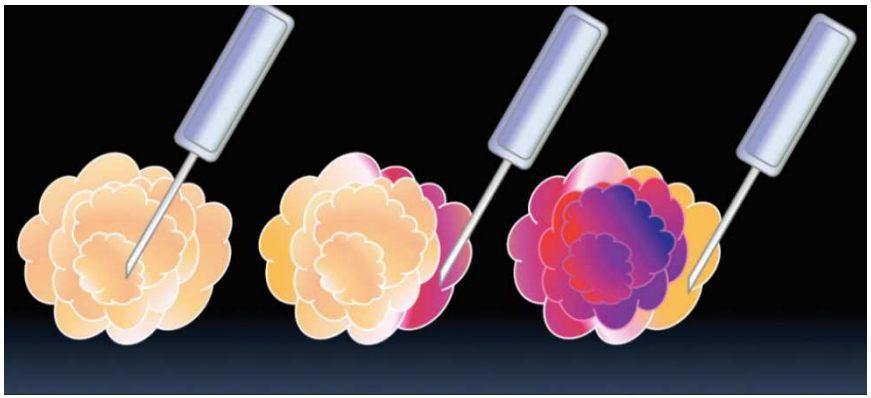
Acquisition d'image utilisant le system hyperion

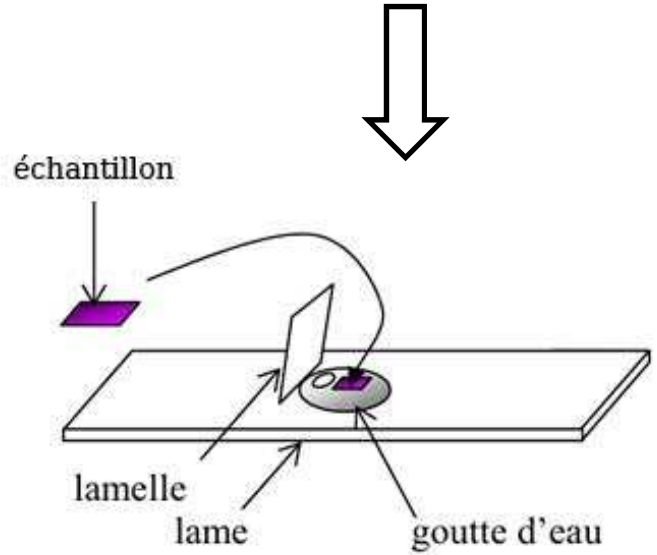


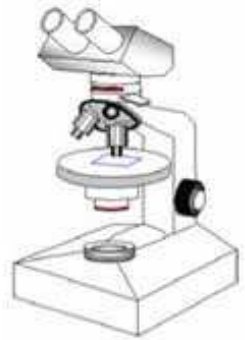
4

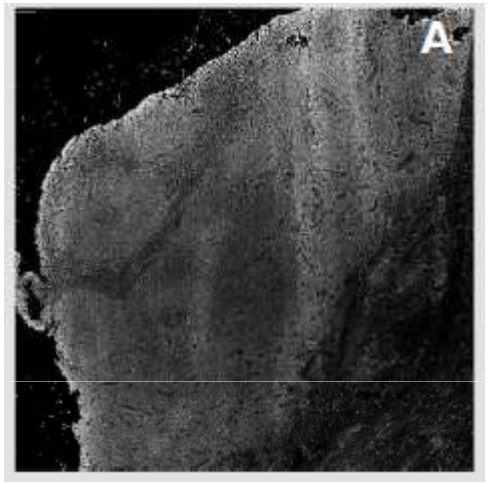
Analyse d'image grâce à des logiciels libre service ou tiers sur plateformes avancées

