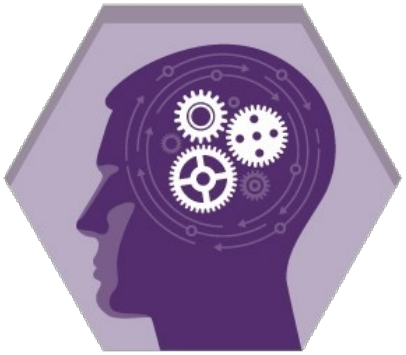
# Workshop in Systems Immunology




*Emanuele de Rinaldis*
*Magnus Fontes*
*Shai Shen-Orr*
*Angela Hadjipanayis*

*June 17th 2024*

# Today's Plan

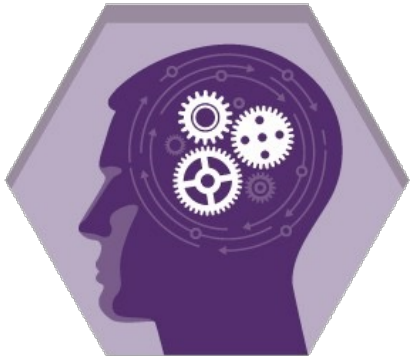
| | |
|---|---|
| 7:30-8:00 am | Continental Breakfast – Salons 10/11 Foyer |
| 8:00-8:10 am | Course Overview & Objectives – Emanuele de Rinaldis, PhD, Sanofi |
| 8:10-9:00 am | Introduction to Systems Immunology – Emanuele de Rinaldis, PhD, Sanofi |
| 9:00-9:45 am | Systems Immunology & Immune Oncology: A Data-Centric View – Magnus Fontes, PhD, Roche |
| 9:45-10:00 am | Coffee Break – Salons 10/11 Foyer |
| 10:00-11:30 am | From Systems Immunology to Novel Therapeutic Insights – Emanuele de Rinaldis, PhD, Sanofi |
| 11:30-12:00 9m | Q&A / Panel Discussion |
| 12:00 pm-1:00 pm | Lunch – Salons 10/11 Foyer |
| 1:00-2:00 pm | Biology is Spatial: A Primer on Spatial Biology - Applications in Onc. and Immun. – Angela Hadjipanayis, MSc, PhD, Sanofi |
| 2:00-2:15 pm | Break |
| 2:15-3:15 pm | Artificial Intelligence: A Primer for Immunologists – Shai, Shen-Orr, Technion – Israel Institute of Technology |
| 3:15-3:30 pm | Coffee Break – Salons 10/11 Foyer |
| 3:30-4:30 pm | Interactive Data Analysis Session – Magnus Fontes, PhD, Roche |
| 4:30-5:00 pm | Wrap Up Notes & Final Remarks – Magnus Fontes, PhD, Roche |

# Introduction to Systems Immunology

## *Technologies, Methods, Applications*

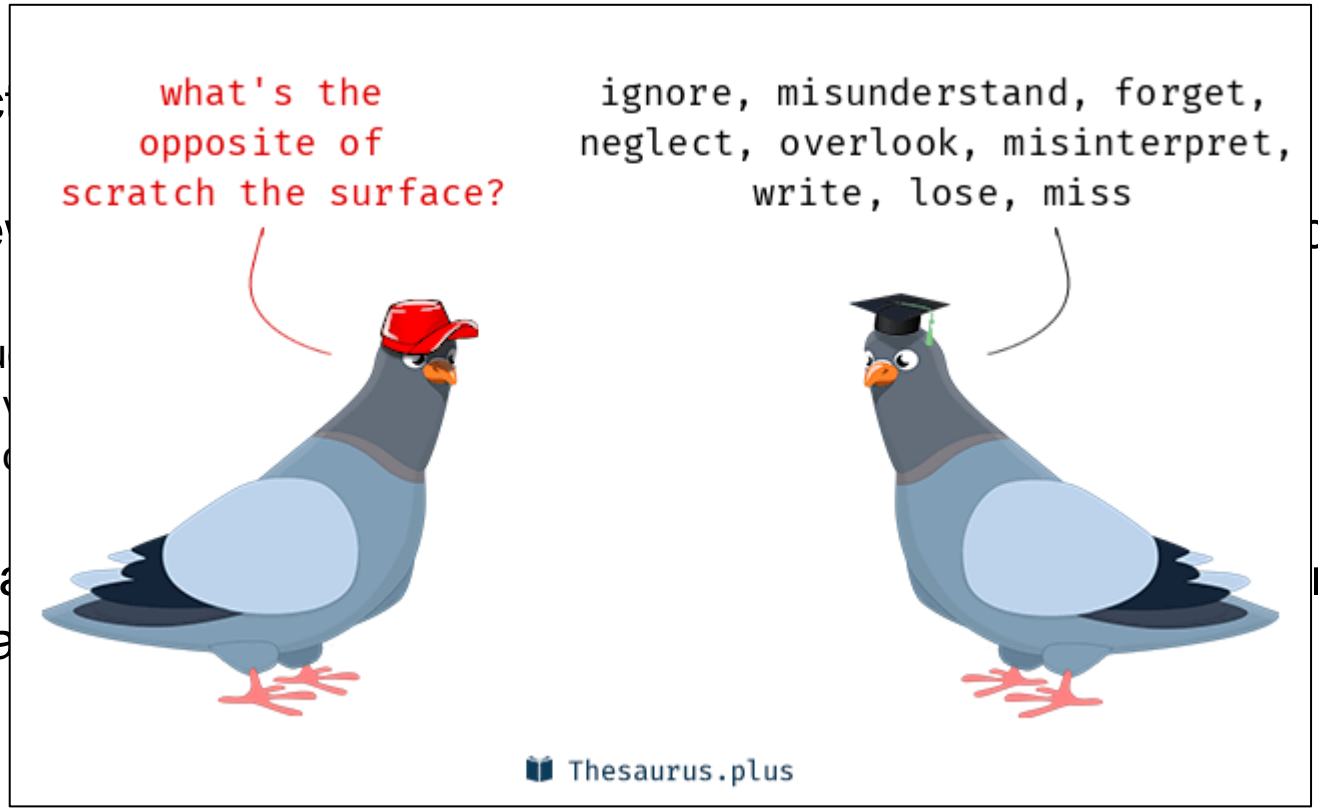
***Emanuele de Rinaldis***

***FOCIS - June 17th, 2024***

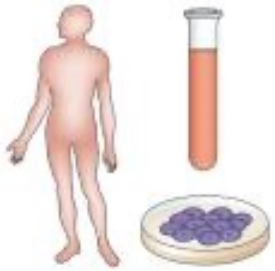


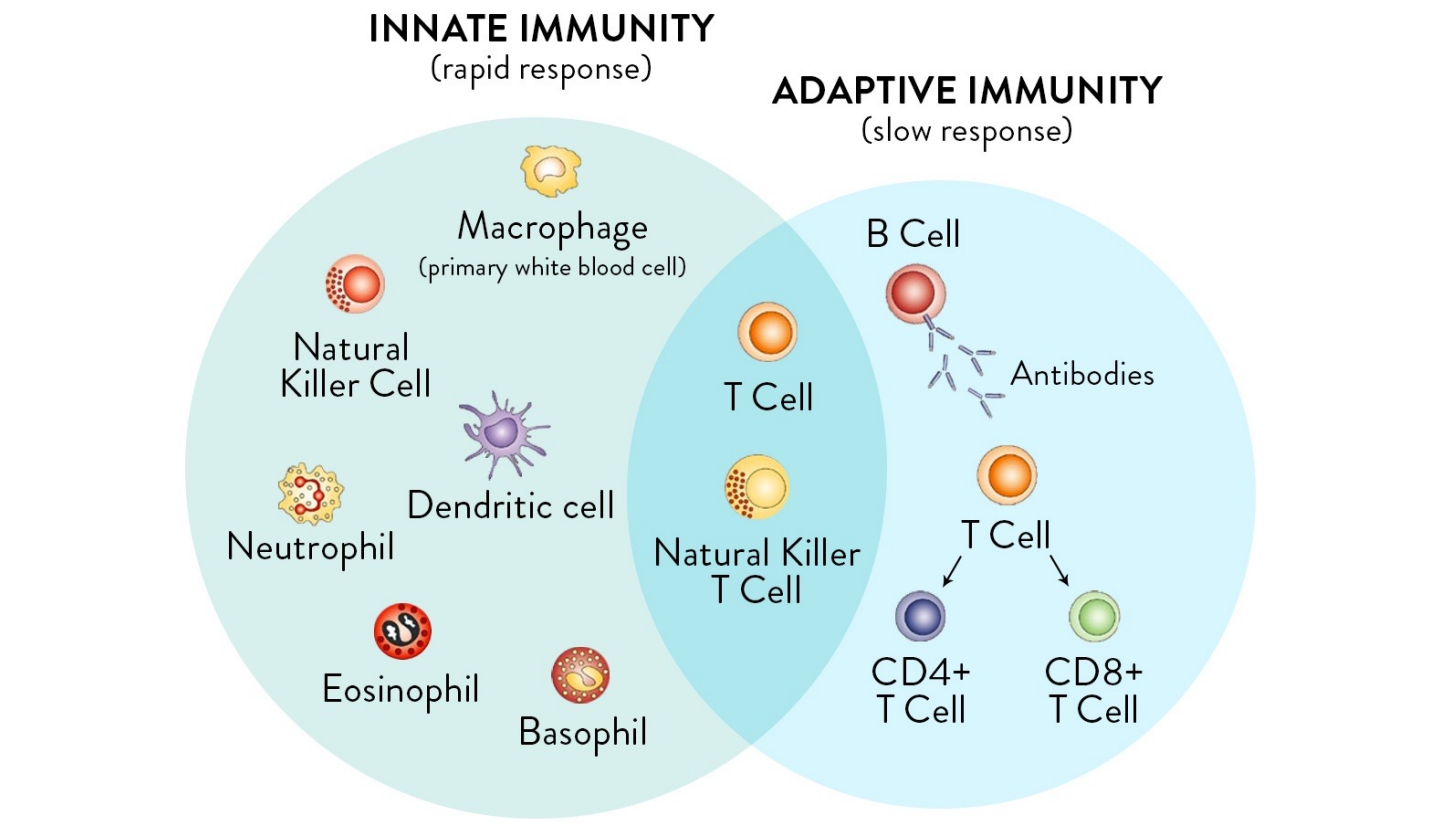
# Outline

- ❑ Introduc
- ❑ Brief vie ons
  - ❑ Sequ
  - ❑ Bulk
  - ❑ Appli
- ❑ Data ana nd visualiza

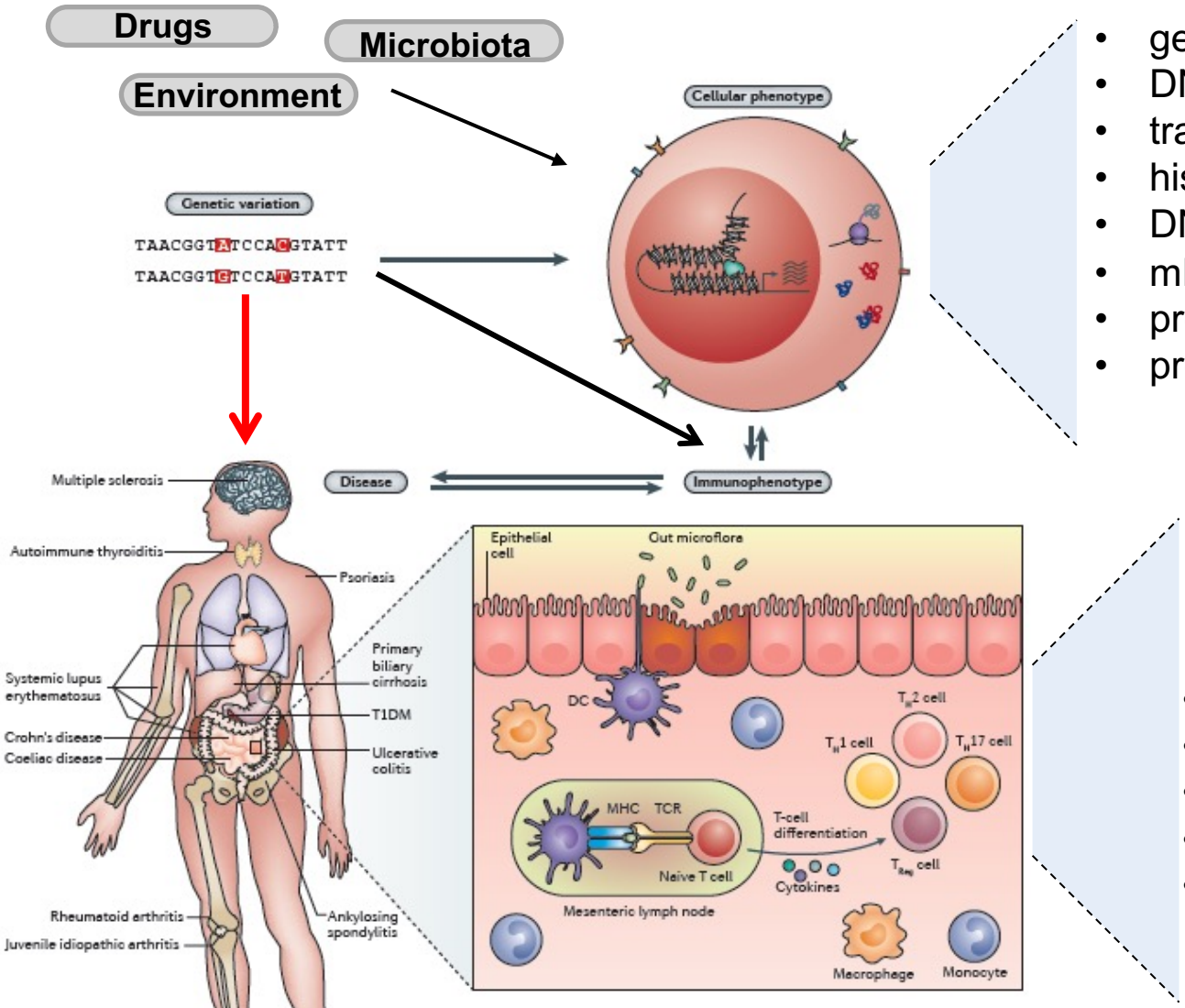


# The Immune System





# Systems Immunology



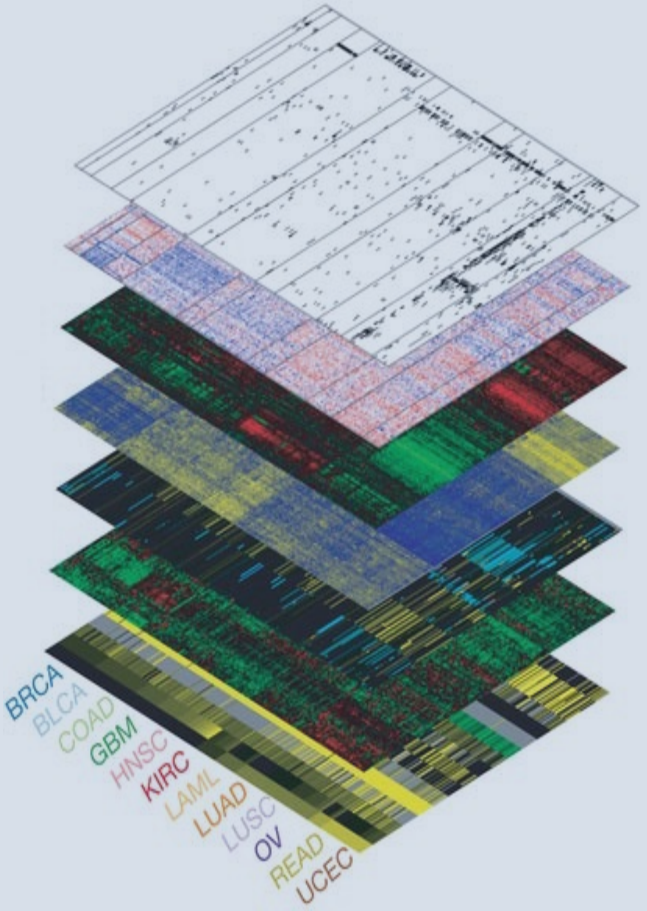

- gene transcription
- DNA–DNA interactions
- transcription factor binding
- histone modifications
- DNA methylation
- mRNA stability and translation
- protein levels
- protein–protein interactions

- signalling response
- cell-type abundances
- cell-cell spatial interactions
- RNA/protein levels
- cytokine production

*Adapted from Nature Reviews Genetics* 17, 160–174 (2016)

# Technologies


# Data

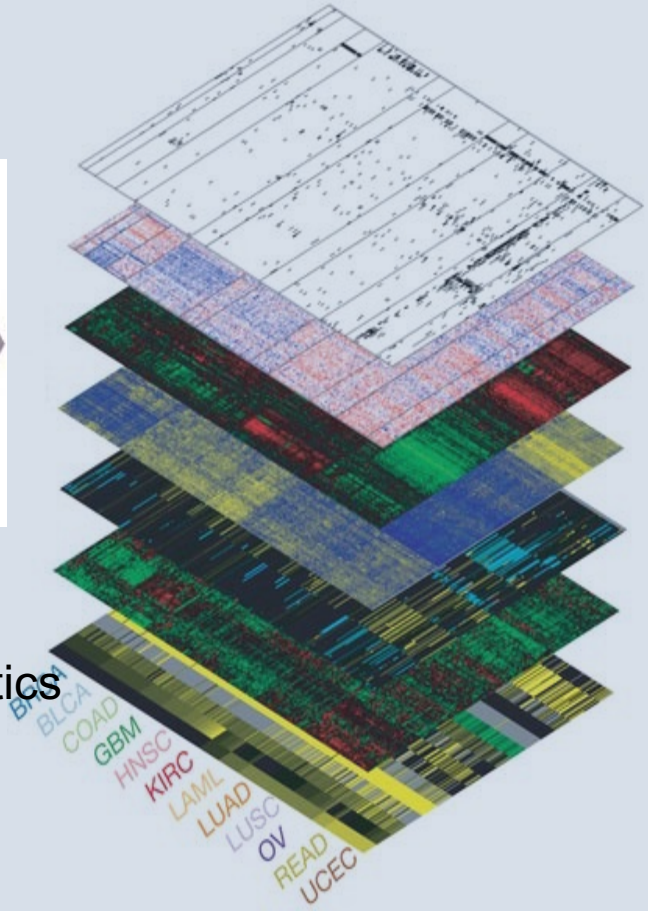


# Technologies


# Analysis

Math
Stats,
Bioinformatics
ML/AI

# Data



# Immune Cell Profiling: Flow Cytometry and Gene Expression

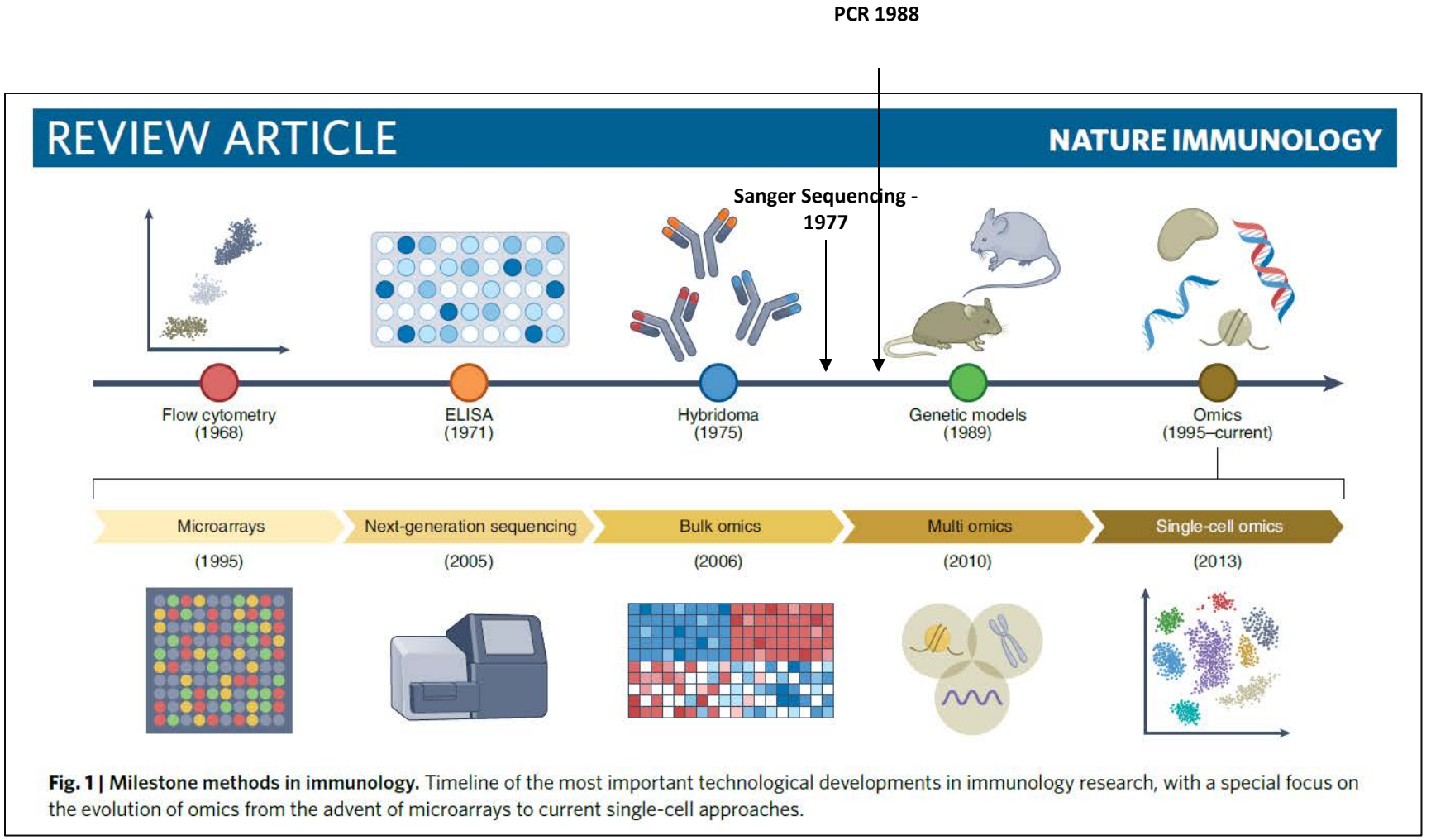
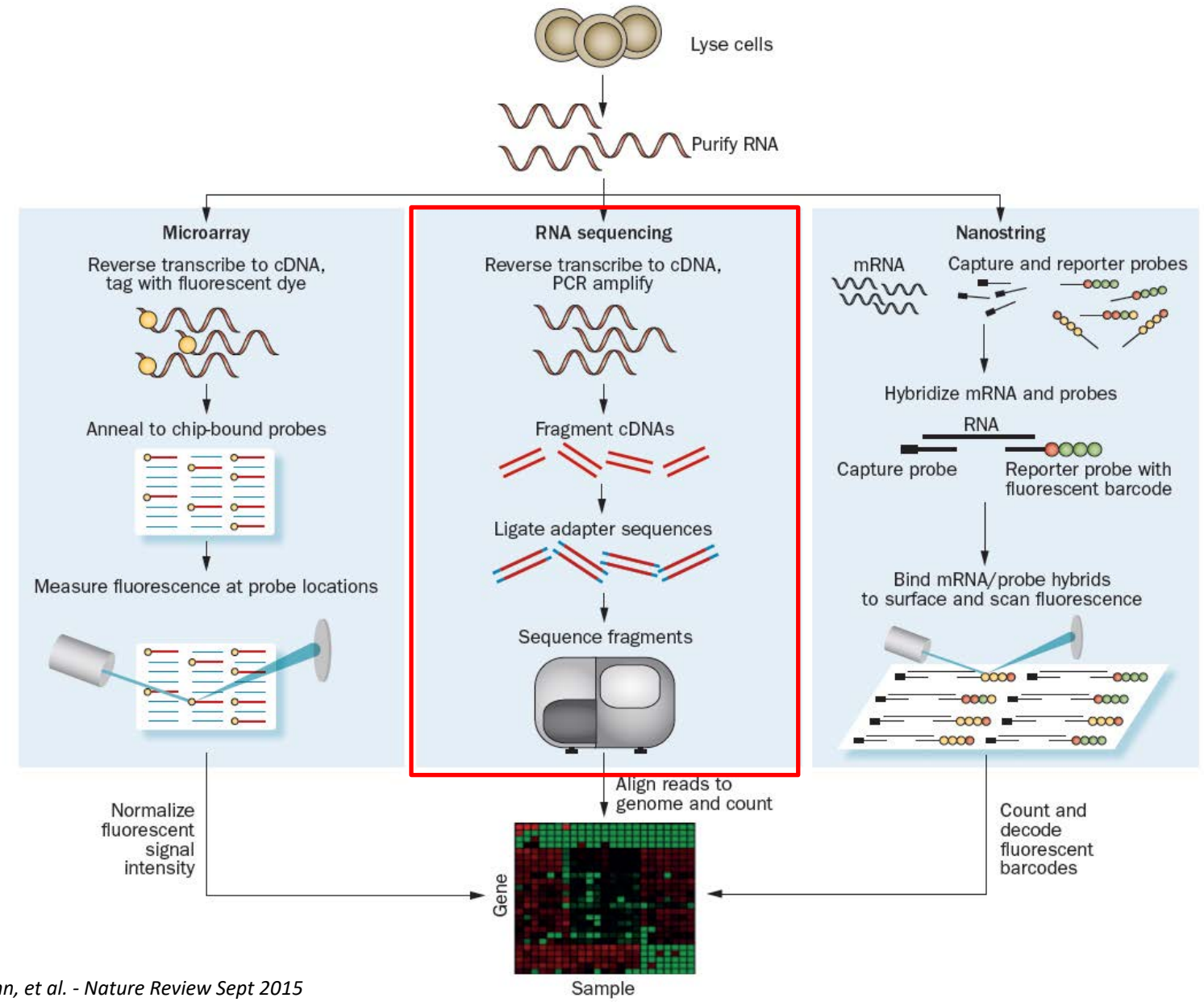



**Fig. 1 | Milestone methods in immunology.** Timeline of the most important technological developments in immunology research, with a special focus on the evolution of omics from the advent of microarrays to current single-cell approaches.

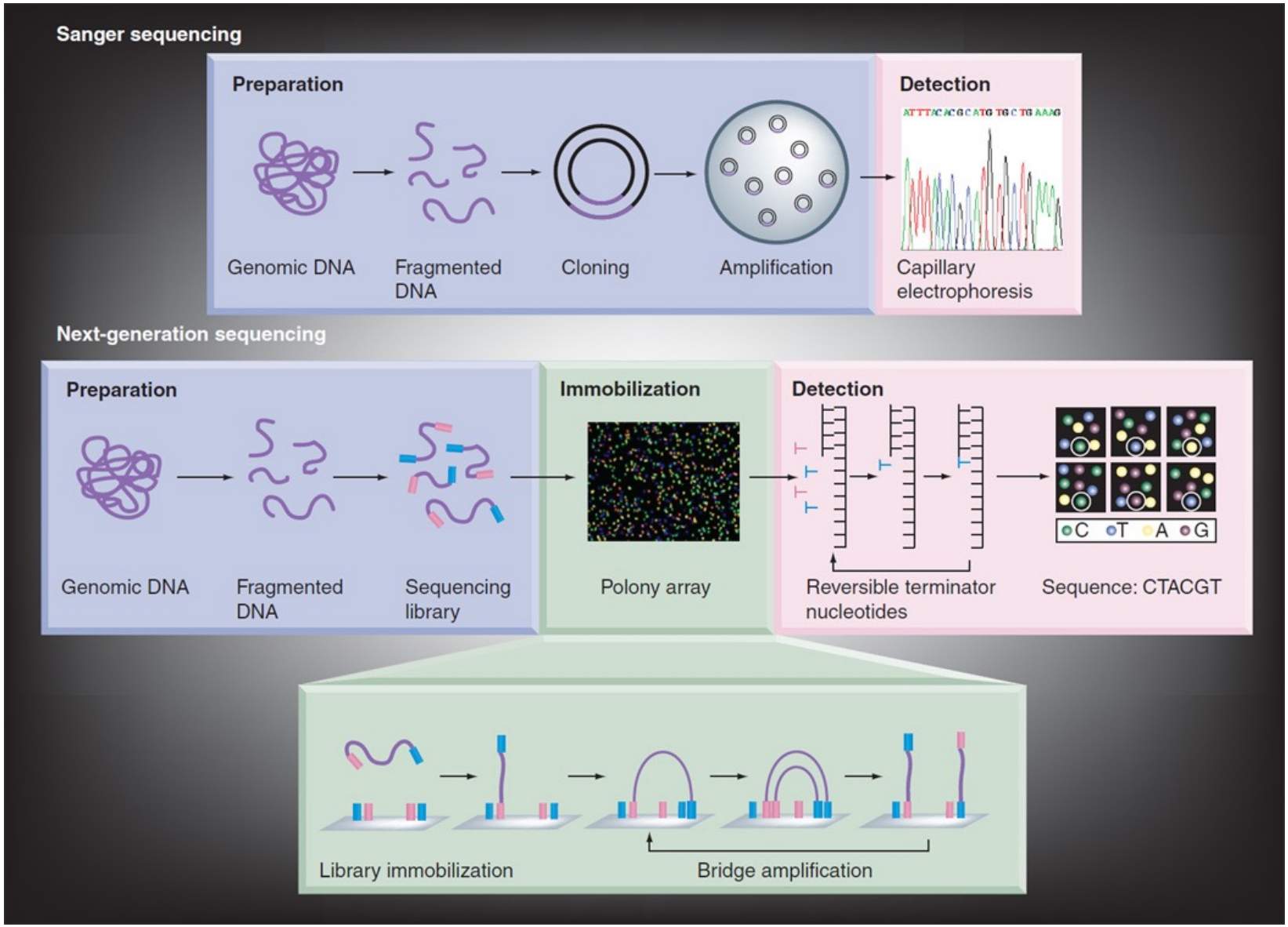
*Nature Immunology volume 23, pages1412–1423 (2022)*

# Gene Expression in “Bulk” Samples




*Joerg Ermann, et al. - Nature Review Sept 2015*

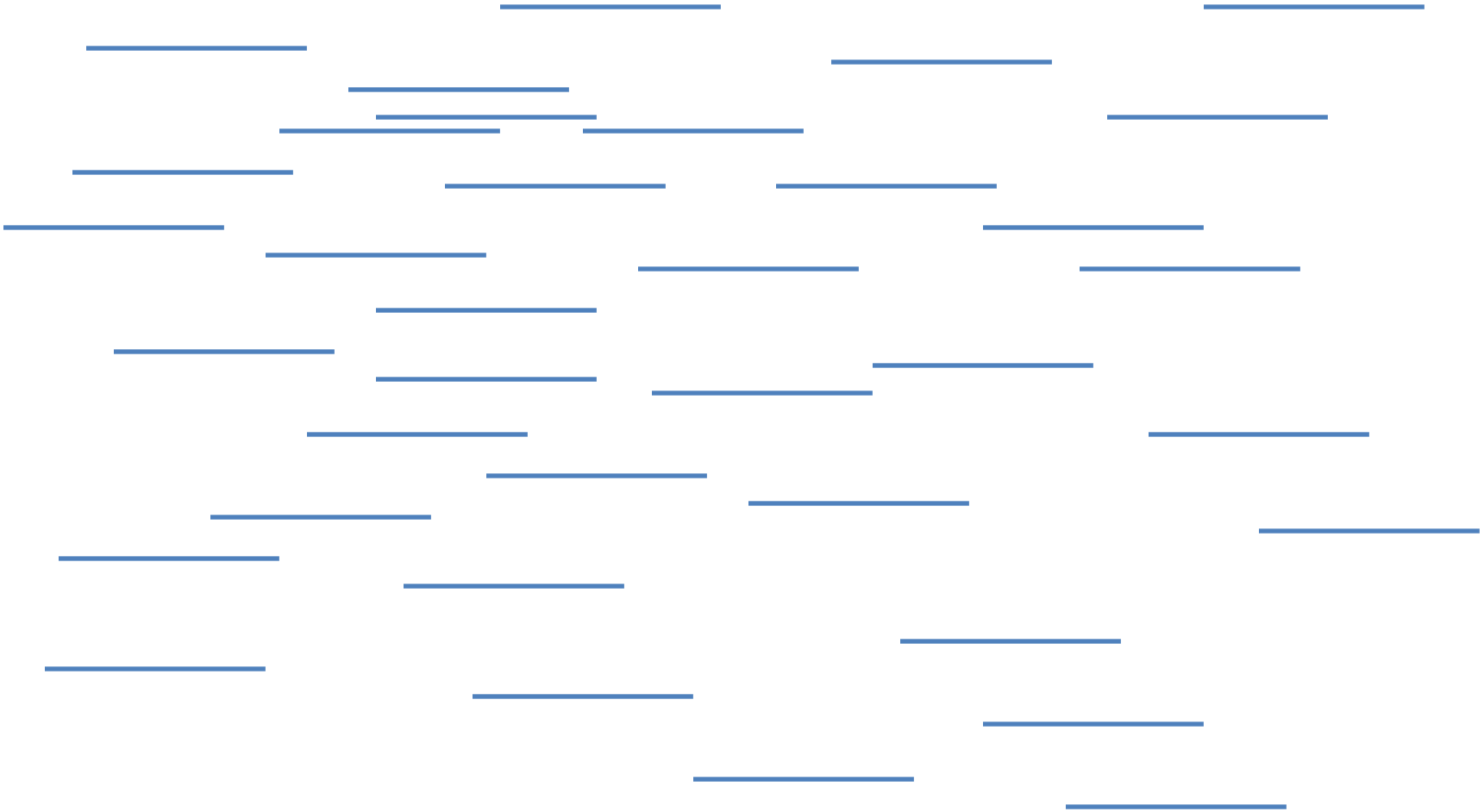
# Next Generation Sequencing – The Illumina Platform




*Per Med. 2011 May 1;8(3):331-345.*

# Lots of sequenced reads…

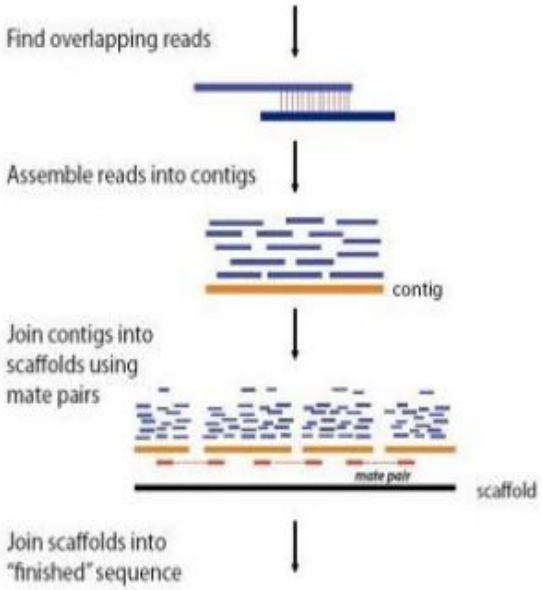
Illumina NovaSeq 6000: up to 20 billion reads, 3.000Gb data, less than 2 days



# What do we do with them ?

Reads

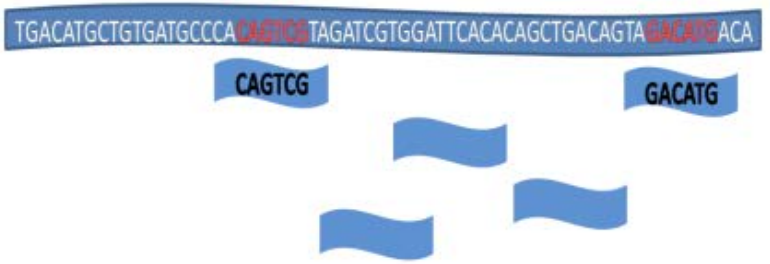
*denovo* Assembly




**Sequencing of a new organism**
**Meta-Genomics**
**Reconstructing cancer genomes**

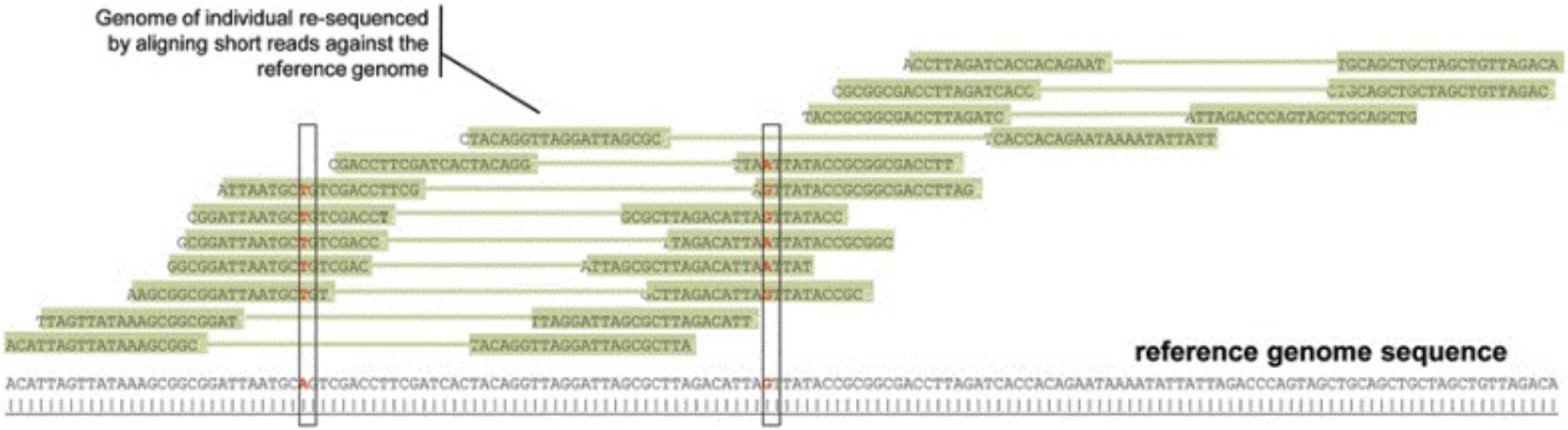
Reference based analysis

Analysis based on a known reference genome

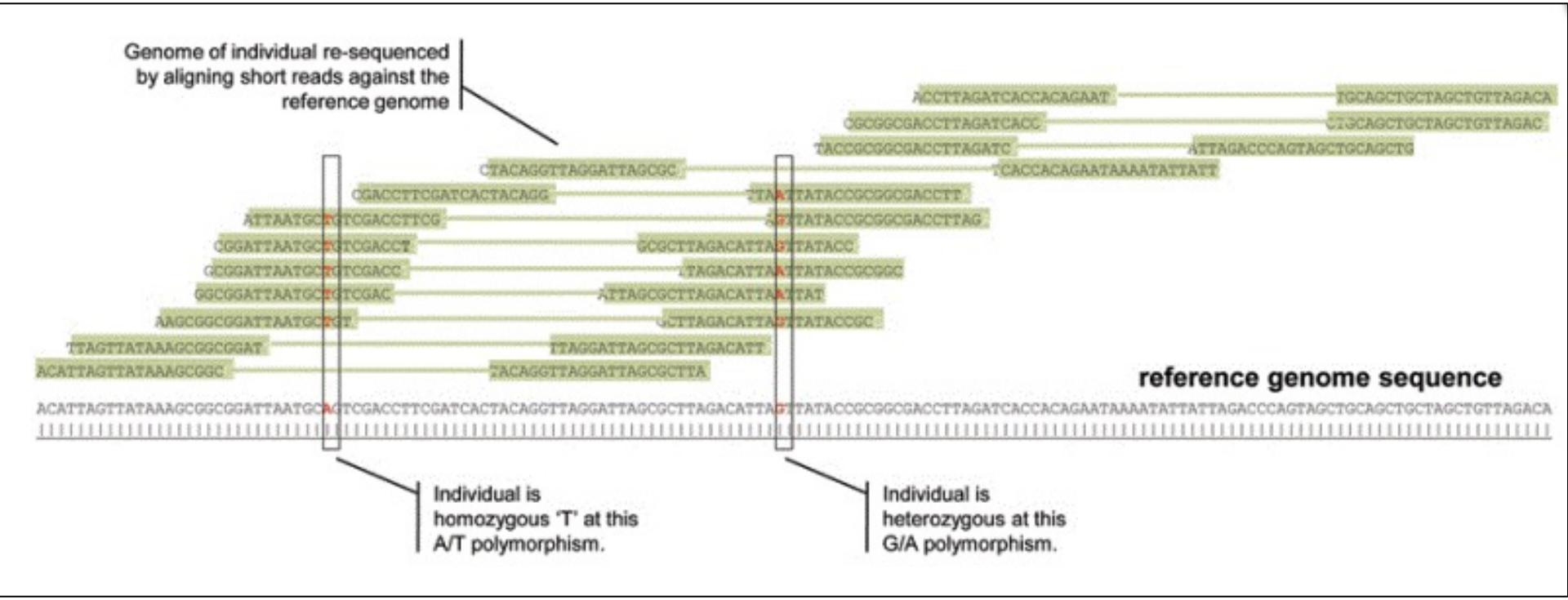



**Organism specific experiments**
**Annotating functional elements**

# Reads Alignment

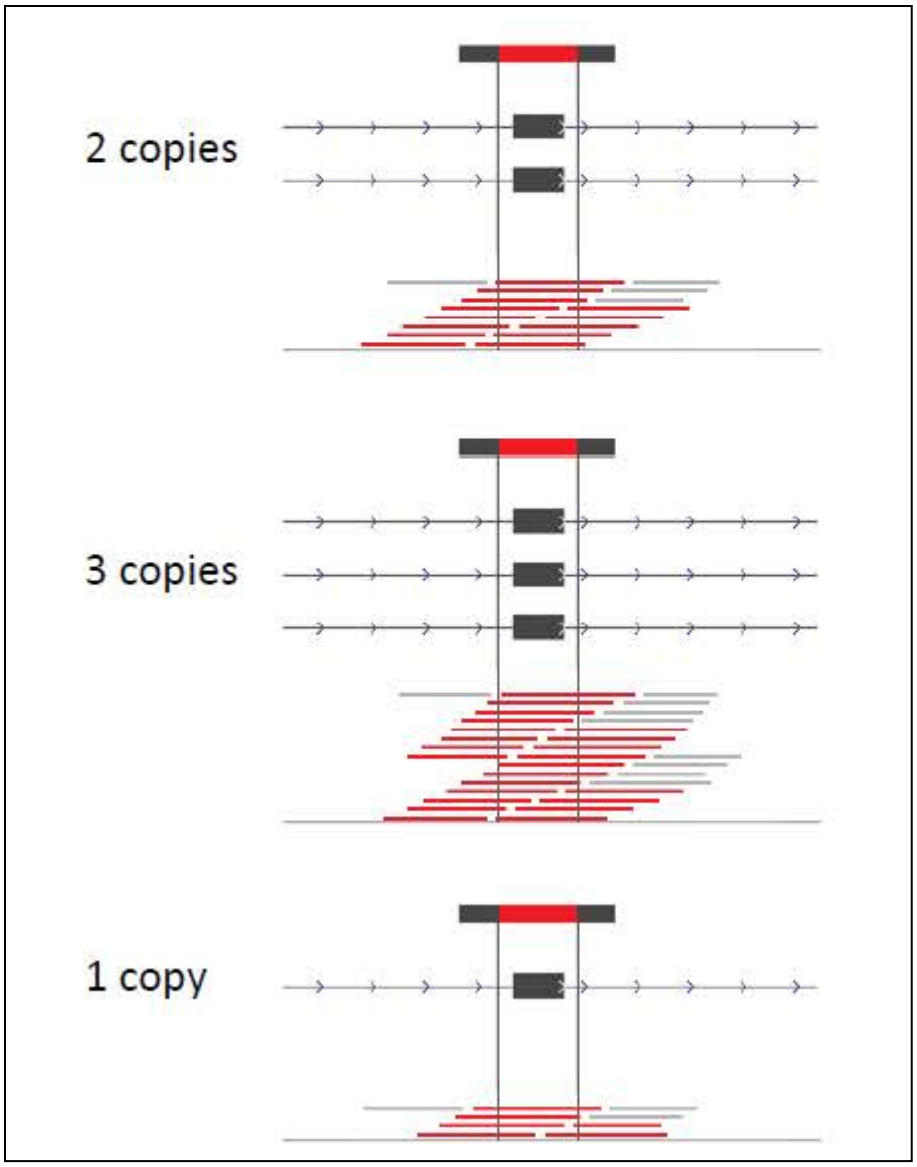


# Genome Sequencing: Variant Calling



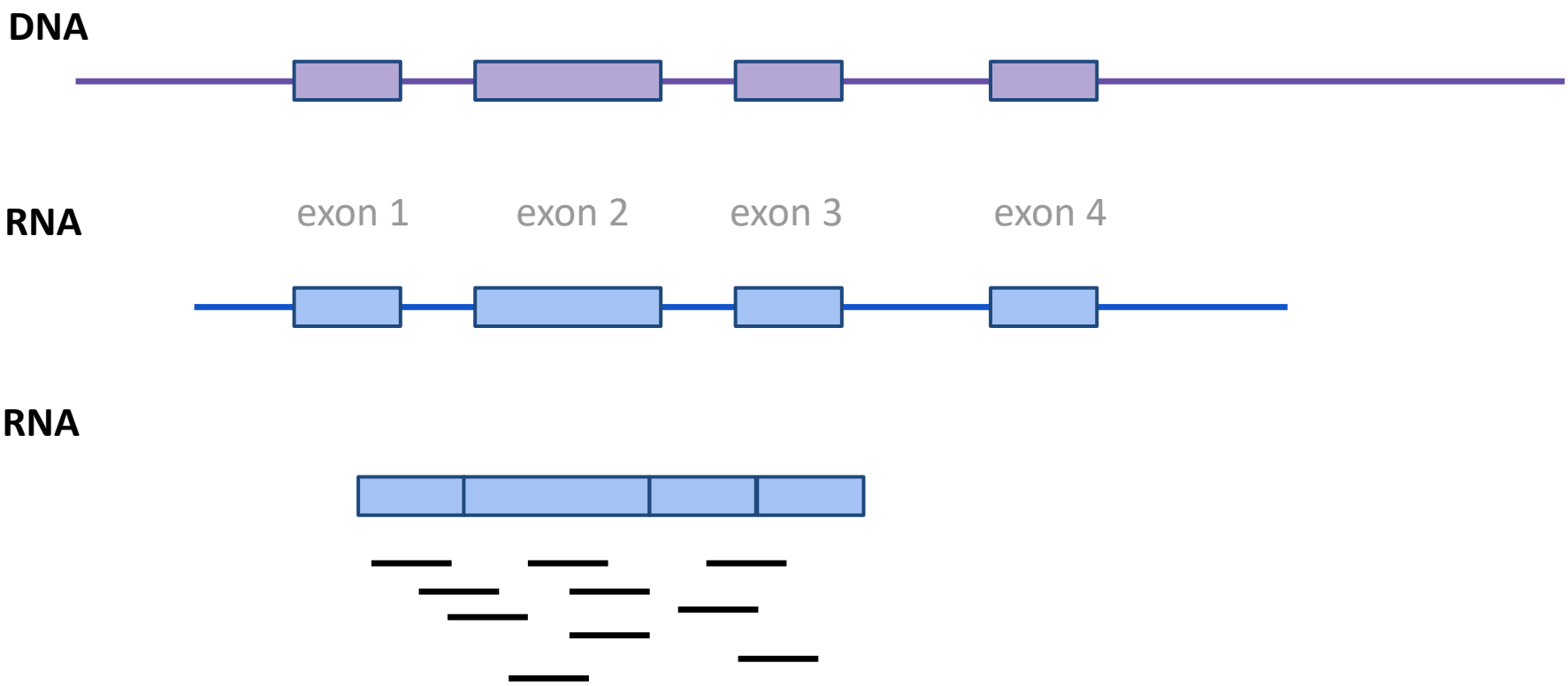

Differences between aligned reads and reference genome can be identified ➔ variants