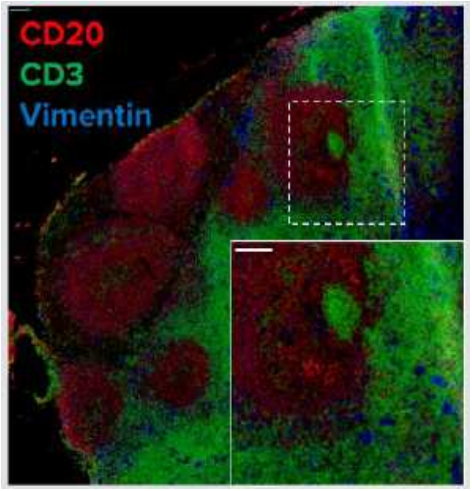
Tissue analysis – IHC/IF



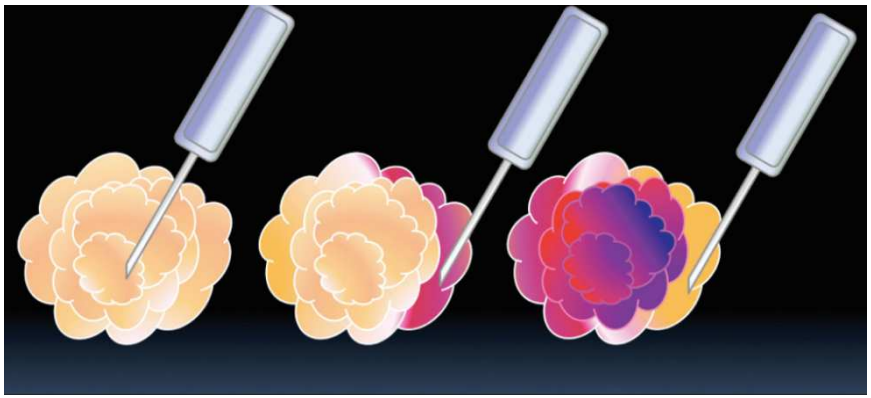


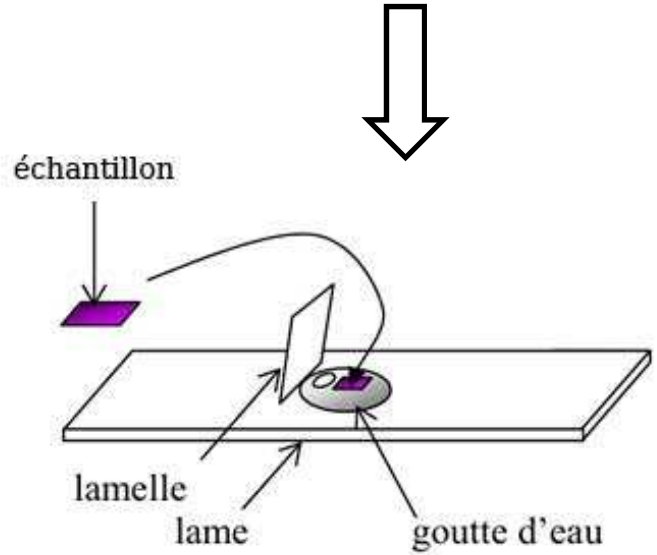


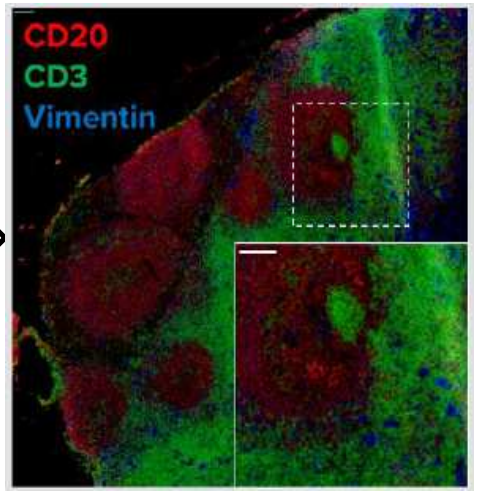