# Genome Sequencing: Copy Numbers



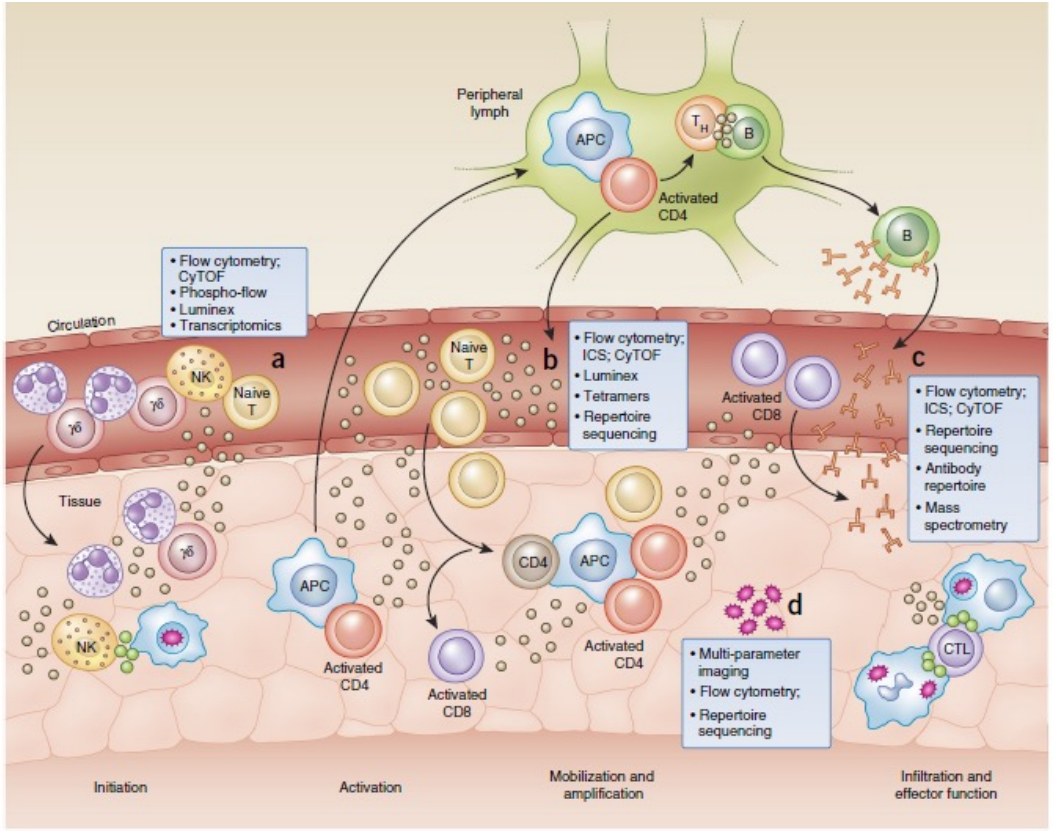

- The number of aligned reads on a given regions is proportional to the number of starting DNA copies of that region
- This information can be used to infer DNA copy number variations (CNVs)

# RNA Sequencing

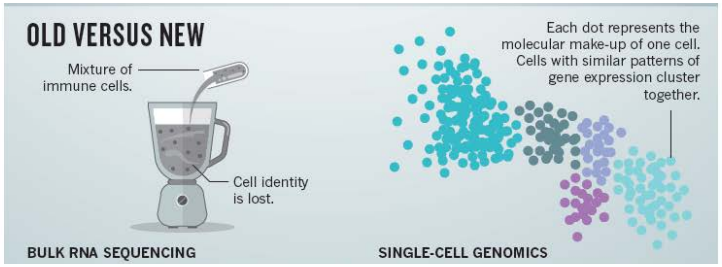



- The number of aligned reads on a given RNA is proportional to the number of its starting molecules
- This information can be used to infer RNA abundances

# Immune Systems Heterogeneity



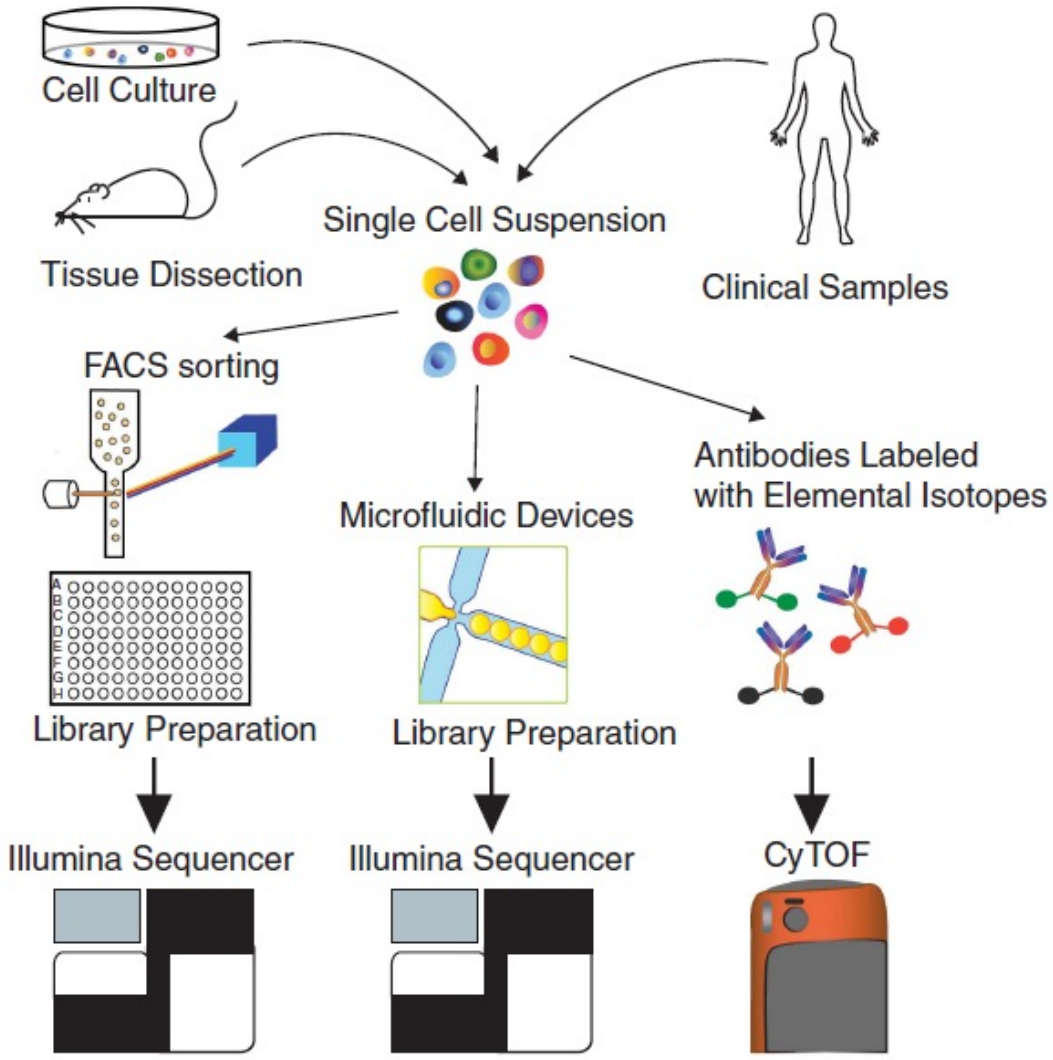

*Davis et al. - Nature Immunology* **18**, 725–732 (2017)




6 JULY 2017 | VOL 547 | NATURE | 27

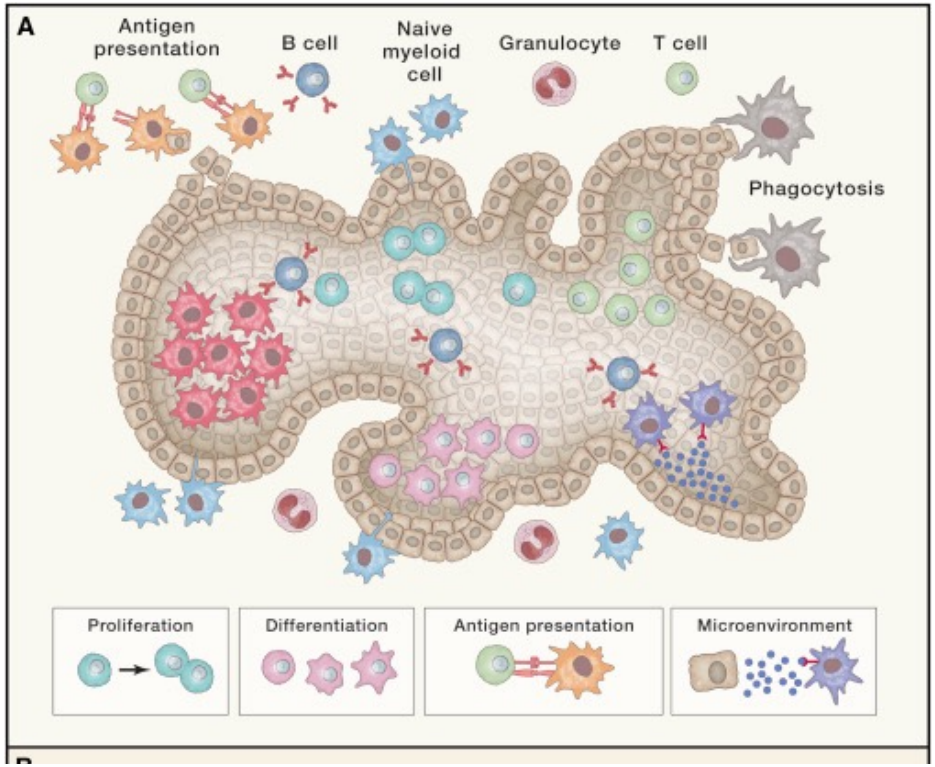
- Average signals are measured and fails to capture sample heterogeneity
- Individual cell properties and interplay between different cell subtypes can be captured

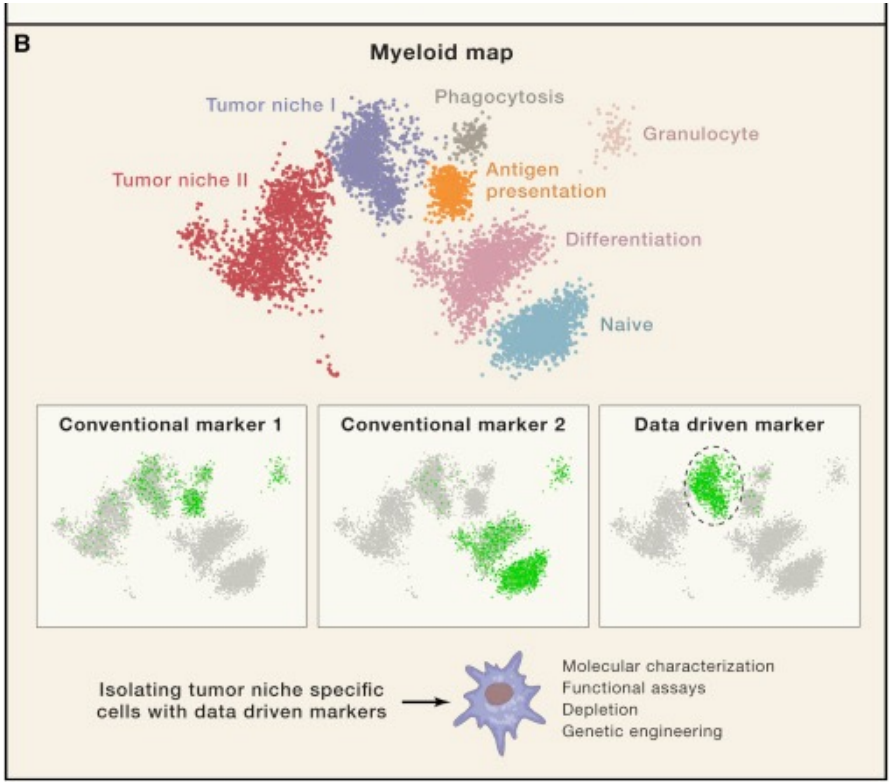
# Single-Cell Analysis




*Imm. and Cell Biol. (2016) 94, 225–229;*

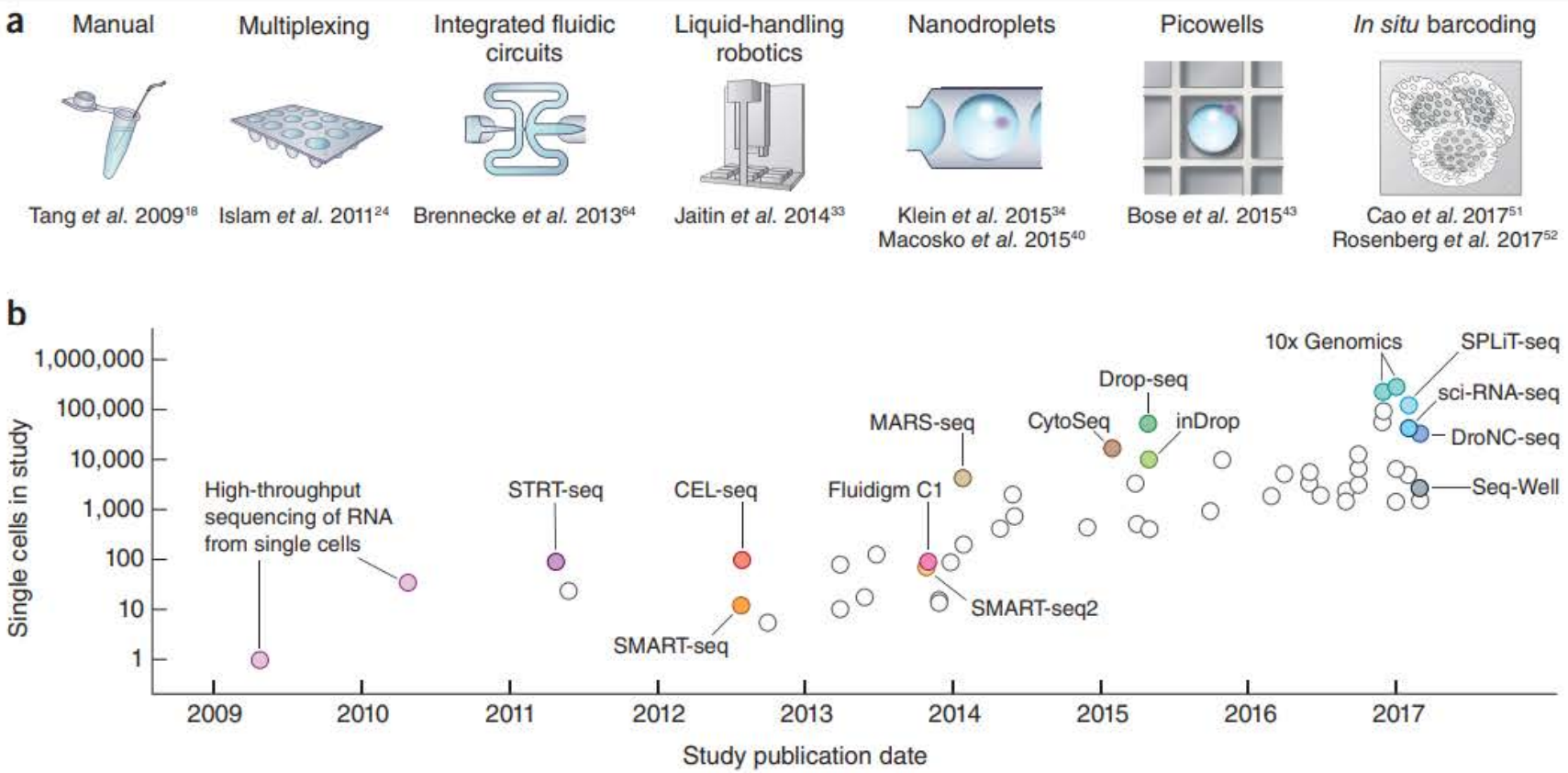
# Dissecting tissue heterogeneity at single-cell level




*Giladi A, Amit I. Cell. 2018 Jan*

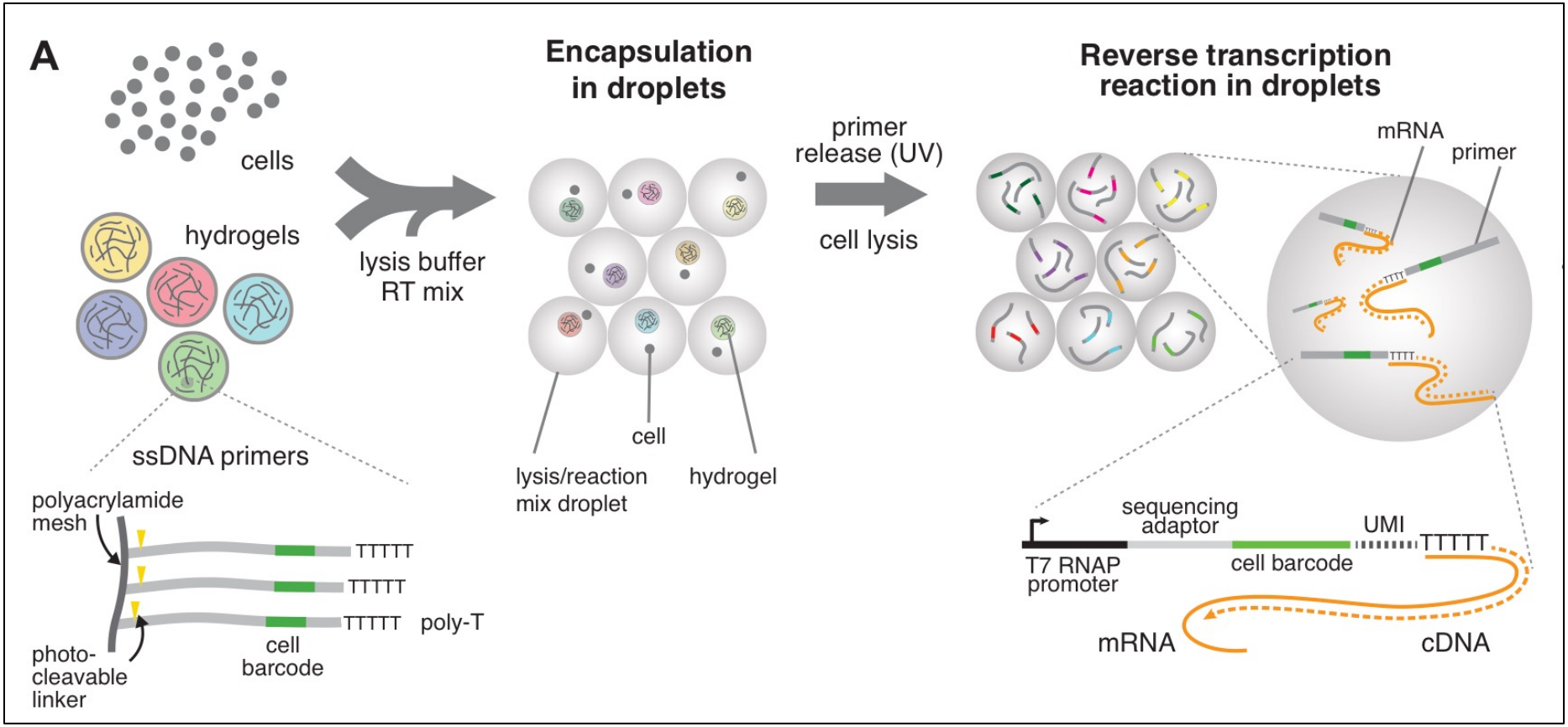
# Development of Single-Cell Technologies




Svensson V, Vento-Tormo R, Teichmann SA.Nat Protoc. 2018

# InDrop: Barcoding process

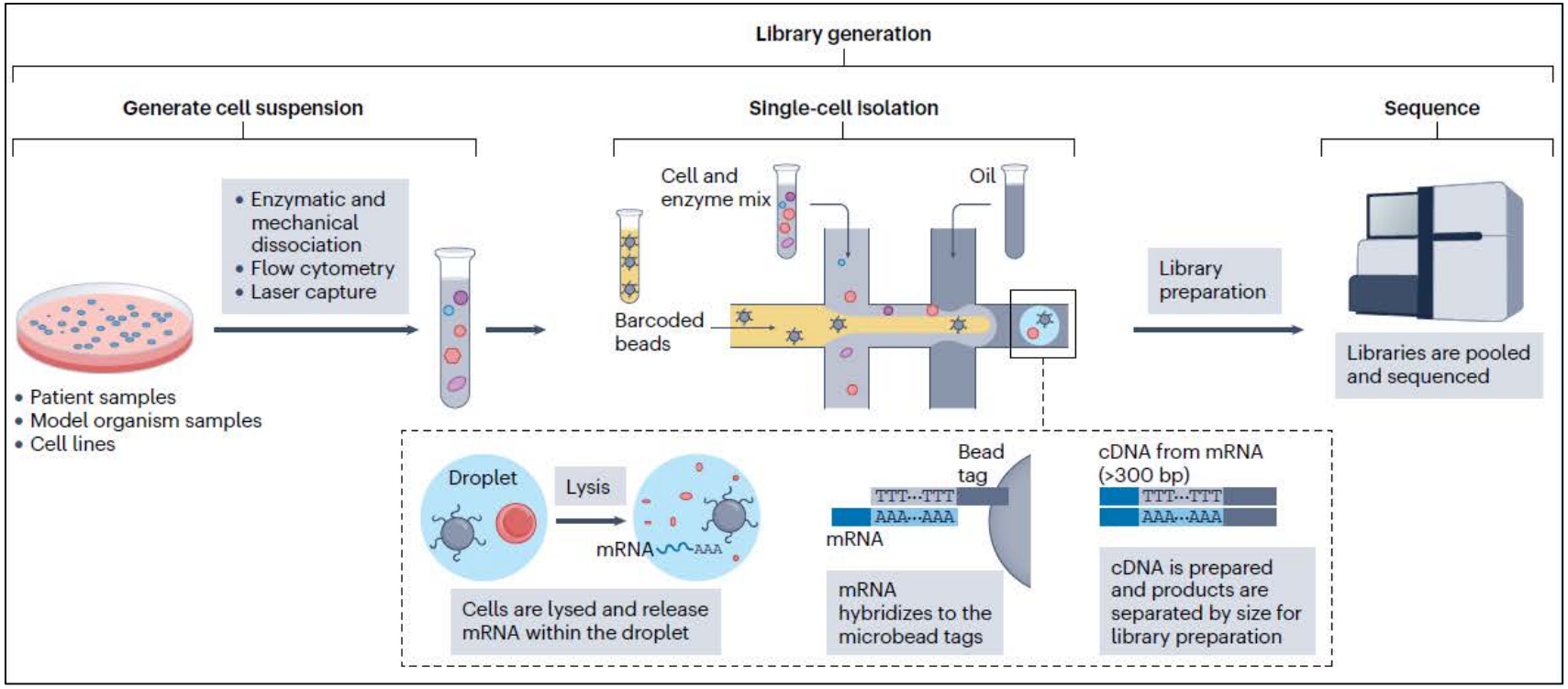
Co-encapsulation of cells, barcodes (delivered by hydrogel bead), and RT–lysis reagents into microfluidic droplets.




Zilionis et al., Nature Protocols 2017

# Basic workflow in 10x Chromium

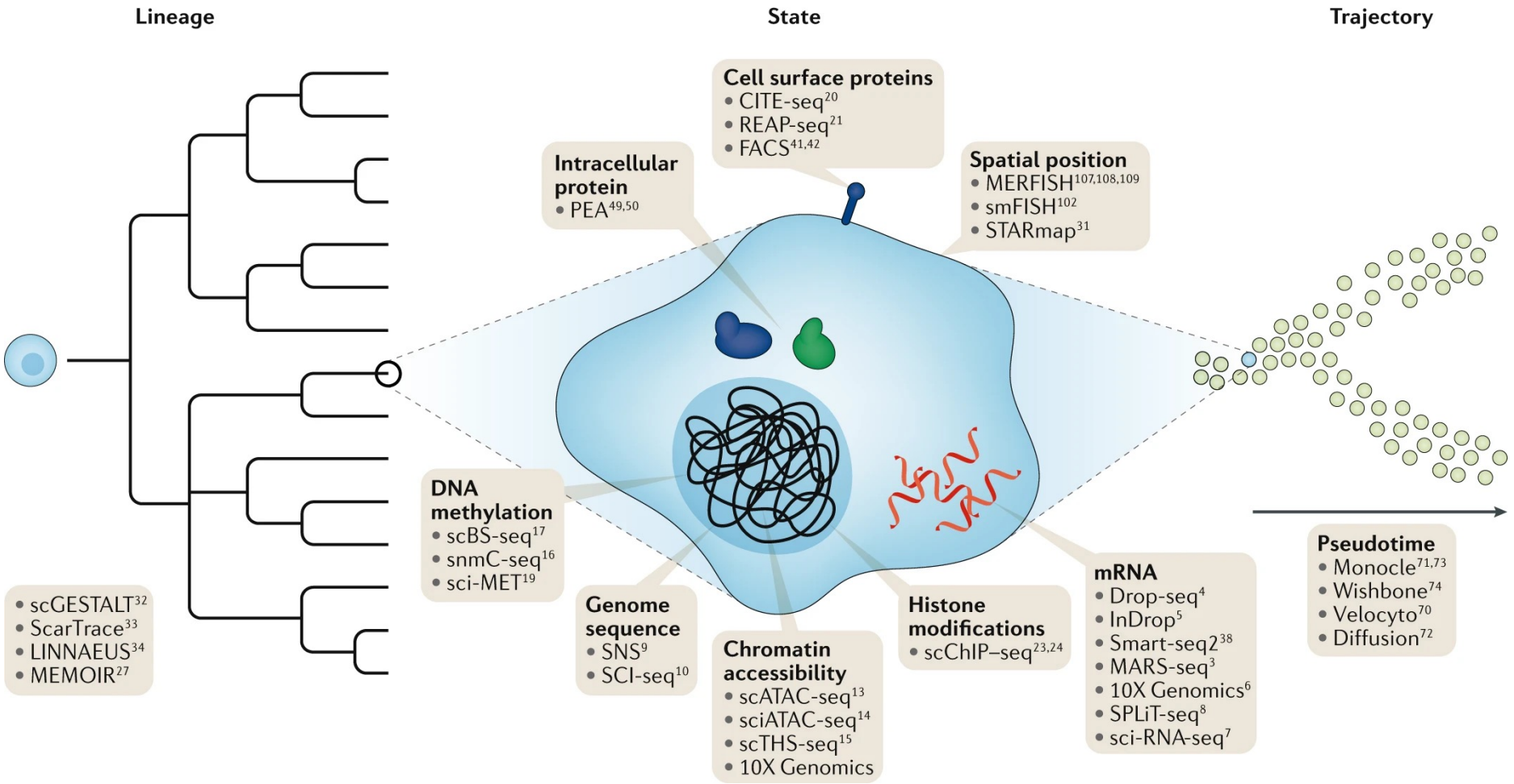
Individual cells must be trapped within a space that is not continuous with spaces containing any other individual cells.




*Nature Reviews Drug Discovery volume 22, pages496–520 (2023)*

- **10X**: combines an aqueous flow of cells, barcoded primers carried in beads, lysis buffer and reverse transcription enzymes with oil to create microdroplet reaction chambers.
- **Plate-based** technologies perform this step in microwells
- Automated **microfluidic devices** use other forms of microchamber.

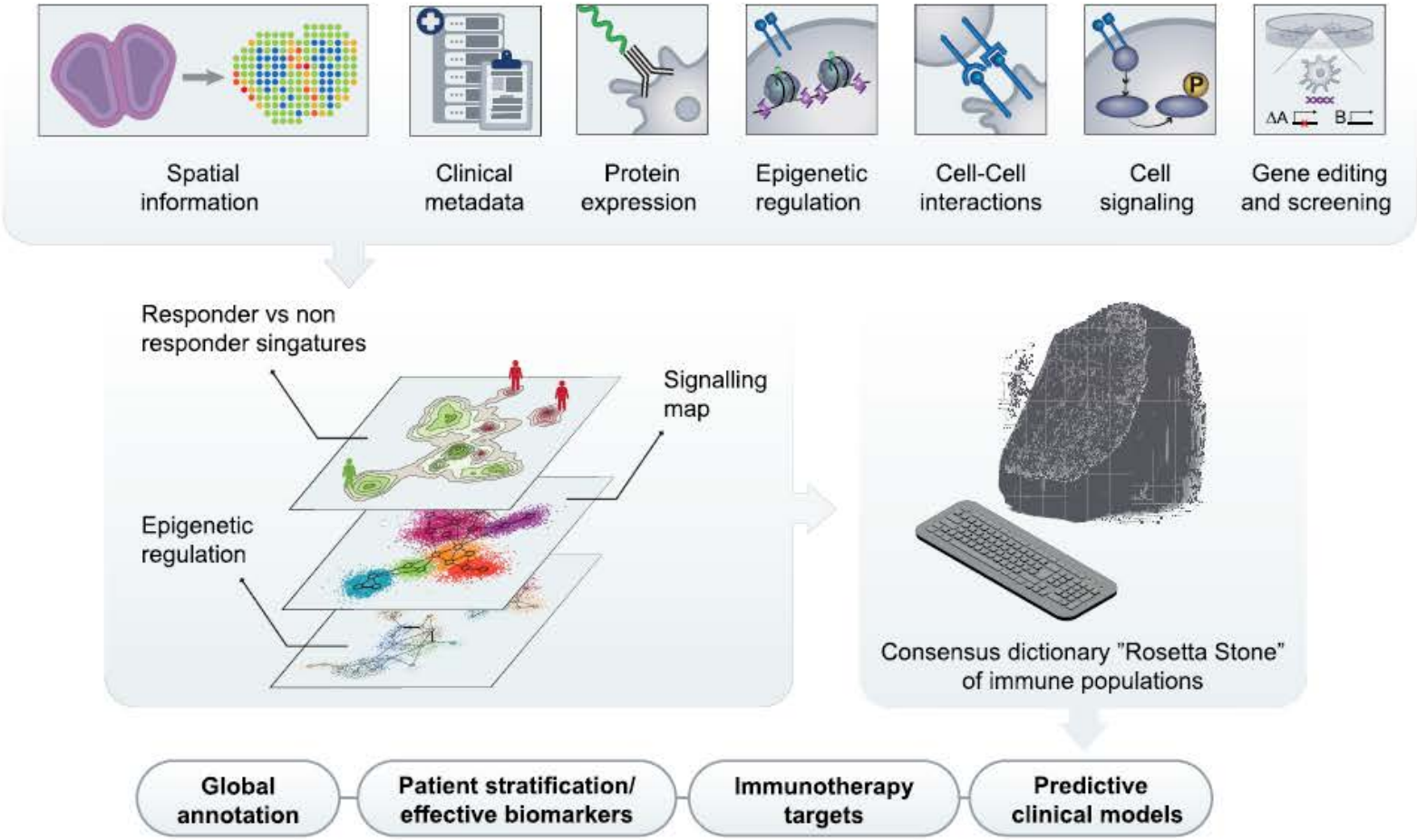
# Single-cell multi-omics




**Stuart T**, Satija R.Nat Rev Genet. 2019

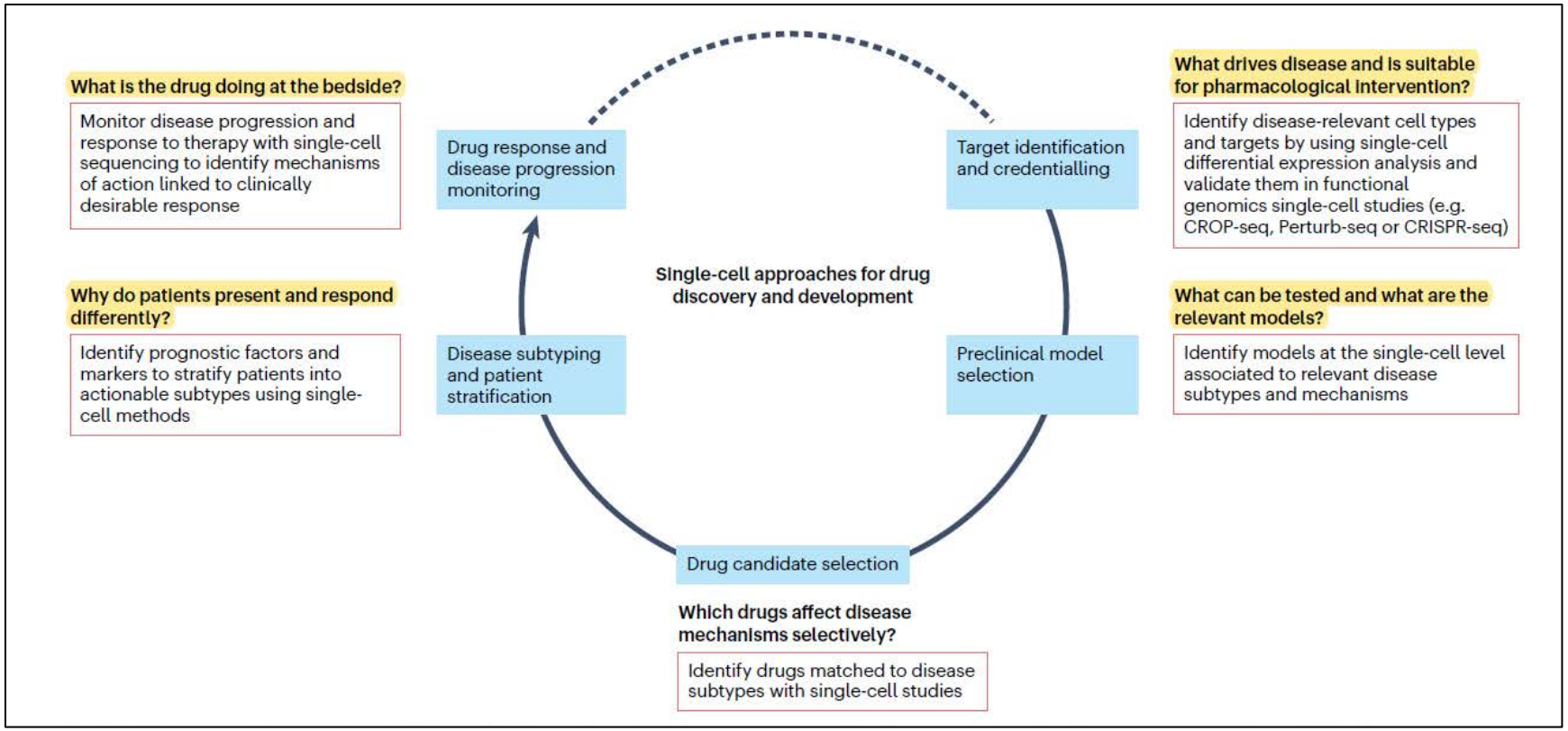
# Systems Biology Through Single-Cell Multi-omics

"Rosetta Stone": **deciphering the different immune populations and states across datasets**




Ginhoux F, Yalin A, Dutertre CA, Amit I. Immunity. 2022

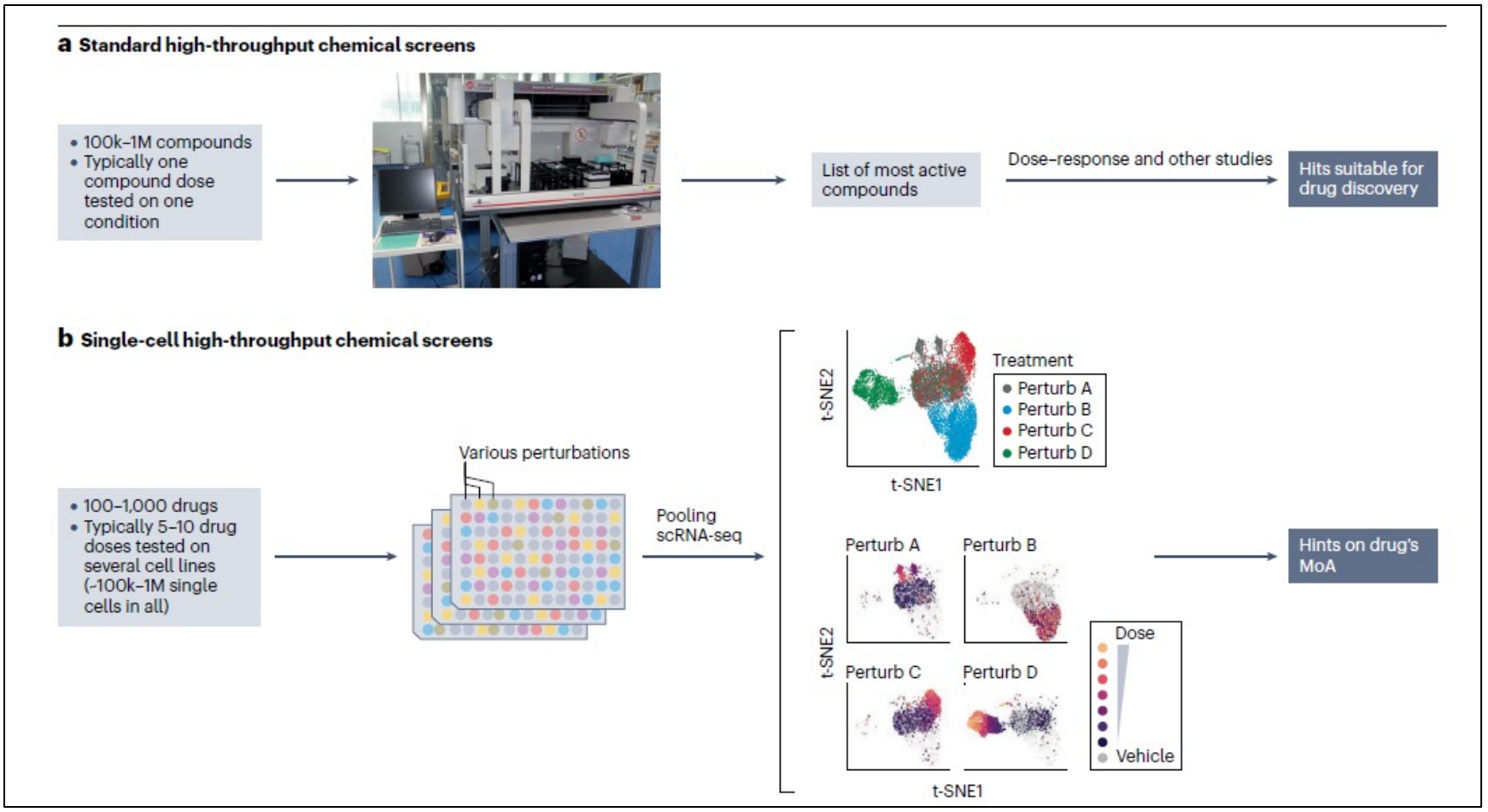
# Applications To Drug Discovery




*Nature Reviews Drug Discovery volume 22, pages496–520 (2023)*

# Applications To Drug Discovery

## Compound screening




*Nature Reviews Drug Discovery volume 22, pages496–520 (2023)*

# Single-Cell for Biomarker Discovery and Patient Stratification

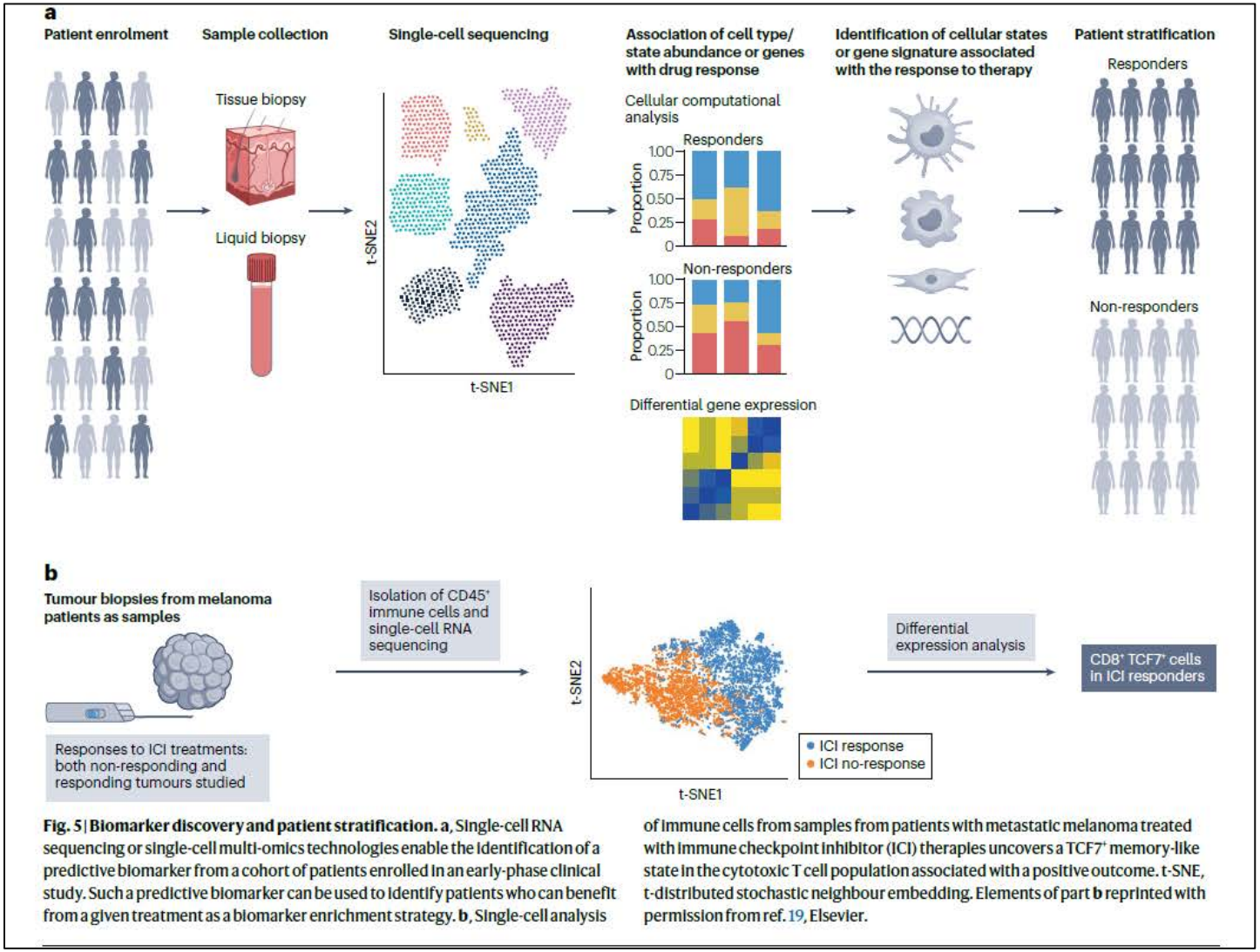
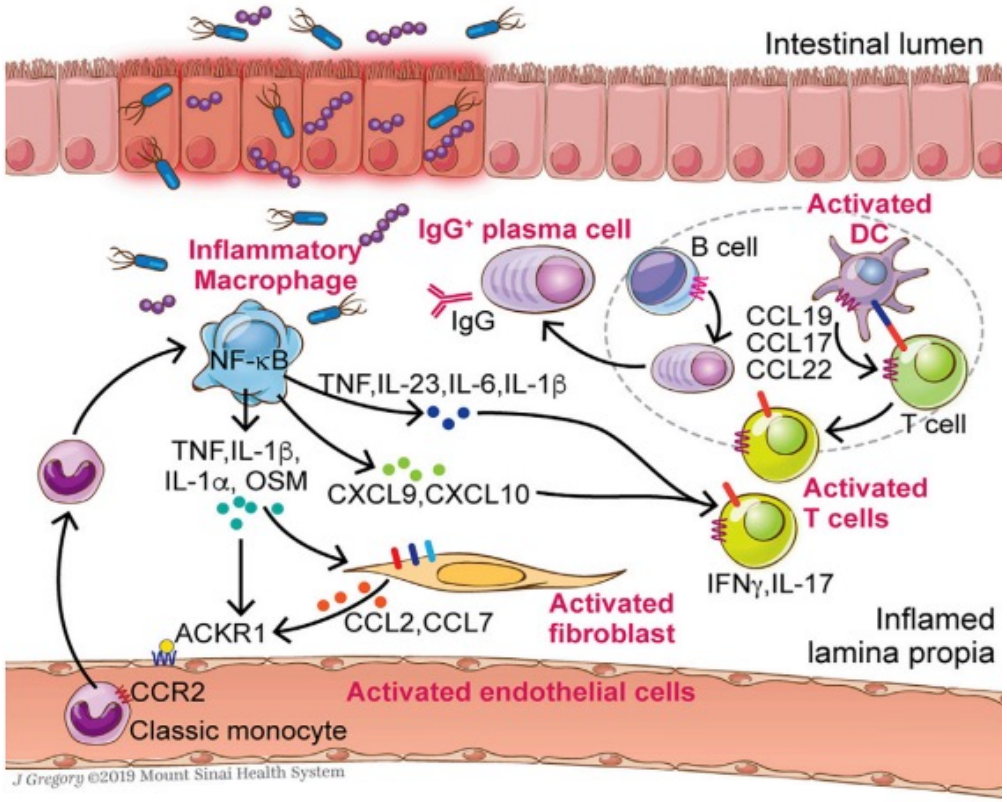



**Fig. 5 | Biomarker discovery and patient stratification. a**, Single-cell RNA sequencing or single-cell multi-omics technologies enable the identification of a predictive biomarker from a cohort of patients enrolled in an early-phase clinical study. Such a predictive biomarker can be used to identify patients who can benefit from a given treatment as a biomarker enrichment strategy. **b**, Single-cell analysis of immune cells from samples from patients with metastatic melanoma treated with immune checkpoint inhibitor (ICI) therapies uncovers a TCF7$^+$ memory-like state in the cytotoxic T cell population associated with a positive outcome. t-SNE, t-distributed stochastic neighbour embedding. Elements of part **b** reprinted with permission from ref. 19, Elsevier.