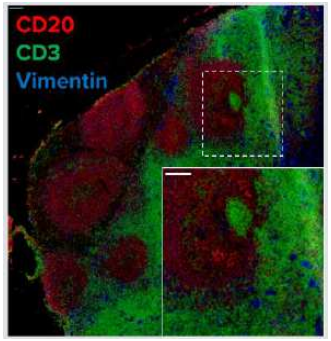


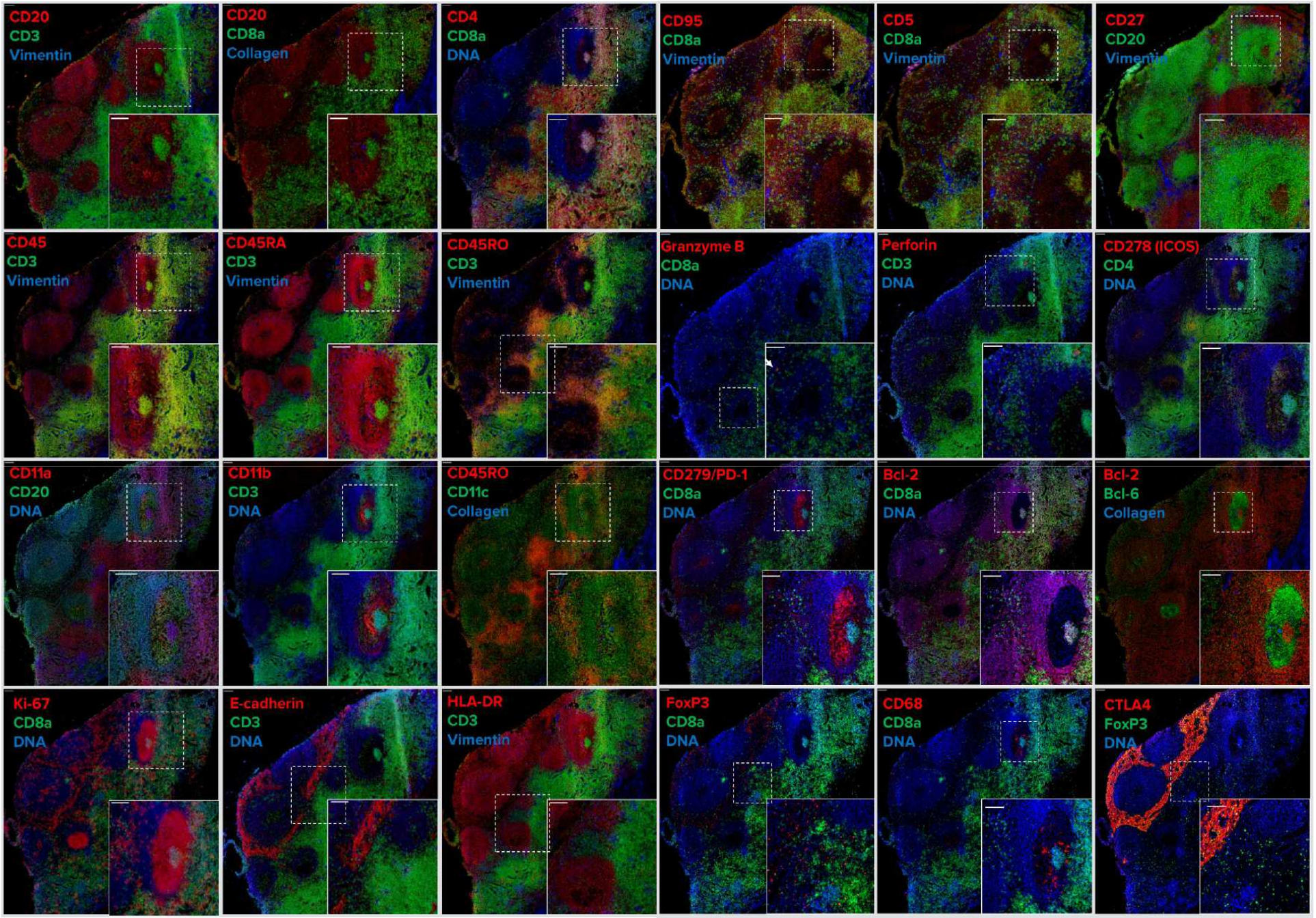
Hyperion – tissue mass imaging



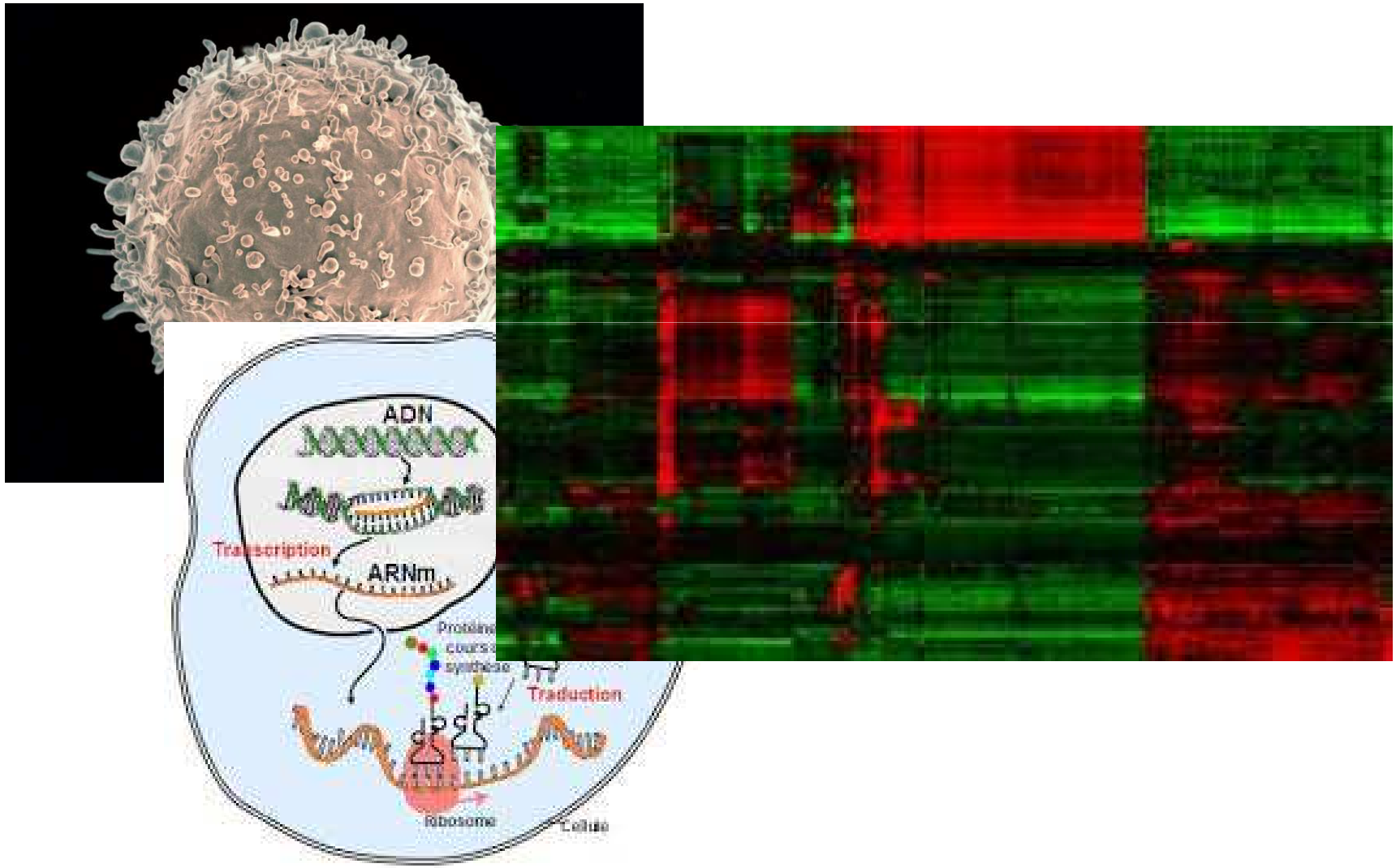


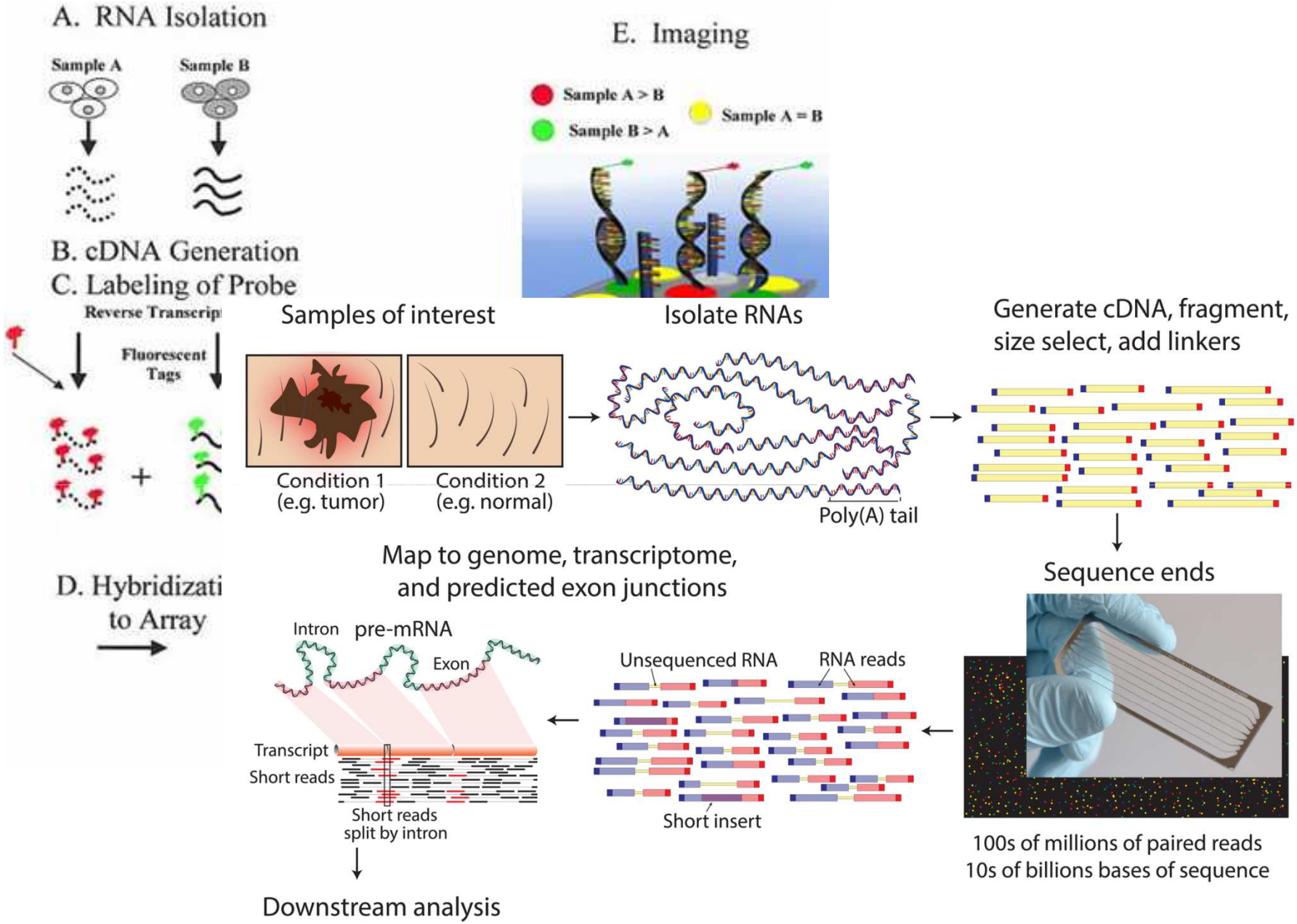



CD20
CD3
Vimentin


CD20
CD3
Vimentin
CD20
CD8a
Collagen
CD4
CD8a
DNA
CD95
CD8a
Vimentin
CD5
CD8a
Vimentin
CD27
CD20
Vimentin
CD45
CD3
Vimentin
CD45RA
CD3
Vimentin
CD45RO
CD3
Vimentin
Granzyme B
CD8a
DNA
Perforin
CD3
DNA
CD278 (ICOS)
CD4
DNA
CD11a
CD20
DNA
CD11b
CD3
DNA
CD45RO
CD11c
Collagen
CD279/PD-1
CD8a
DNA
Bcl-2
CD8a
DNA
Bcl-2
Bcl-6
Collagen
Ki-67
CD8a
DNA
E-cadherin
CD3
DNA
HLA-DR
CD3
Vimentin
FoxP3
CD8a
DNA
CD68
CD8a
DNA
CTLA4
FoxP3
DNA