*Nature Reviews Drug Discovery volume 22, pages496–520 (2023)*

# Single-Cell-Based Biomarker of response in anti-TNF therapy (CD)

GIMATS$^{high}$ Module in CD inflamed ileums associates with resistance to anti-TNF responder CD patients

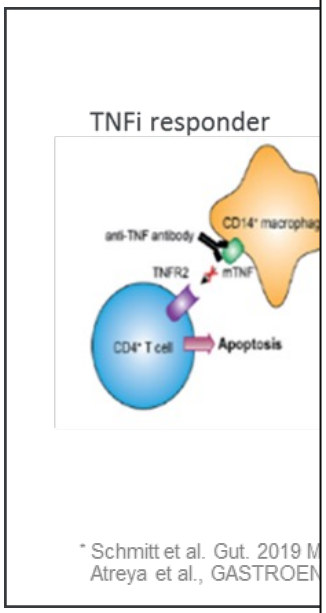



- GIMATS=IgG plasma cells, inflammatory mononuclear phagocytes, activated T cells, and stromal cells
- Existence of two qualitatively distinct subsets of disease, with distinct responses to anti-TNF therapy.



Martin et al., 2019, Cell 178, 1493–1508

# Understanding dual targeting: TNF/IL23 in Chron's Disease



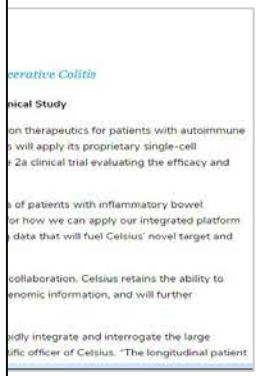

* Schmitt et al. Gut. 2019 M
Atreya et al., GASTROEN

Results of Novel Clinical Study Show Adults with Moderately to Severely Active Ulcerative Colitis Achieved Higher Rates of Clinical Response, Clinical Remission, and Endoscopic Improvement at 12 Weeks with Guselkumab and Golimumab Combination Therapy Versus Either Monotherapy Alone

The VEGA Phase 2a proof-of-concept study shows 83.1 percent of patients who received combination therapy achieved the primary endpoint of clinical response and 36.6 percent of patients achieved clinical remission at week 12

The VEGA study represents a first-of-its-kind biologic combination assessment of an interleukin (IL)-23p19 subunit antagonist with a tumor necrosis factor-alpha (TNFα) antagonist in ulcerative colitis

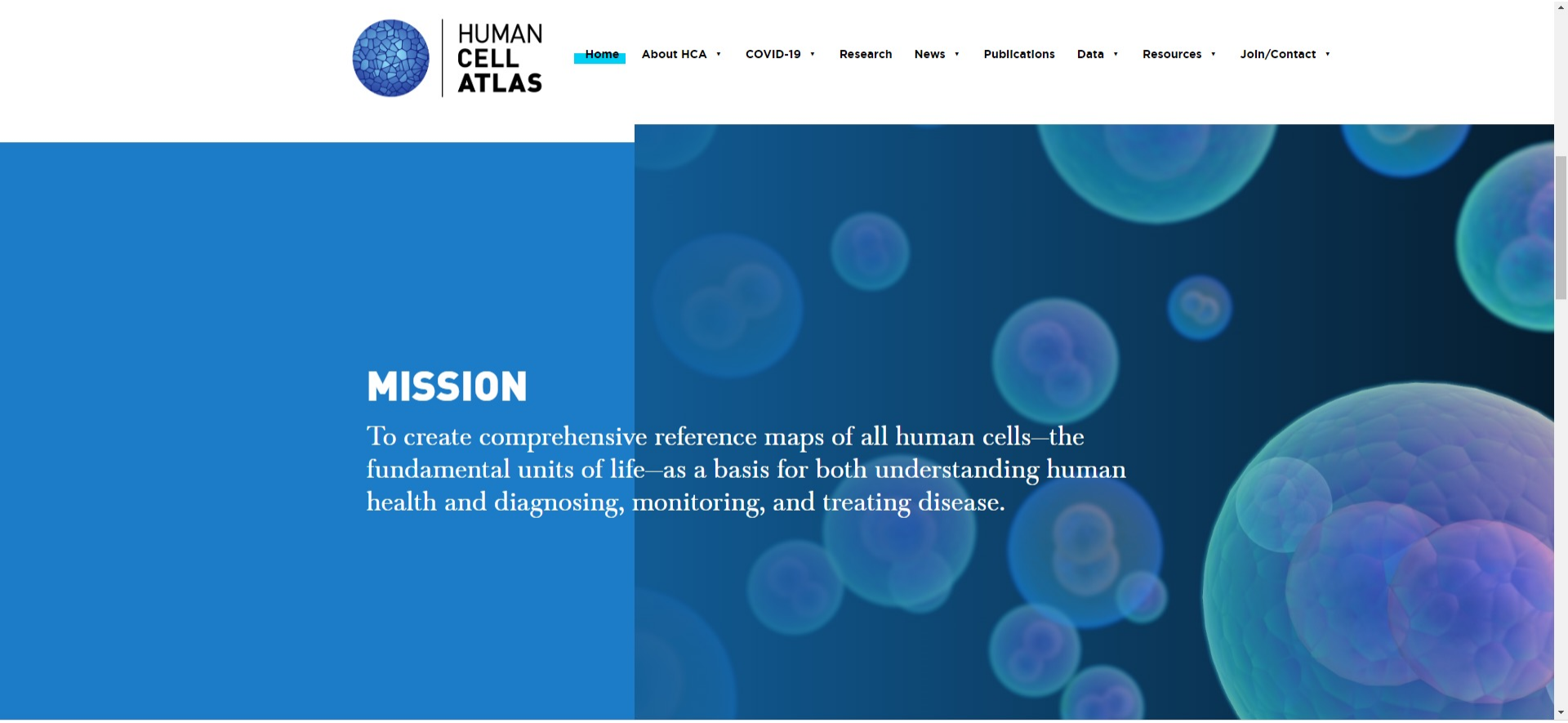
f single-cell
in Phase 2a
apy



Working Hypo non-responders

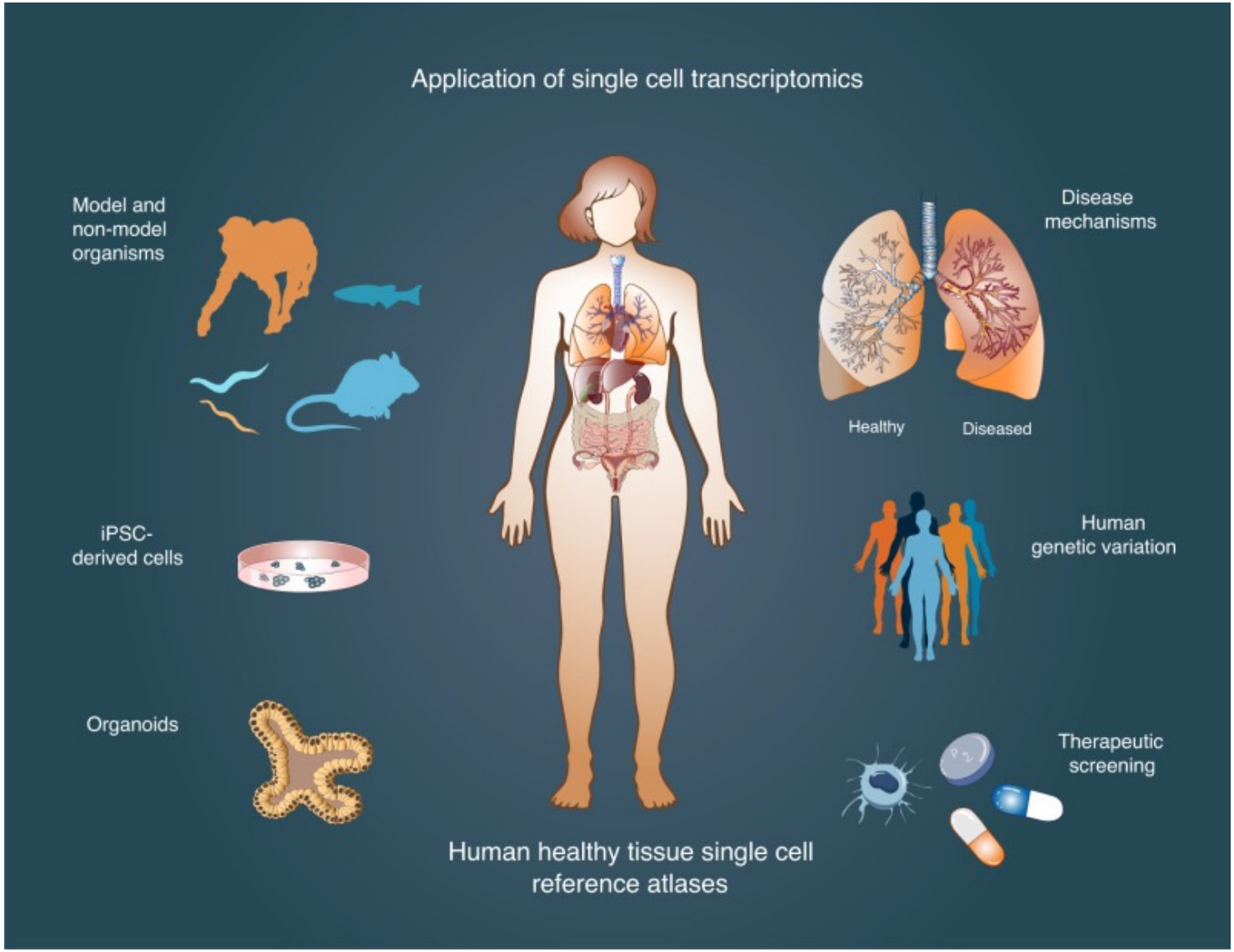
Feagan BG, Sands BE, Sandborn WJ et al. VEGA Study Group. Gusel kumab plus golimumab combination therapy versus guselkumab or golimumab monotherapy in patients with ulcerative colitis (VEGA): a randomised, double-blind, controlled, phase 2, proof-of-concept trial. *Lancet Gastroenterol Hepatol.* 2023;8(4):307–320

# Human Cell Atlas



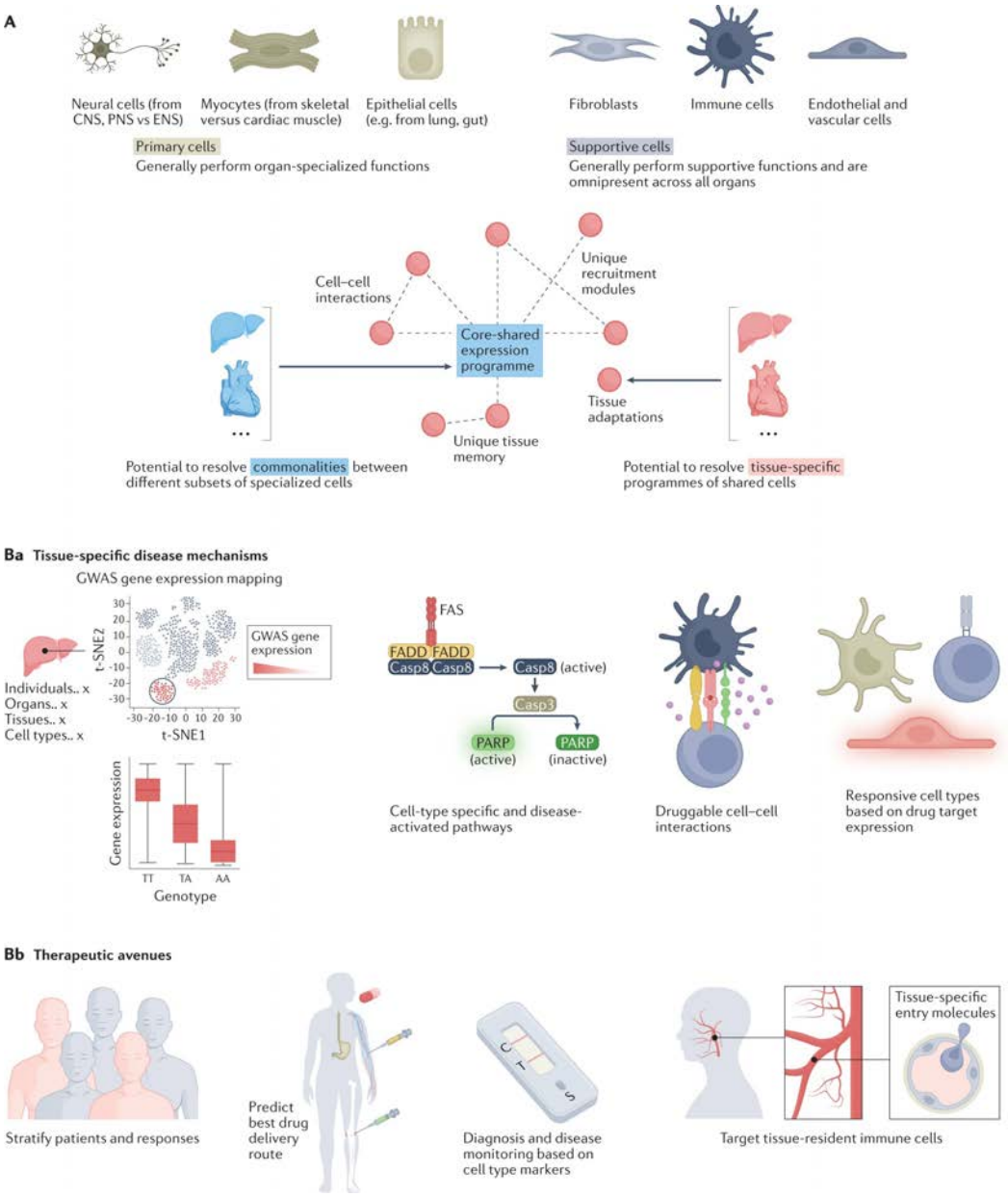

https://www.humancellatlas.org/

# Single-Cell Atlas



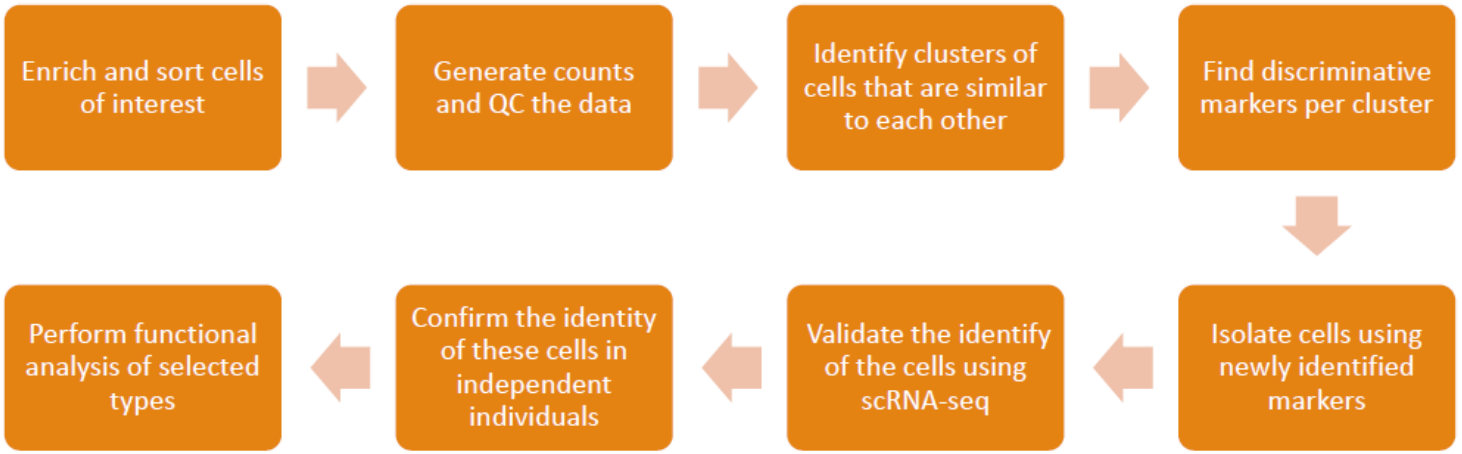

Aldridge S, Teichmann SA.Nat Commun. 2020 Aug 27;

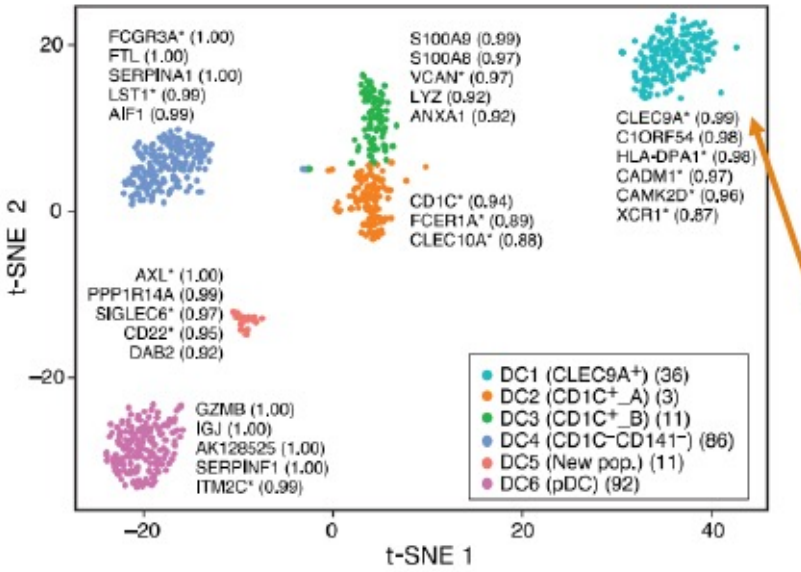
# Single-Cell Cross-Tissue Comparisons



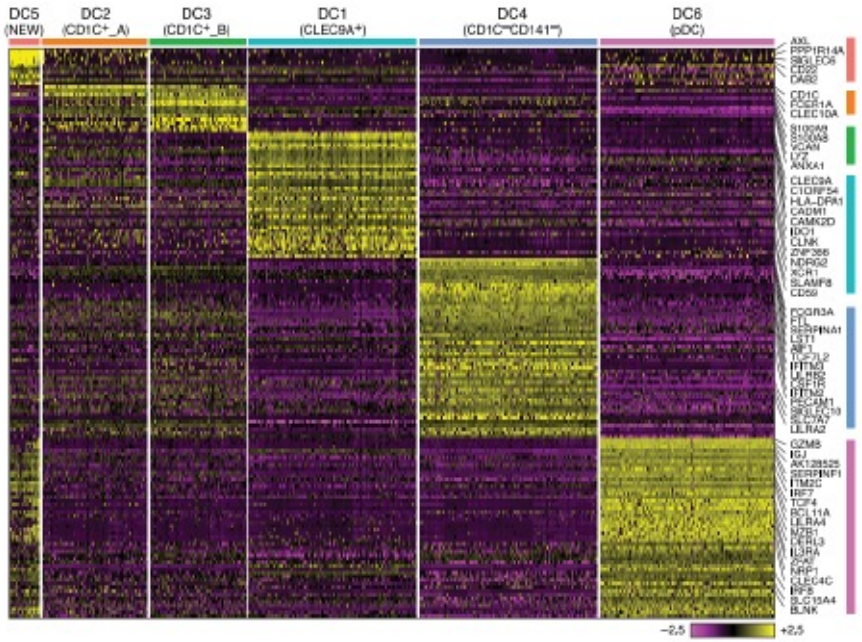

Elmentaite R, Domínguez Conde C, Yang L, Teichmann SA.Nat Rev Genet. 2022 Feb 25.

# Immune System Heterogeneity: Reclassification of DCs and monocytes by scRNA-Seq




AUC

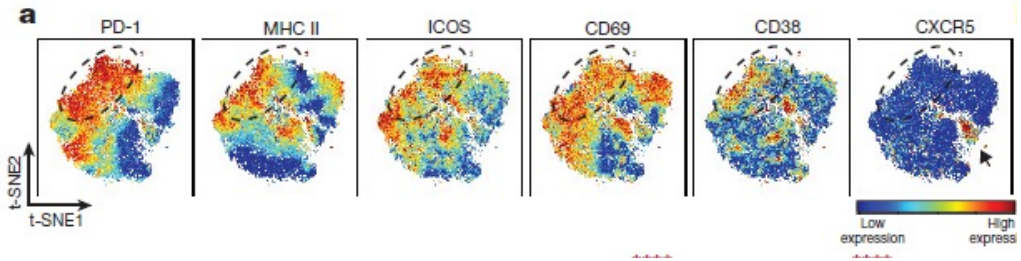



**2.400 HLA-DR$^{+}$lineage$^{-}$ cells**

*Villani et al. Science 21 Apr 2017*

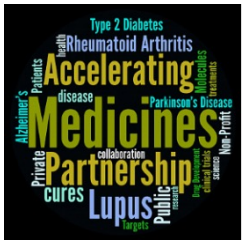
# Novel pathological cell types: expanded peripheral Th subsets in RA

PD-1$^{hi}$ CXCR5$^{-}$ CD4$^{+}$

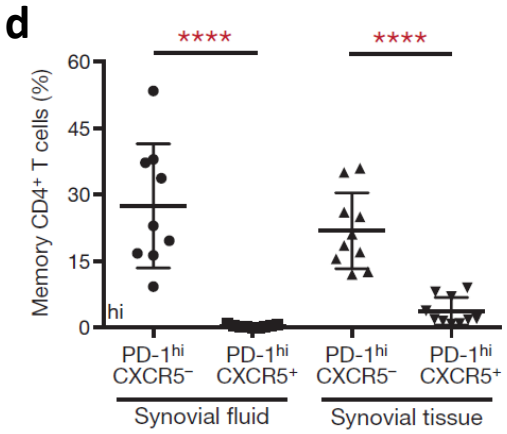



**Pathologically expanded peripheral T helper cell subset drives B cells in RA**

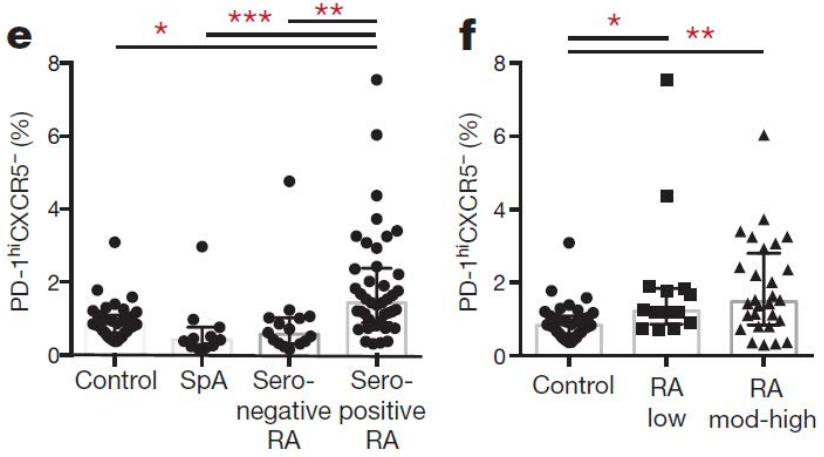
**Synovial PD-1hi CXCR5−CD4+ T cells express factors associated with B-cell help (ICOS, IL21, MAF).**




*synovial fluid*

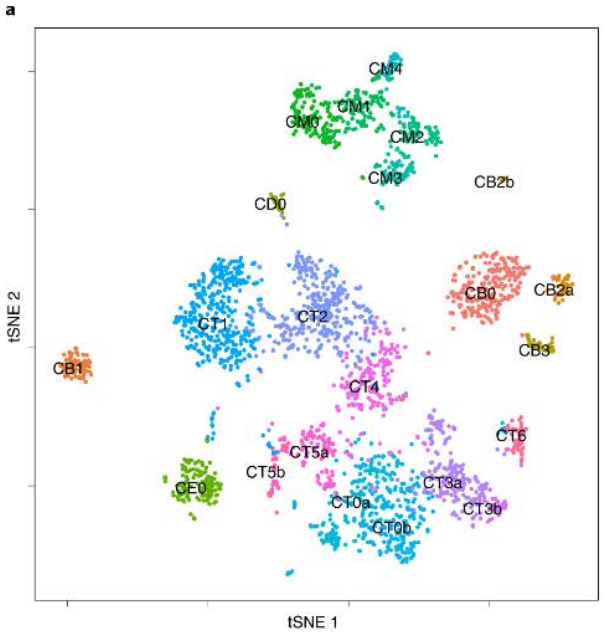



*blood*

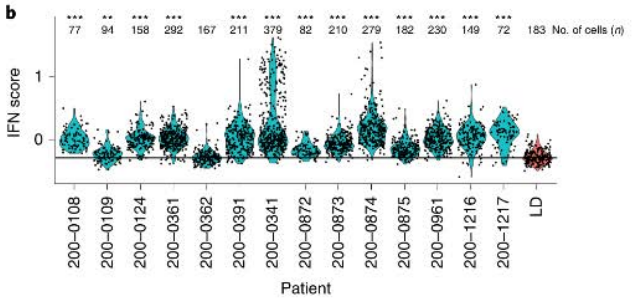


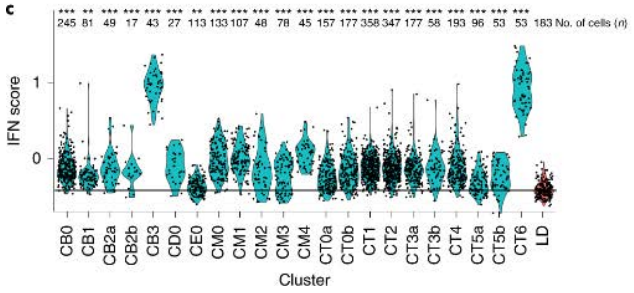

*Rao et al. - Nature. 2017 Feb 1;542(7639)*

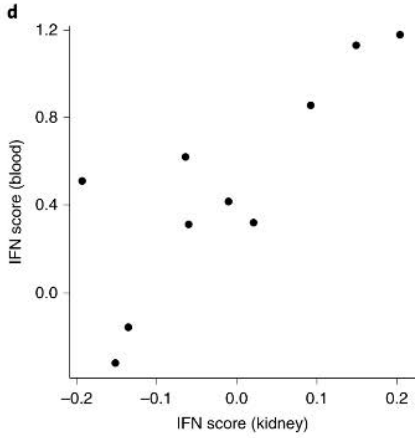
# Immune cell landscape in kidneys of patients with lupus nephritis



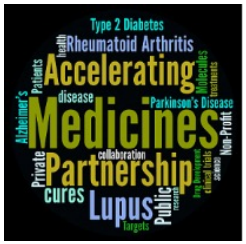

CM0: Inflammatory CD16$^{+}$ macrophages
CM1: Phagocytic CD16$^{+}$ macrophages
CM2: Tissue-resident macrophages
CM3: cDCs
CM4: M2-like CD16$^{+}$ macrophages
CT0a: Effector memory CD4$^{+}$ T cells
CT0b: Central memory CD4$^{+}$ T cells
CT1: CD56$^{dim}$CD16$^{+}$ NK cells
CT2: CTLs
CT3a: $T_{reg}$ cells
CT3b: TFH-like cells
CT4: GZMK$^{+}$ CD8$^{-}$ T cells
CT5a: Resident memory CD8$^{+}$ T cells
CT5b: CD56$^{bright}$CD16$^{-}$ NK cells
CT6: ISG-high CD4$^{+}$ T cells
CB0: Activated B cells
CB1: Plasma cells and plasmablasts
CB2a: Naive B cells
CB2b: pDCs
CB3: ISG-high B cells
CD0: Dividing cells
CE0: Epithelial cells




- First single-cell dissection of LN
- 24 patients, 10 ctrls
- 21 subsets of leukocytes, including clusters of myeloid cells, T-cells, NK, B-cells
- CXCR4, CX3CR1 broadly expressed
- Use of urine liquid biopsies and kidney samples

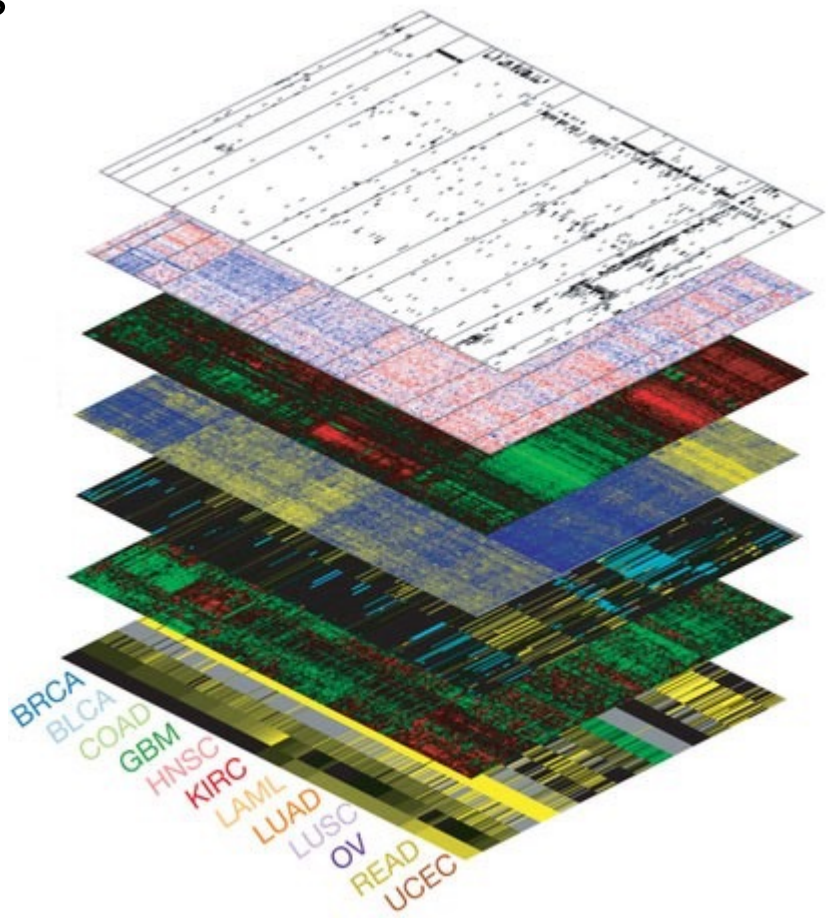



*Arazi et al. Nat Imm 2019*

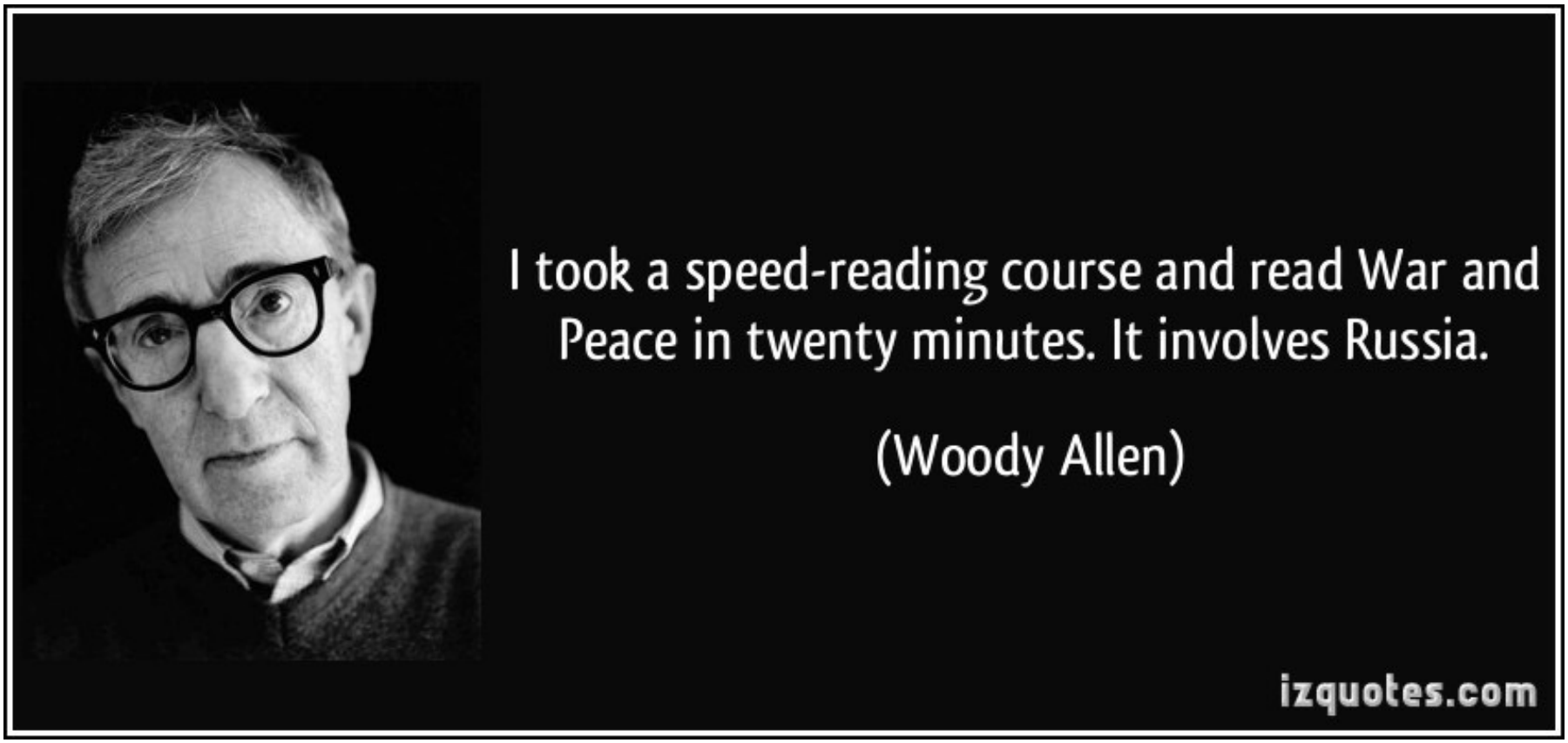
# DATA AGGREGATION AND VISUALIZATION

# Data Dimensionality

- Gene/protein expression
- Methylation
- Epigenetics markers
- Genetics SNPs
- Metabolomics
- …..



**Data aggregation** is any process in which information is gathered and expressed in a summary form, for purposes such as statistical analysis



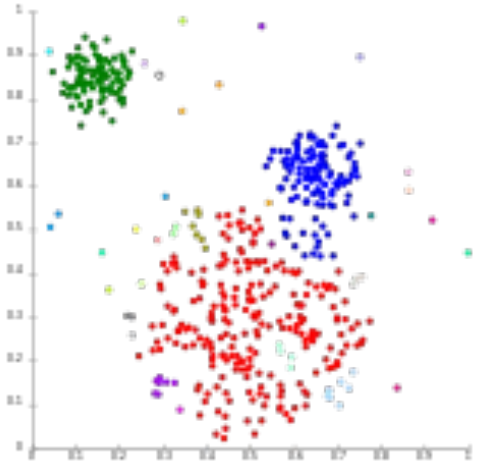
# Data Aggregation

- Clustering and Geometrical Representation of Data
- Dimensions Reduction
- Pathways and Gene Sets

# Clustering

Finding a partition such that:

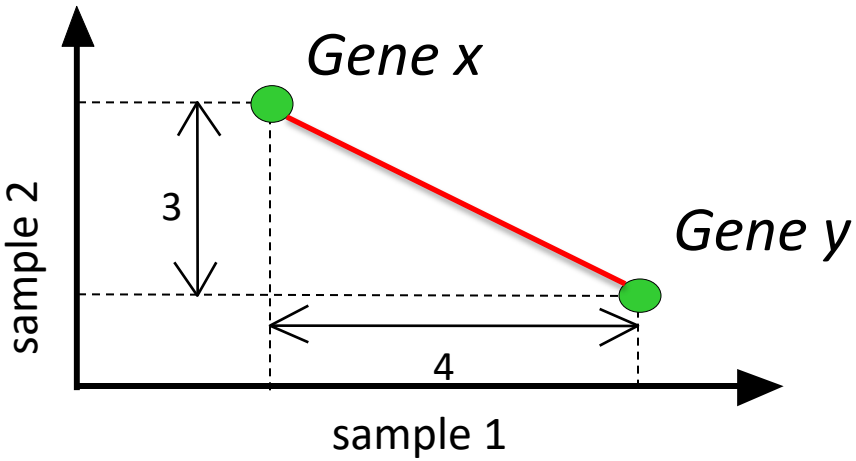
- Distance between objects within the same cluster is **minimised**
- Distance between objects from different clusters is **maximised**



**Requires defining a <u>similarity measure</u>**

# Geometrical Distances as measures of similarity

| | Sample 1 | Sample 2 |
|---|---|---|
| Gene X | 2 | 3 |
| Gene Y | 5 | 1 |



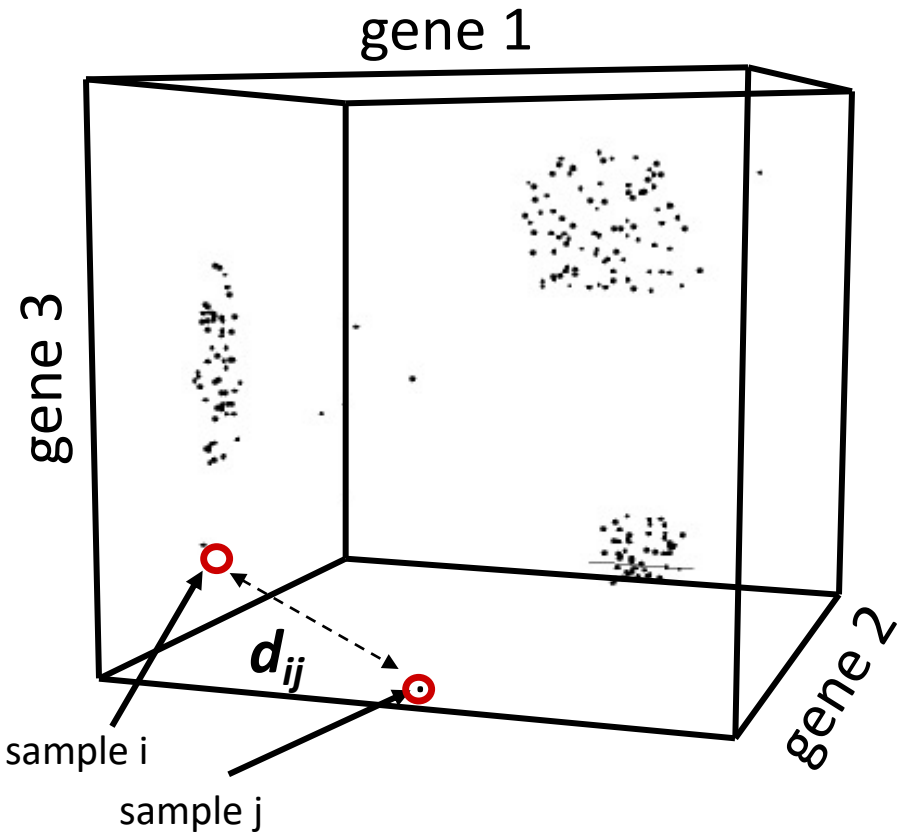

- Euclidean distance : $\sqrt[2]{4^2 + 3^2} = 5$
- Manhattan distance : $4 + 3 = 7$
- "sup" distance : $\max\{4,3\} = 4$

• Similarity among genes/samples is expressed as a mathematical distance

• Genes/samples close in the “expression space” have similar expression profiles

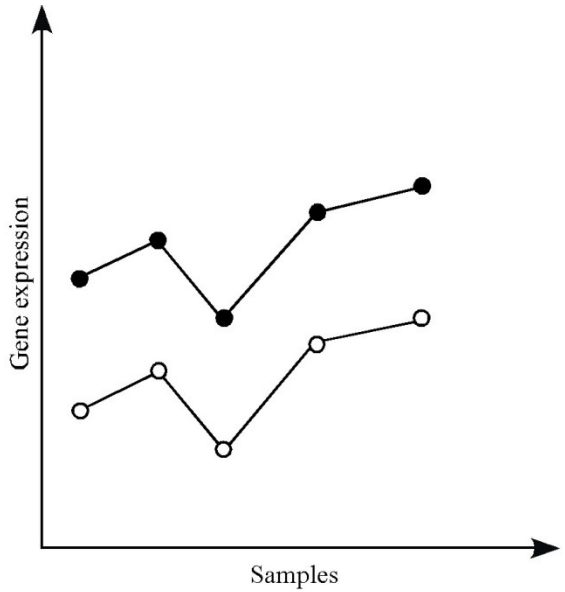
# Geometrical Distances as measures of similarity

| | Sample i | Sample j |
|---|---|---|
| Gene 1 | 2 | 3 |
| Gene 2 | 5 | 1 |
| Gene 3 | 7 | 4 |

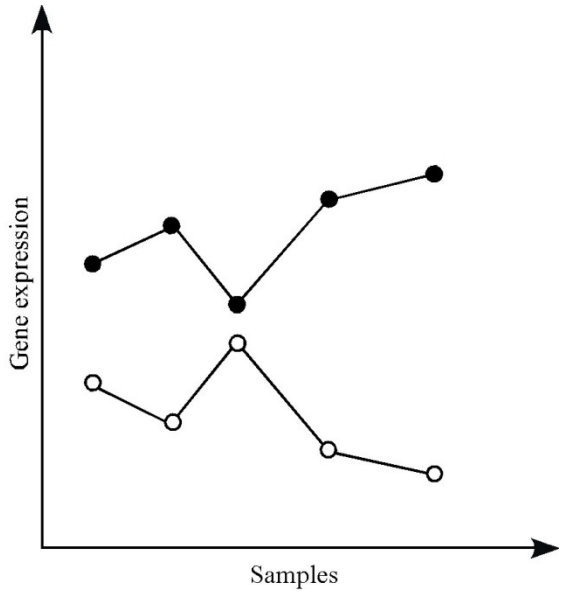



- N genes = N dimensions
- each sample can be represented as a point in the N-dimensional space

# Similarity based on correlation




positive correlation




negative correlation

$$\sigma(x, y) = \mathrm{E}\left[(x - \mathrm{E}[x])(y - \mathrm{E}[y])\right],$$

$$\text{- correlation distance} : \frac{\mathrm{cov}(a,b)}{std(a) \cdot std(b)}$$

# Unsupervised Methods: Hierarchical Clustering

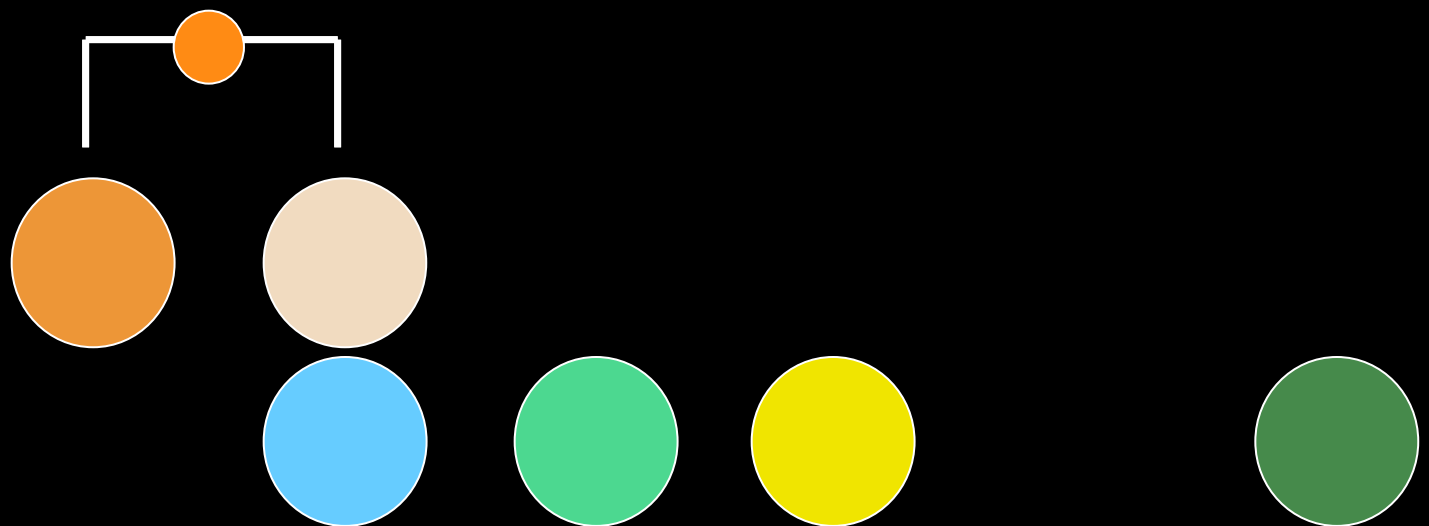
At the beginning, each object (gene) is a cluster. In each of the subsequent steps, the two *closest* clusters are merged into one cluster until there is only one cluster left.