Analyses transcriptomiques



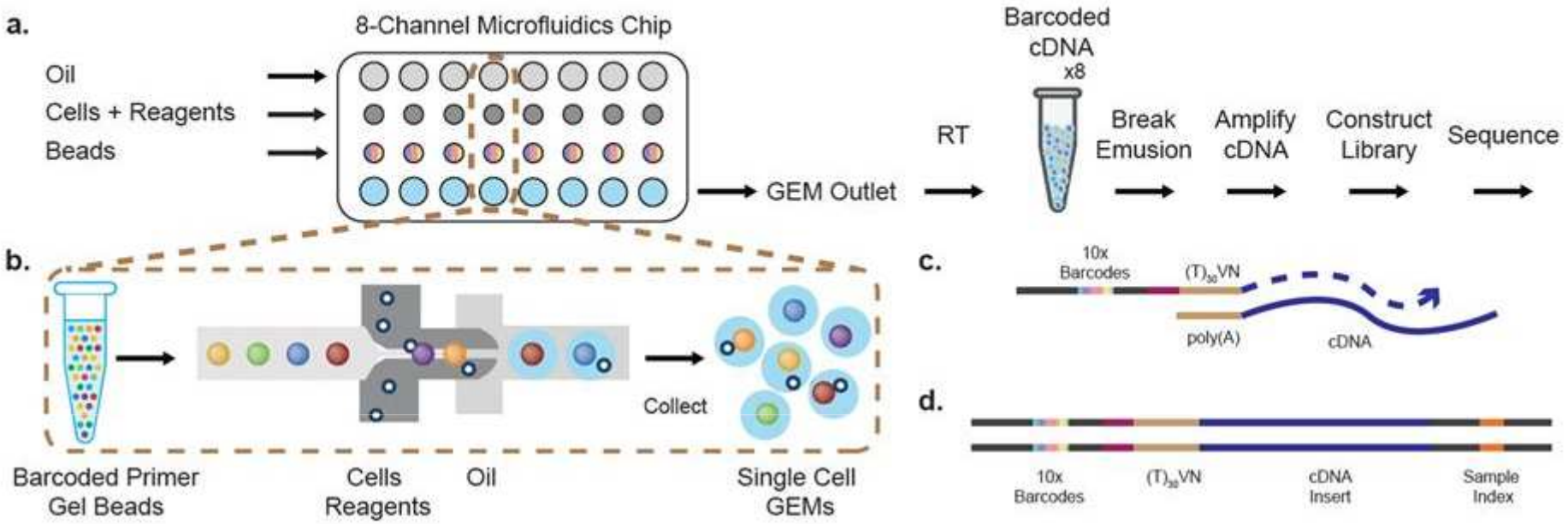

A. RNA Isolation
Sample A
Sample B
B. cDNA Generation
C. Labeling of Probe
Reverse Transcrip
Fluorescent Tags
D. Hybridizati
to Array
E. Imaging
Sample A > B
Sample B > A
Sample A = B
Samples of interest
Isolate RNAs
Condition 1
(e.g. tumor)
Condition 2
(e.g. normal)
Poly(A) tail
Generate cDNA, fragment,
size select, add linkers
Sequence ends
100s of millions of paired reads
10s of billions bases of sequence
Unsequenced RNA
RNA reads
Short insert
Map to genome, transcriptome,
and predicted exon junctions
Intron
pre-mRNA
Exon
Transcript
Short reads
Short reads
split by intron
Downstream analysis

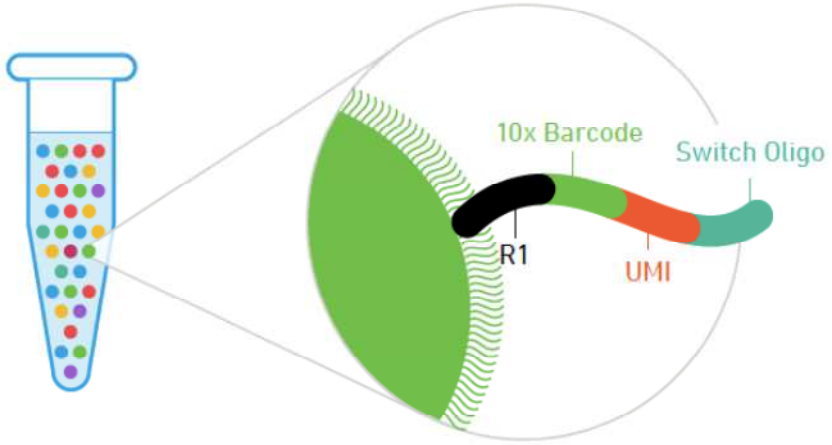
Down to the single-cell level

- Mass cytometry-based technologies
  - CyTOF (circulating cells): 50 markers
  - Hyperion (imaging mass cytometer): 40 markers on tissue

- Single-cell RNA-Seq (10X technology)
  - Single-cell transcriptomics (~2000 genes in ~10,000 cells)
  - Ig gene sequencing (paired VDJ sequences): B-cell repertoire
  - On the same cell
  - Circulating cells and tissue-eluted cells

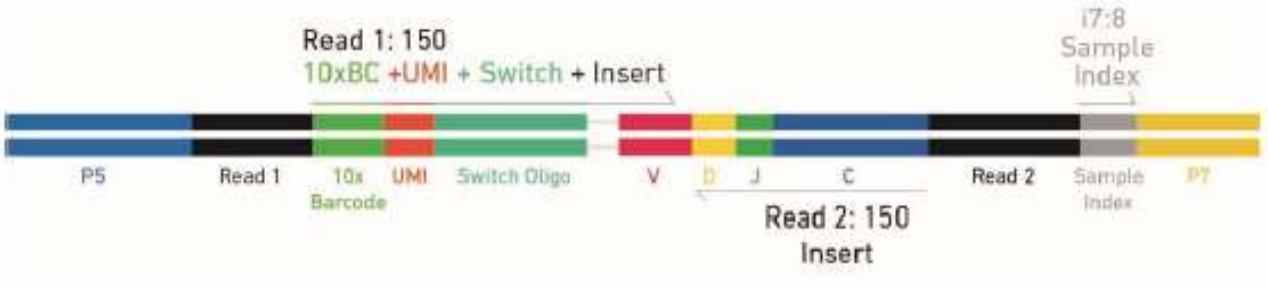
single cell RNAseq





i. Partial Illumina Read 1 Sequence (22 nucleotides (nt))
ii. 16 nt 10x™ Barcode
iii. 10 nt Unique Molecular Identifier (UMI)
iv. 13 nt Switch Oligo

V(D)J Enriched Library Structure:



5' Gene Expression Library Structure:

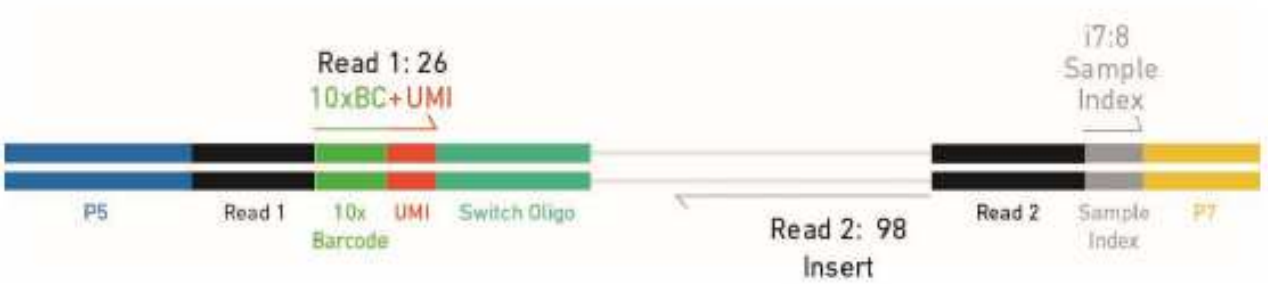
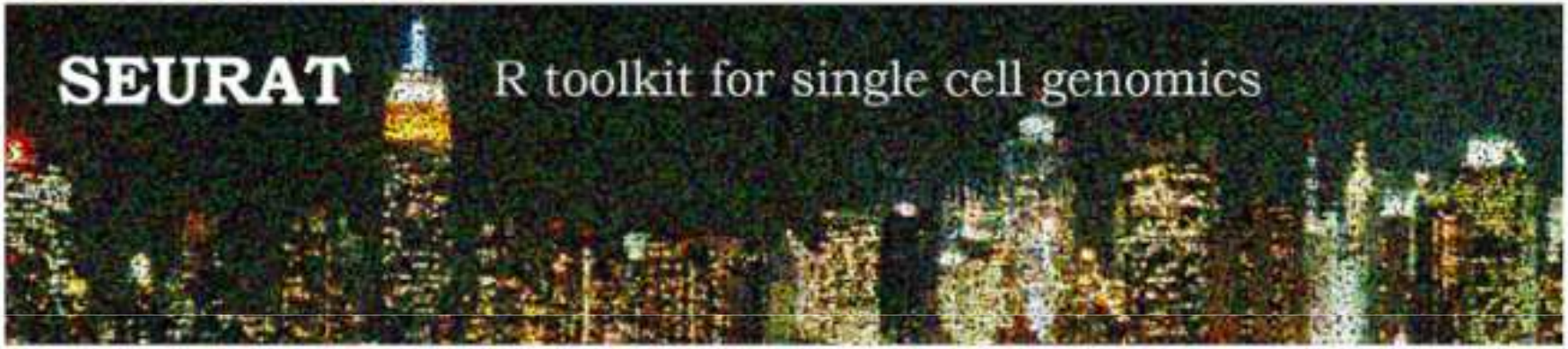


Figure 2. Schematic of final library constructs and recommended sequencing run parameters for the Single Cell V(D)J Protocol options.

Seurat
https://satijalab.org/seurat/

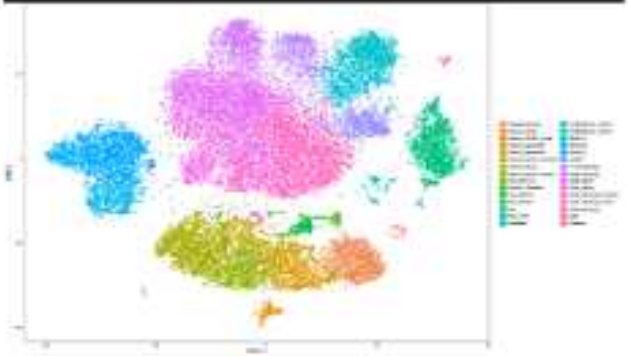
SATIJA LAB

HOME NEWS PEOPLE RESEARCH PUBLICATIONS SEURAT JOIN/CONTACT

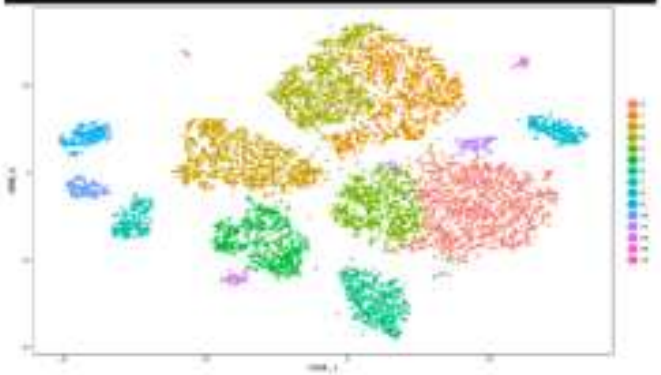


About | Install | Get Started | Frequently Asked Questions | Frequently Requested Vignettes | Contact