# Hierarchical Clustering

Similarity distance: color

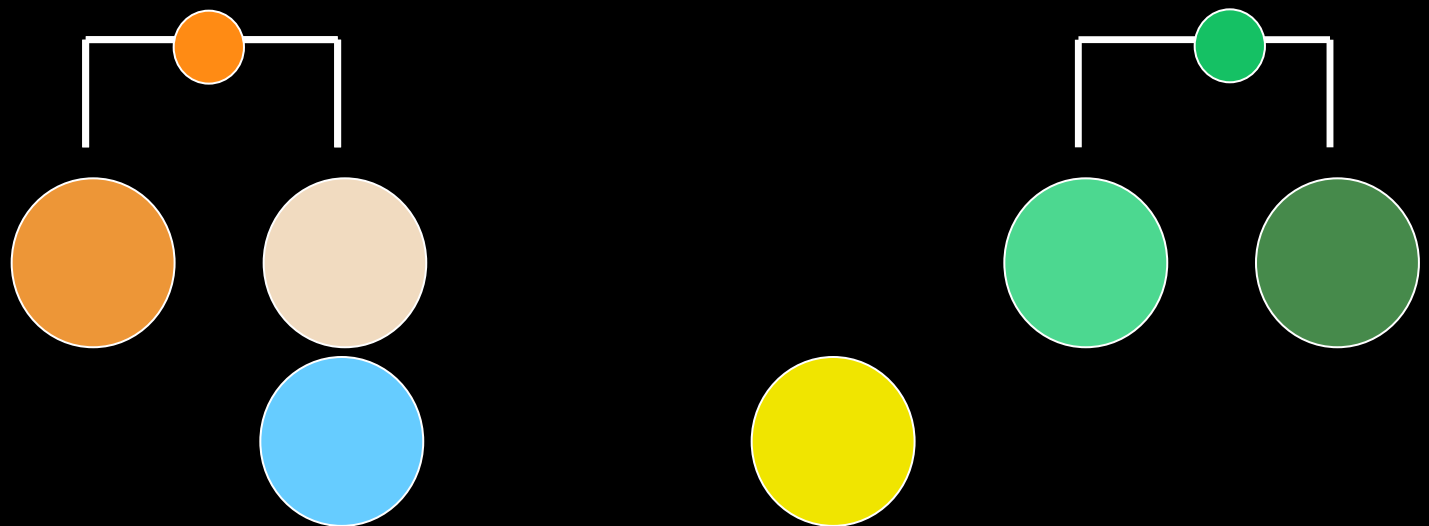
# Hierarchical Clustering

Similarity distance: color



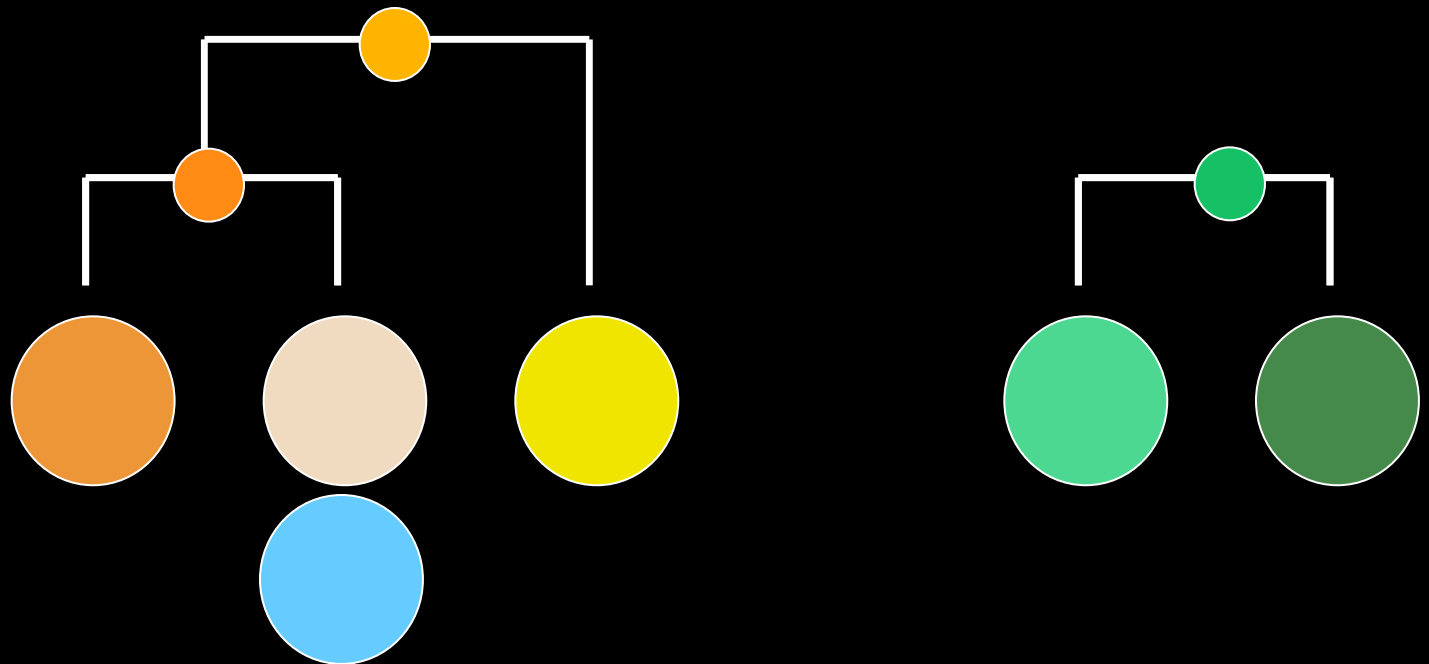
# Hierarchical Clustering

Similarity distance: color



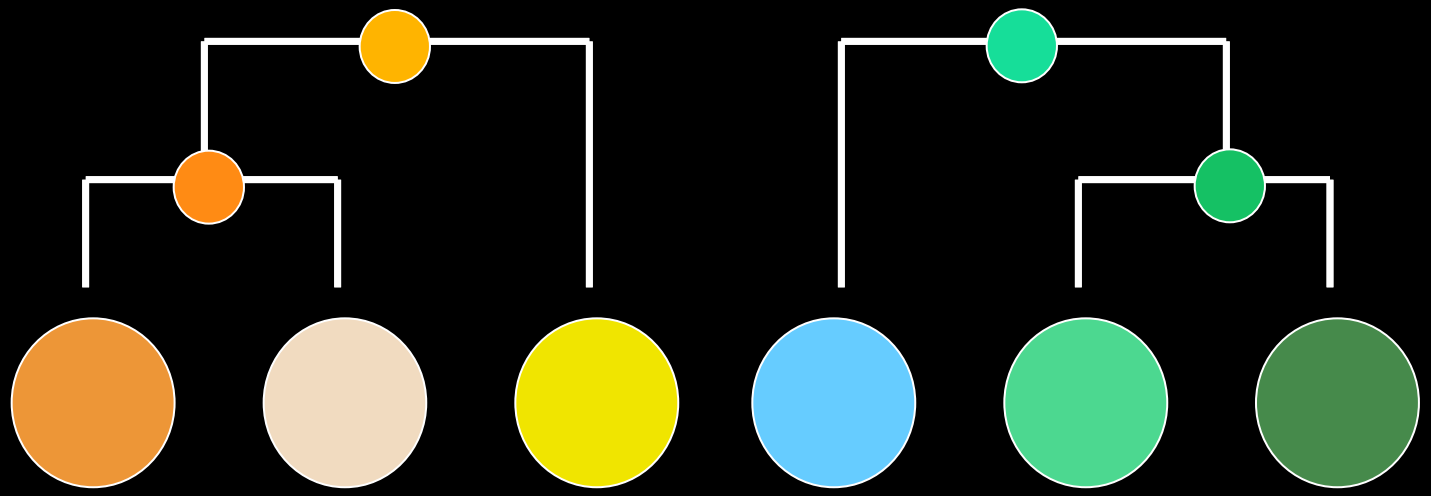
# Hierarchical Clustering

Similarity distance: color



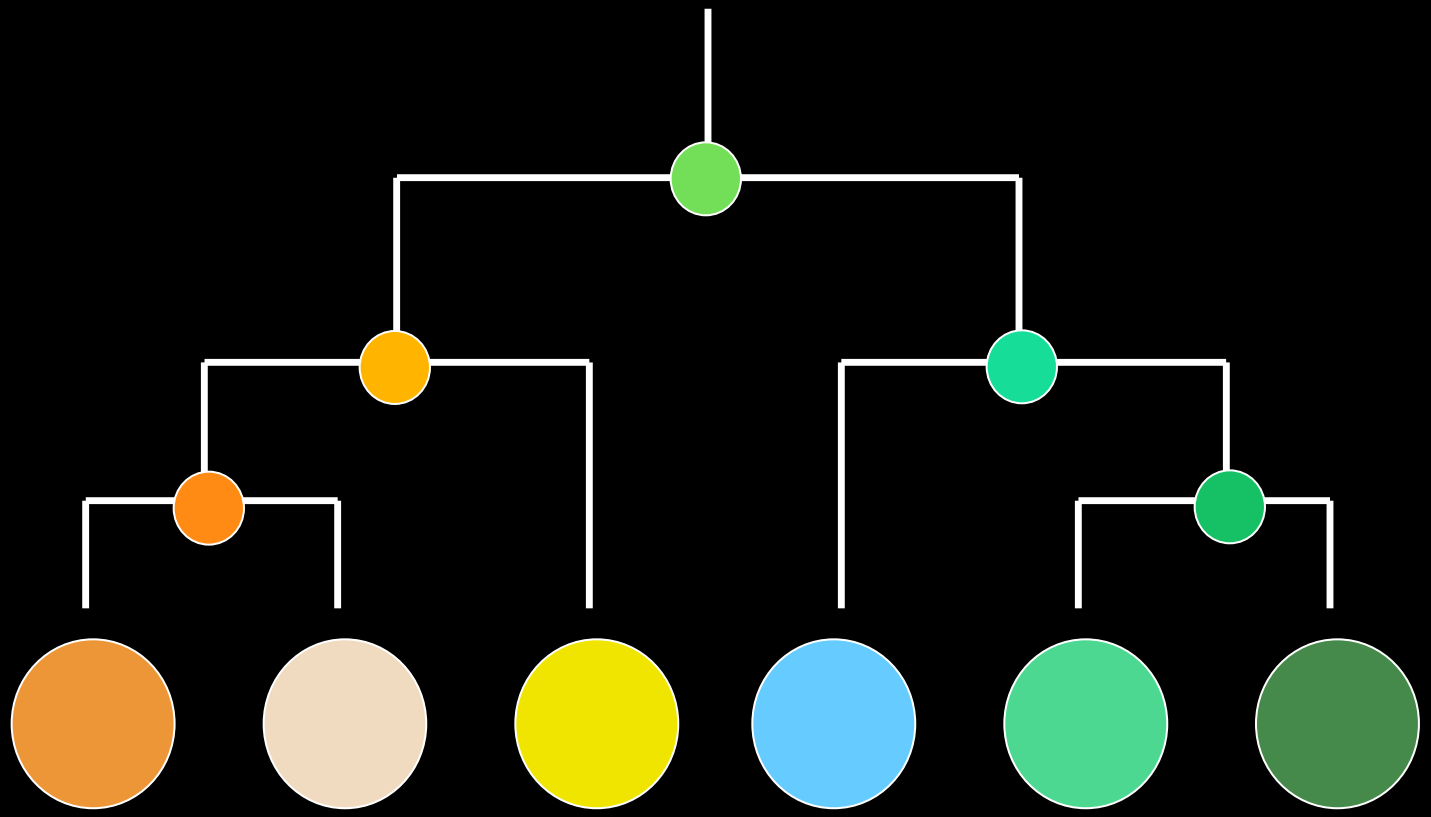
# Hierarchical Clustering

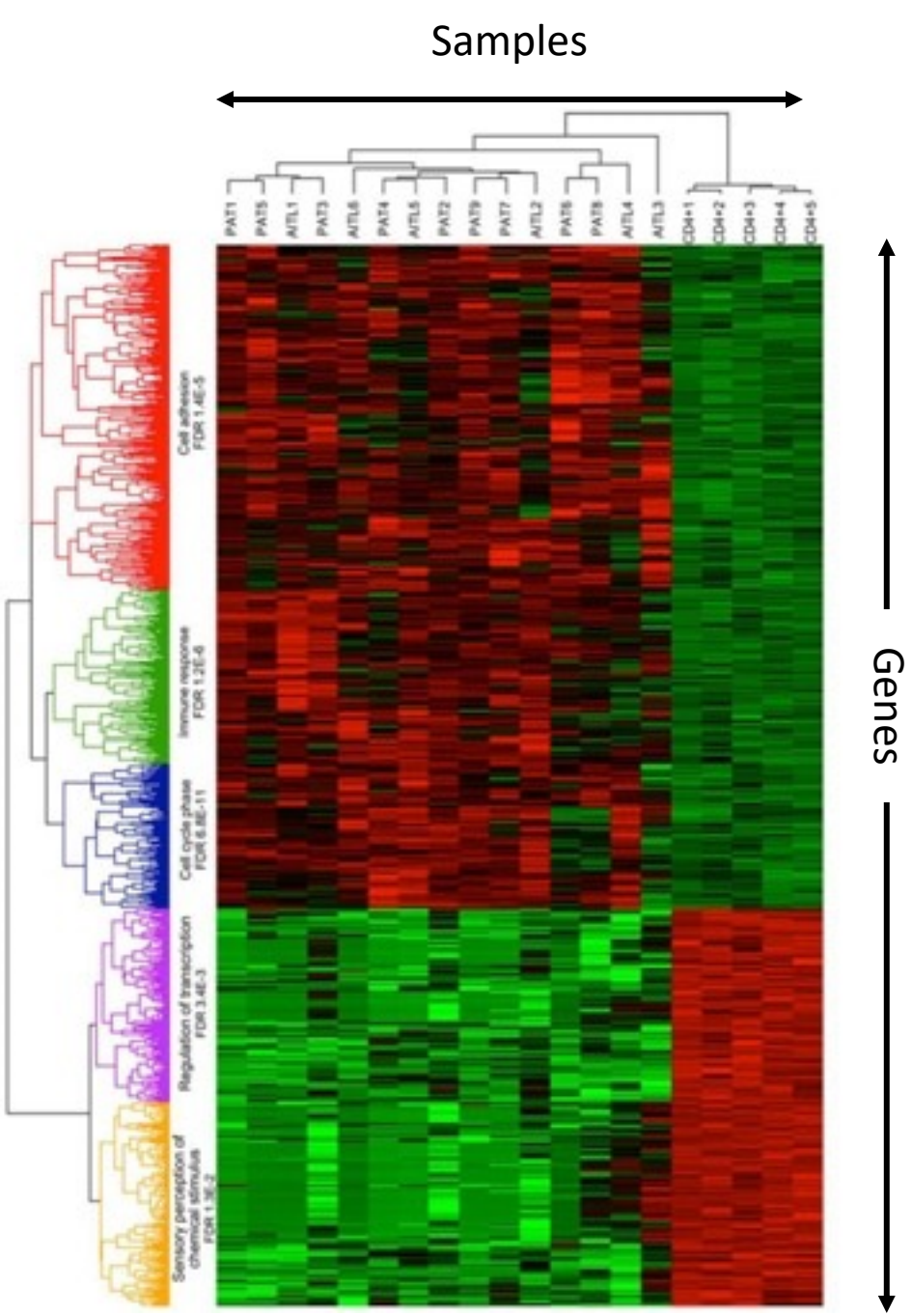
Similarity distance: color



# Hierarchical Clustering

Similarity distance: color




Samples
Genes
PAT1
PAT5
AITL1
PAT3
AITL6
PAT4
AITL5
PAT2
PAT9
PAT7
AITL2
PAT6
PAT8
AITL4
AITL3
CD4+1
CD4+2
CD4+3
CD4+4
CD4+5
Cell adhesion
FDR 1.4E-5
Immune response
FDR 1.2E-6
Cell cycle phase
FDR 6.8E-11
Regulation of transcription
FDR 3.4E-3
Sensory perception of
chemical stimulus
FDR 1.1E-2

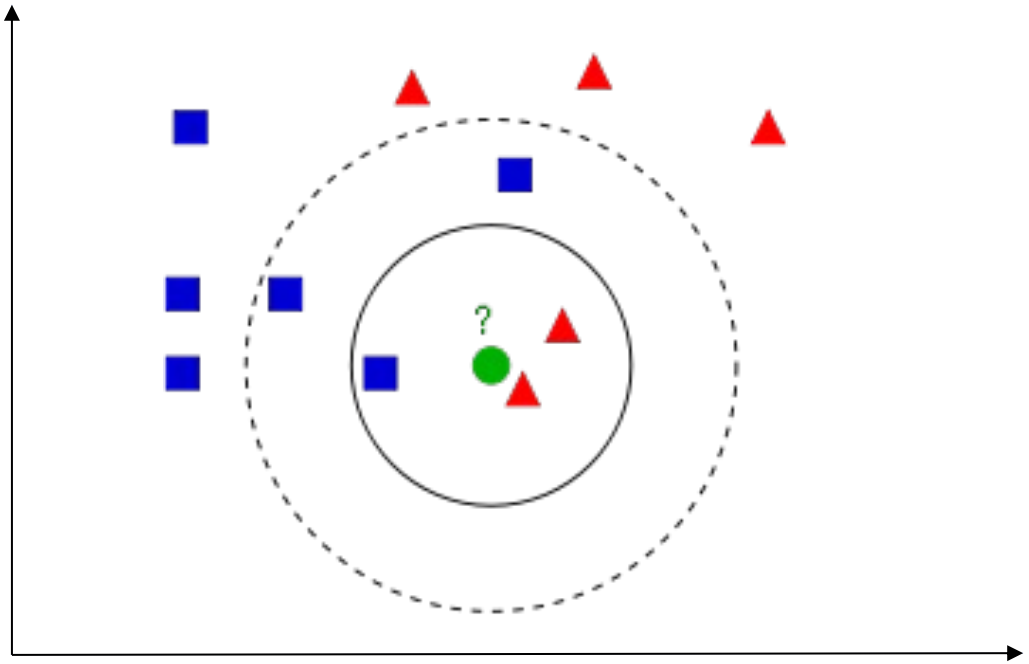
# Supervised Clustering (Classification)

- use pre-existing biological information (e.g. tumor type, immune cell type, responders/non-responders etc.)
- Are used to infer which class an unknown sample belongs to
- Machine learning methods: **k-nearest neighbors,** SVM, Random forests, Bayesian networks, Deep Learning

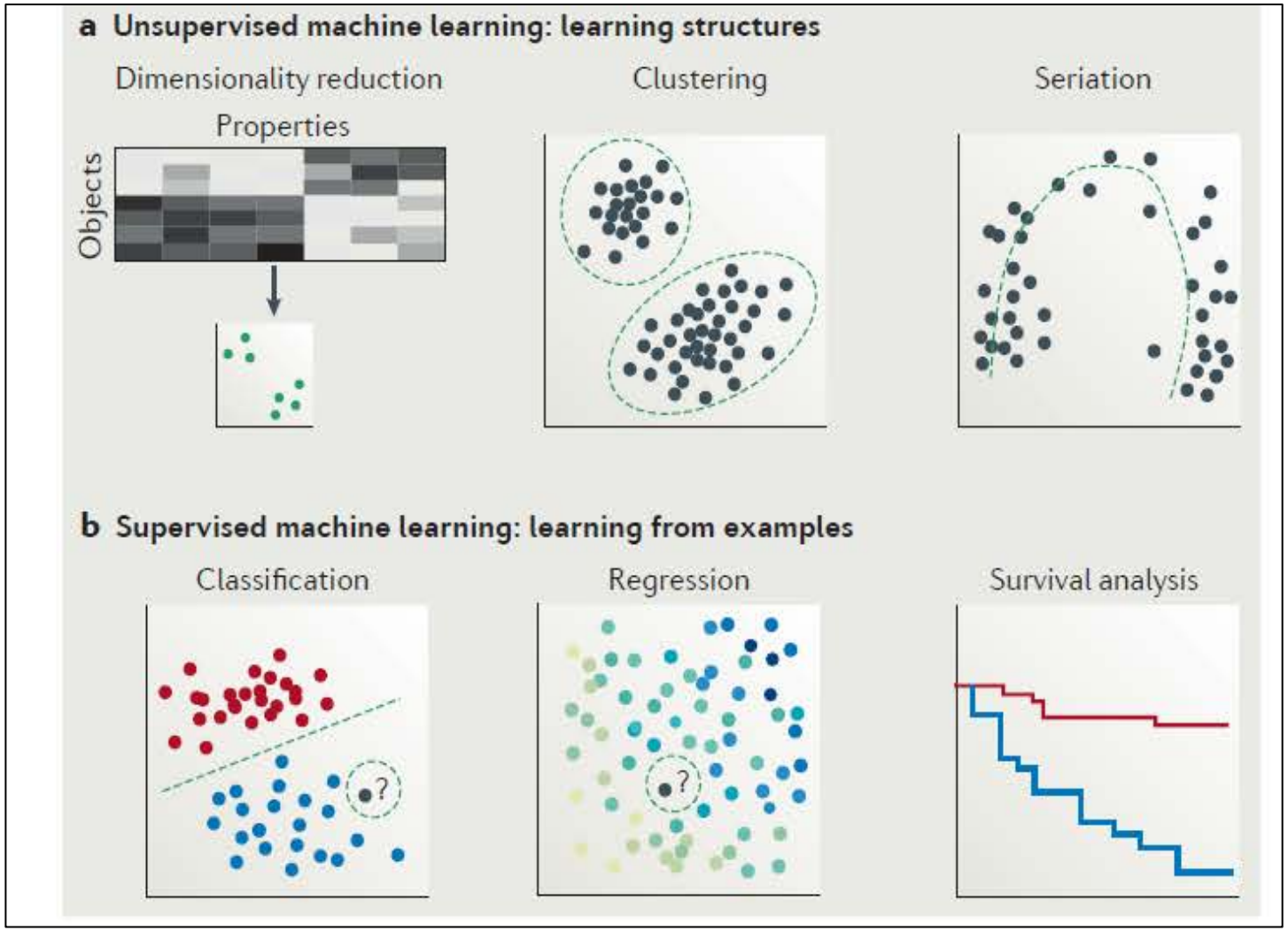
# k-nearest neighbors classification (k-NN)

An object is classified by a majority vote of its neighbours, with the object being assigned to the class most common among its k nearest neighbours (k is a positive integer, typically small).

If k = 1, then the object is simply assigned to the class of that single nearest neighbour.