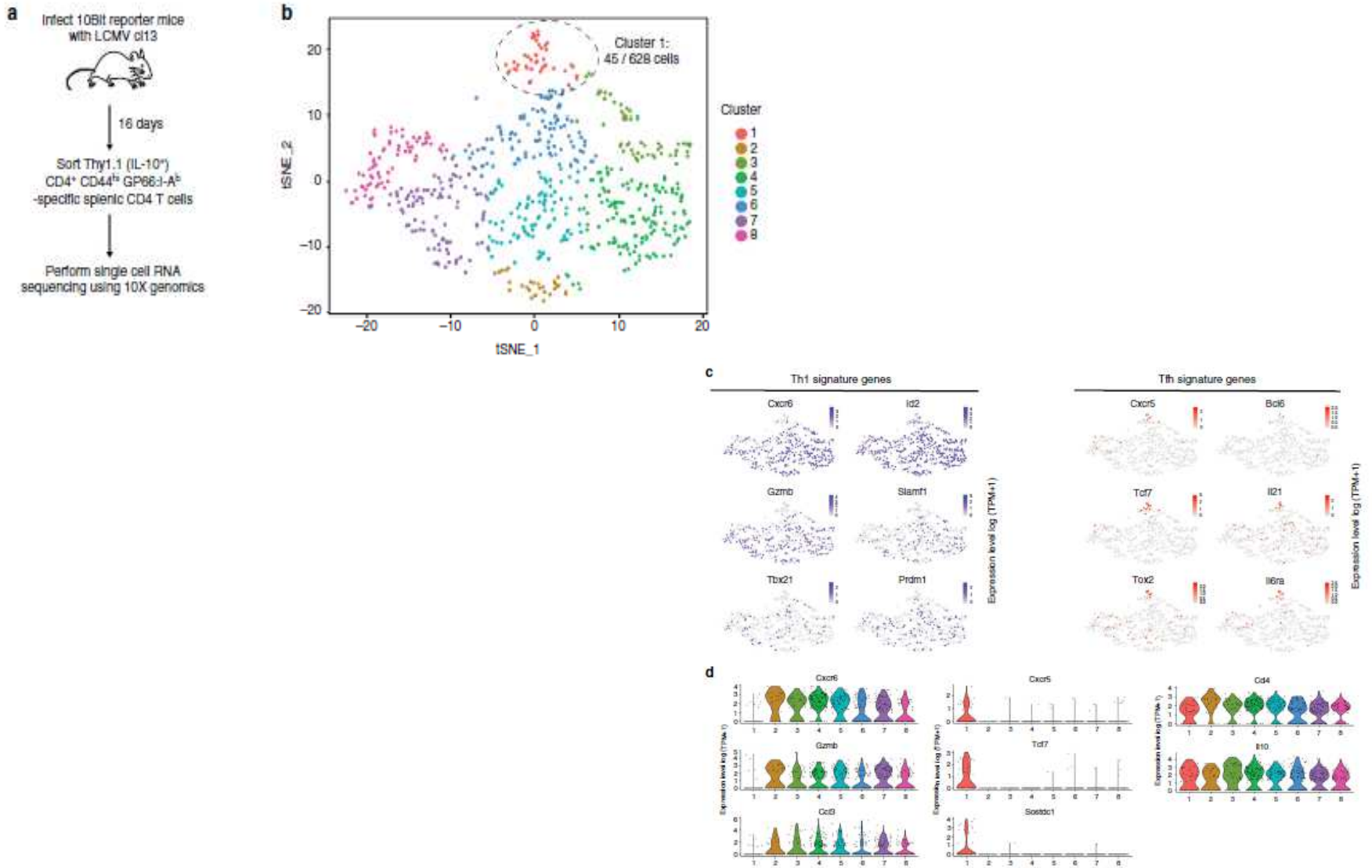
Command List --- 33,000 PBMCs



Command List --- 8,500 Pancreas Cells



Gang Xin.Single-cell RNA sequencing unveils an IL-10- producing helper subset that sustains humoral immunity during persistent infection. Nature communication 2018



Association of scRNA-Seq and mass cytometry-based analyses

- Discovery of subtile cell subsets based on their transcriptomic programm
- Confirmation at the protein level
- Can be performed
  - on circulating cells: scRNA-Seq + CyTOF
  - on target tissue: scRNA-Seq (eluted cells from a half fresh SG) + Hyperion (on the second half of the same SG)

La technologie Hyperion®, Une révolution en imagerie multiparamétrique

Patrice Hemon · Pierre Pochard · Nadège Marec