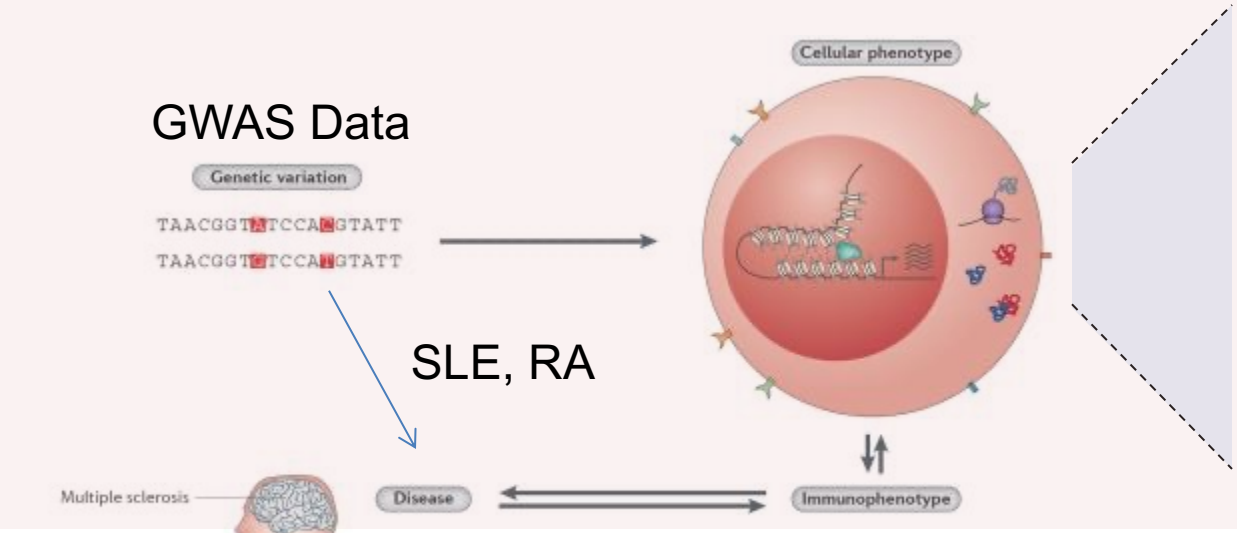


# Supervised and Unsupervised Machine Learning

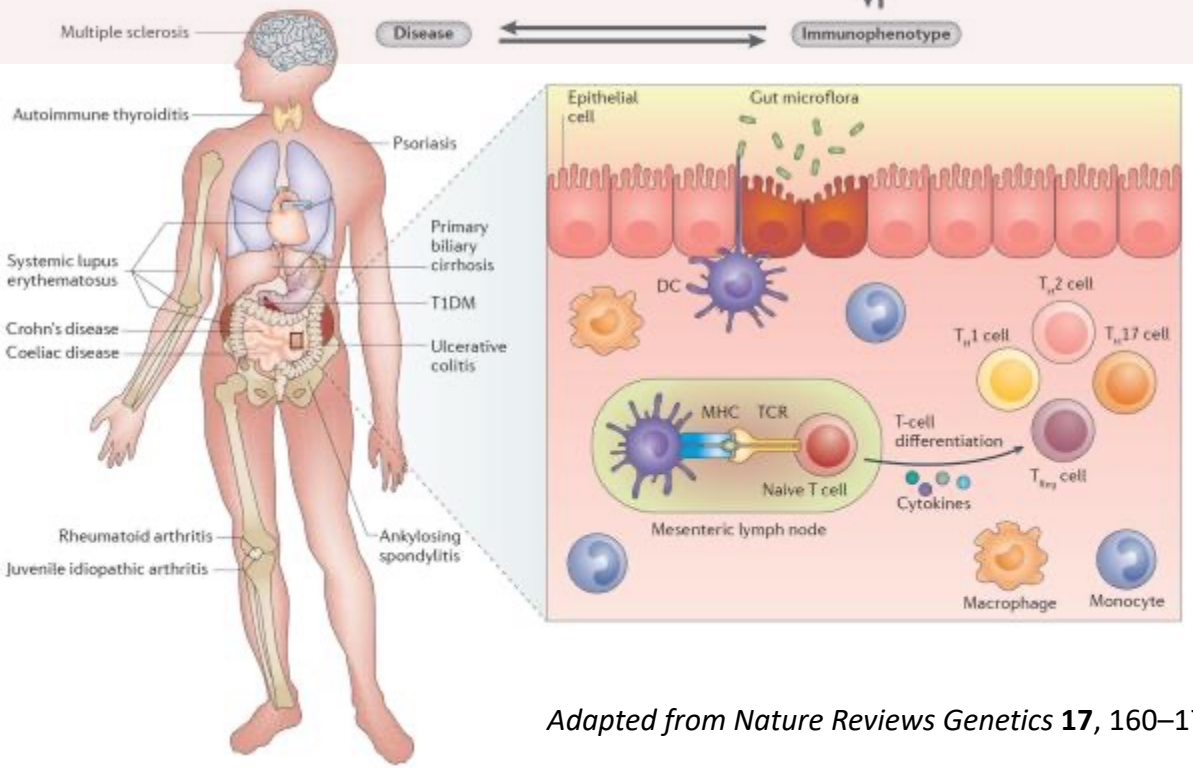



*Nature Reviews Immunology 16, 449–462 (2016)*

# Exploring connections between Genetics, Immune Phenotypes and Clinical Phenotypes




- Gene transcription
- Pathway activation
- Histone modifications
- protein–protein interactions




*Adapted from Nature Reviews Genetics* **17**, 160–174 (2016)

# Q & A


FOCIS 2024
June 18-21
San Francisco
CALIFORNIA
ANNUAL MEETING
CASTRO

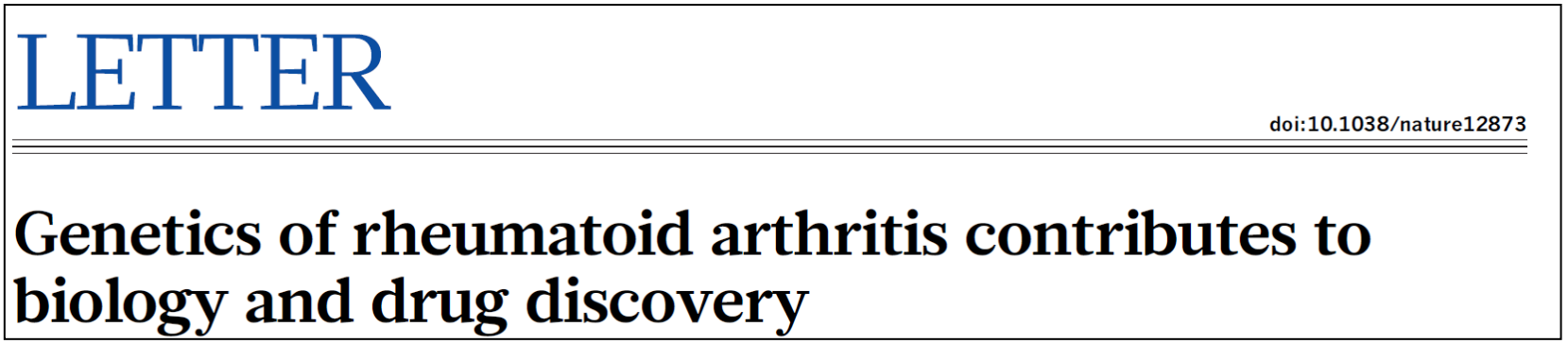
# Deep Dive into Case Studies:

# From Systems Immunology to Novel Therapeutic Insights

*Emanuele de Rinaldis*

*Workshop in Systems Immunology*
*June 17th$^{th}$, 2024*

**Leveraging on integration of orthogonal data sets to identify genes of therapeutic interest in RA (R. Plenge's)**

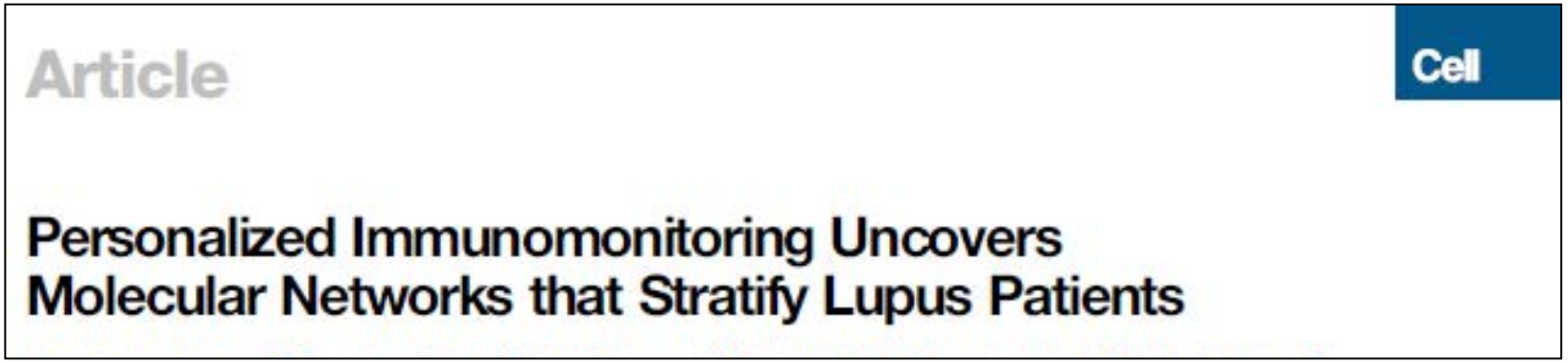

LETTER

doi:10.1038/nature12873

Genetics of rheumatoid arthritis contributes to biology and drug discovery

Nature. 2014 Feb 20;506(7488):376-8

**Understanding SLE biology and stratifying patients using blood bulk gene expression data (V. Pascual's)**


Article

Cell

Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients

Cell. 2016 Apr 21;165(3):551-6

# Genetics and Drug Discovery in RA – Study Workflow

LETTER

doi:10.1038/nature12873

**Genetics of rheumatoid arthritis contributes to biology and drug discovery**

Identification of SNPs associated to RA

From SNPs to causal genes through gene-mapping

Characterization of results

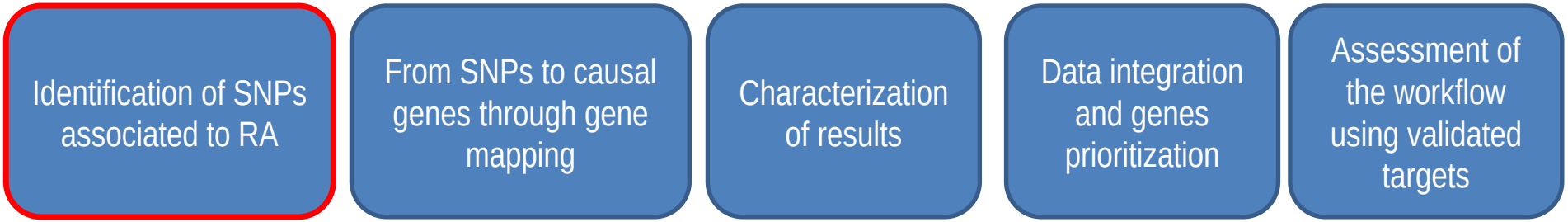
Data integration and genes prioritization

Assessment of the workflow using validated targets

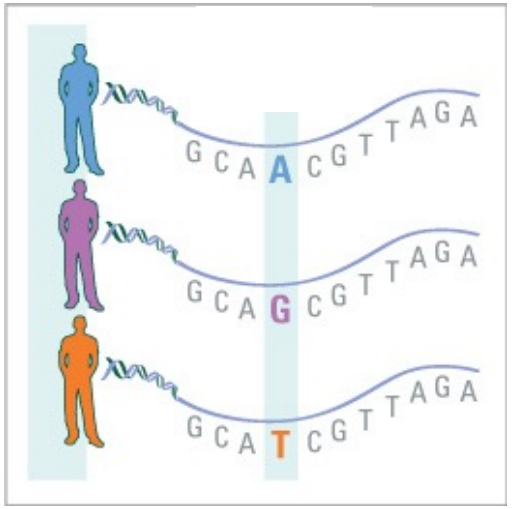
- Novel loci associated to RA
- New hints on disease biology
- Novel candidate targets
- Repositioning of existing drug targets

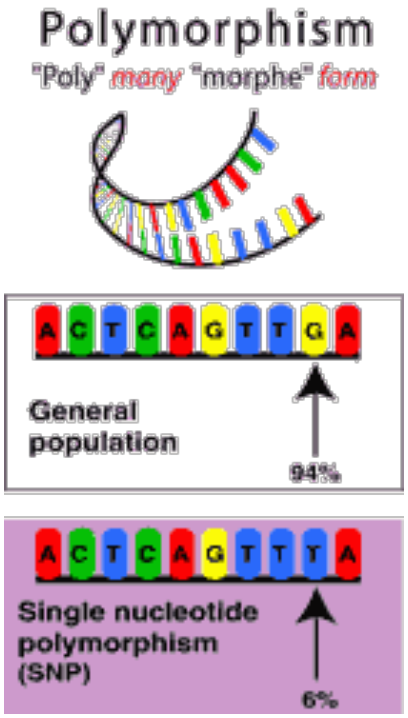
# Toolbox

- **Genome Wide Association Studies (GWAS) and Meta-Analysis**
  - Genome variability and SNPs
  - Logistic Regression
  - Linkage Disequilibrium
  - Imputation
  - Manhattan Plots
- **Multiple Testing**
- **Network Analysis**
- **Fine-mapping and data integration**
  - Epigenetics data
  - Transcriptional data ➔ eQTLs
- **Statistical enrichment**

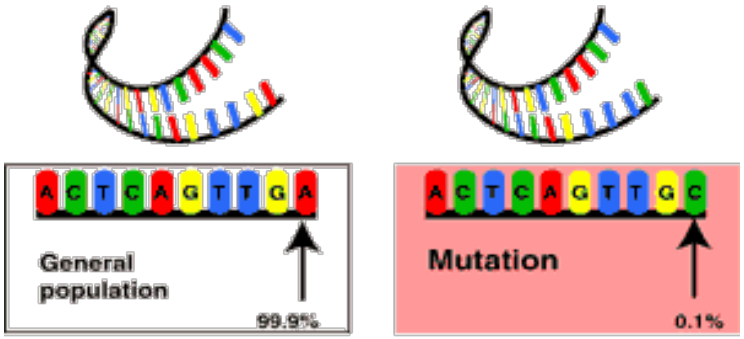

Identification of SNPs associated to RA
From SNPs to causal genes through gene mapping
Characterization of results
Data integration and genes prioritization
Assessment of the workflow using validated targets

# Genetic Variability



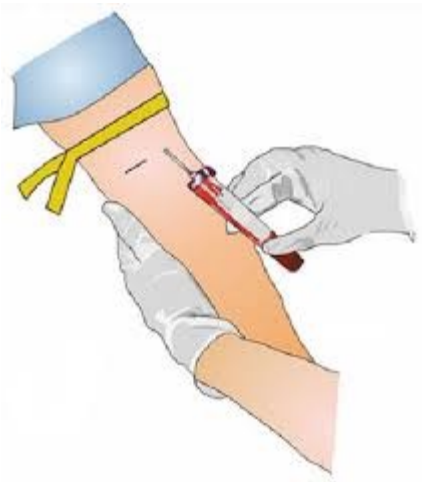




**SNP**: common (>1%) variant of one **S**ingle **N**ucleotide

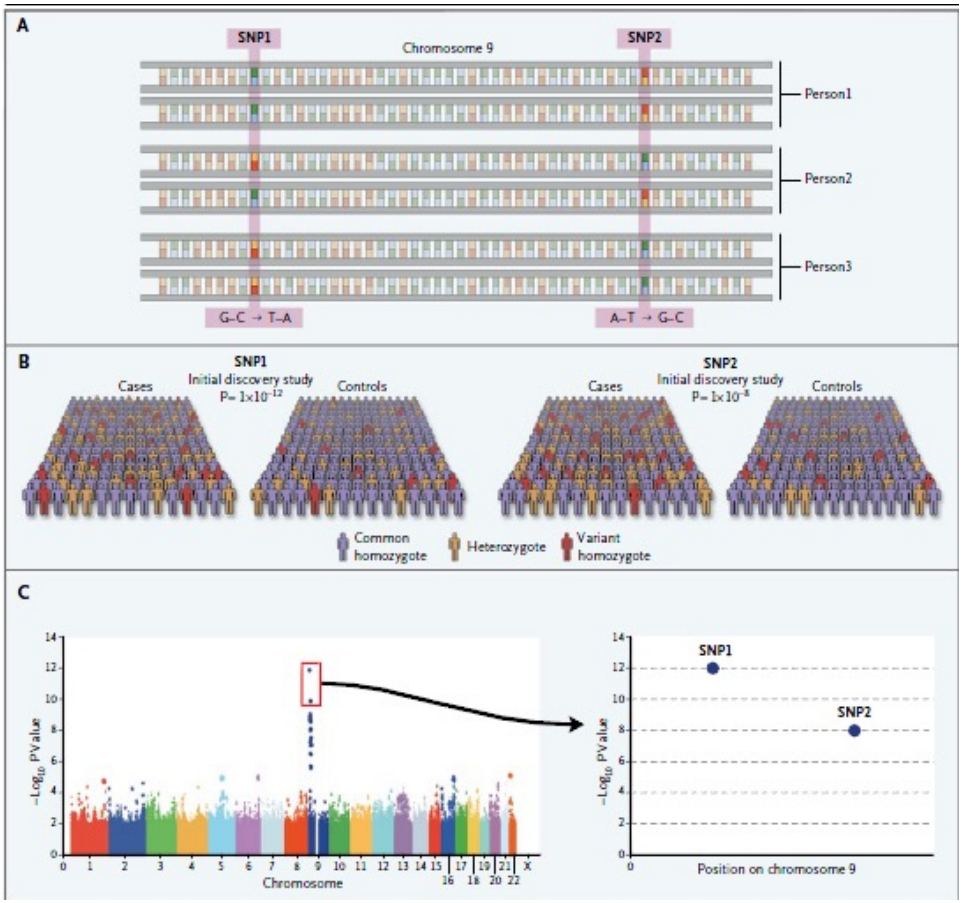



**Mutation: typically a** rare variant, associated with a disease

# Genome-Wide Association Studies (GWAS)



**Extraction of germline DNA (e.g. blood)**



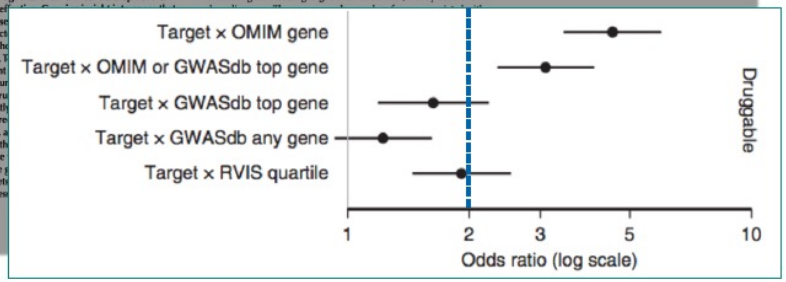

*N Engl J Med. 2010 Nov 18;363(21):2076-7.*

***Genome-wide association study:*** An approach used in genetics research to look for associations between many - typically hundreds of thousands - specific genetic variations - most commonly single-nucleotide polymorphisms - and particular diseases

# Why Pharma Is Investing in Genetics

The support of human genetic evidence for approved drug indications

~2-fold increase in success for genetic targets




RESEARCH ARTICLE

Are drug targets with genetic support twice as likely to be approved? Revised estimates of the impact of genetic support for drug mechanisms on the probability of drug approval

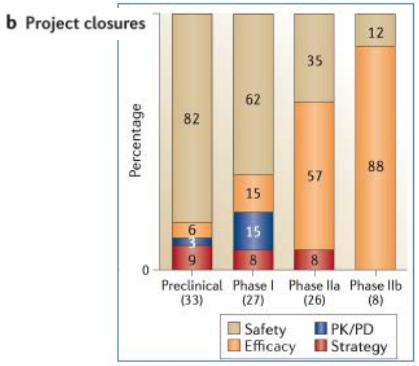
**Emily A. King*, J. Wade Davis, Jacob F. Degner**

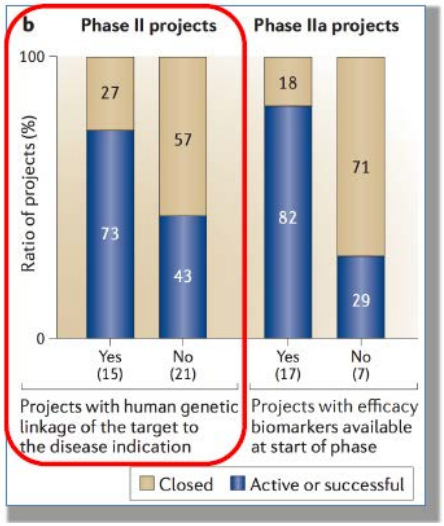
Department of Computational Genomics, AbbVie, North Chicago, Illinois, United States of America

* emily.king@abbvie.com

Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework

*David Cook, Dearg Brown, Robert Alexander, Ruth March, Paul Morgan, Gemma Satterthwaite and Menelas N. Pangalos*




- ❑ When causality is clear (Mendelian traits or coding variants) fold increase for success greater than 2 folds
- ❑ Limited contribution to GWAS genetic evidences not in OMIM ➔ undetermined function

# Genetic Dataset

SNPs

Samples

| | | | | |
|---|---|---|---|---|
| 0 | 1 | … | 1 | 1 |
| 1 | 2 | … | 2 | 0 |
| 0 | 1 | … | 1 | 2 |
| 2 | 2 | … | 2 | 2 |
| 0 | 0 | … | 0 | 1 |
| 0 | 1 | … | 1 | 2 |
| 0 | 2 | … | 2 | 1 |
| 0 | 1 | … | 1 | 1 |
| 0 | 2 | … | 1 | 1 |
| 2 | 2 | … | 2 | 2 |
| 0 | 0 | … | 0 | 1 |
| 0 | 1 | … | 1 | 2 |

Number of minor alleles for a given SNP

# Single test for association

Do people carrying a certain genotype have an increased probability of having the disease?

| SNP | S_IBD1 | S_IBD2 | S_IBD3 | S_Cont1 | S_Cont2 | S_Cont3 |
|---|---|---|---|---|---|---|
| rsxxxxx | 0 | 0 | 1 | 0 | 1 | 0 |
| rsxxxx0 | 0 | 0 | 0 | 0 | 0 | 0 |
| rs... | 1 | 0 | 0 | 0 | 2 | 1 |
| rs... | 0 | 0 | 0 | 1 | 0 | 1 |
| rs... | 0 | 0 | 1 | 1 | 1 | 1 |
| PC1 | .. | | | | | |
| PC2 | | | .. | | | |
| PC .. | | | | | | .. |

$$Log(\frac{p}{1-p}) = \beta_0 + \beta_1 snp + \beta_2 pc1 + \beta_3 pc2 + \beta_4 pc3 + \ldots$$

The tool used was "plink", What we get back is:
the value of B1, and
a p-value for B1 being different than zero.

11

Other variable of interest like ethnicity, age, sex can be added to the model

Logistic regression

# Multiple tests in the same cohort

Test all SNPs for association independently

Correct for multiple testing

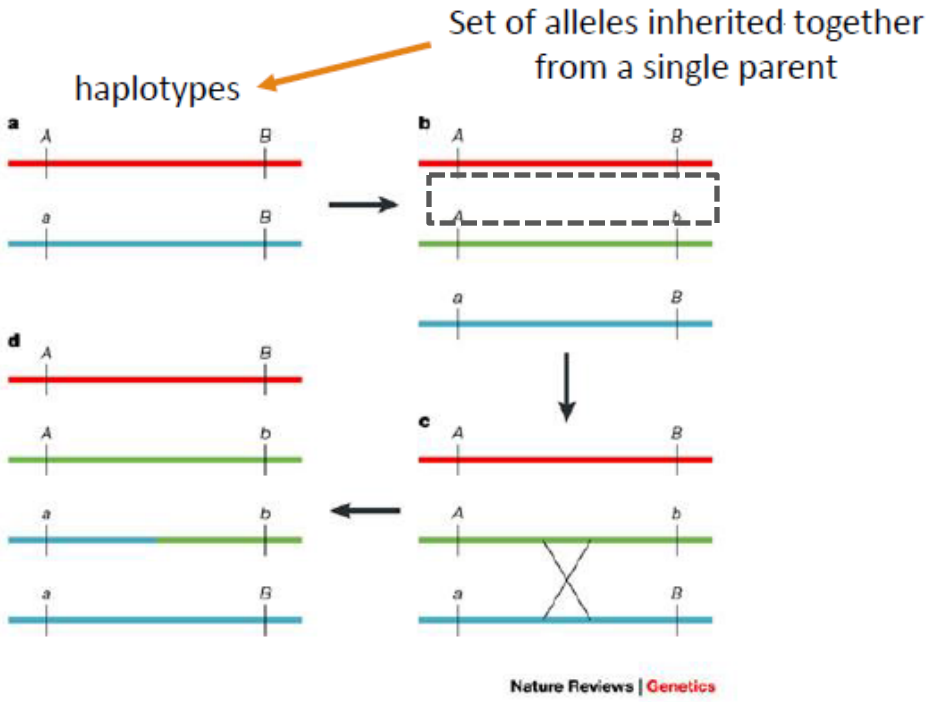
Instead of using 0.05 as a threshold for significance divide it by the total number of independent tests ($5x10^{-8}$ for genome-wide studies)

# Linkage Disequilibrium

- early in the lifetime of the mutation, only three out of the four possible haplotypes will be observed in the population. **The b allele will always be found on a chromosome with the A allele at the adjacent locus**
- The association between alleles at the two loci will gradually be disrupted by recombination between the loci.
- This will result in the creation of the fourth possible haplotype and an eventual decline in LD among the markers in the population as the recombinant chromosome

Non-random association of alleles at different loci (genetic positions)

Genotypes are not independent but correlated




K. G. Ardlie, L. Kruglyak, M. Seielstad, *Nature reviews Genetics* 3, 299-309 (2002)

# Multiple tests in the same cohort

Test all SNPs for association independently

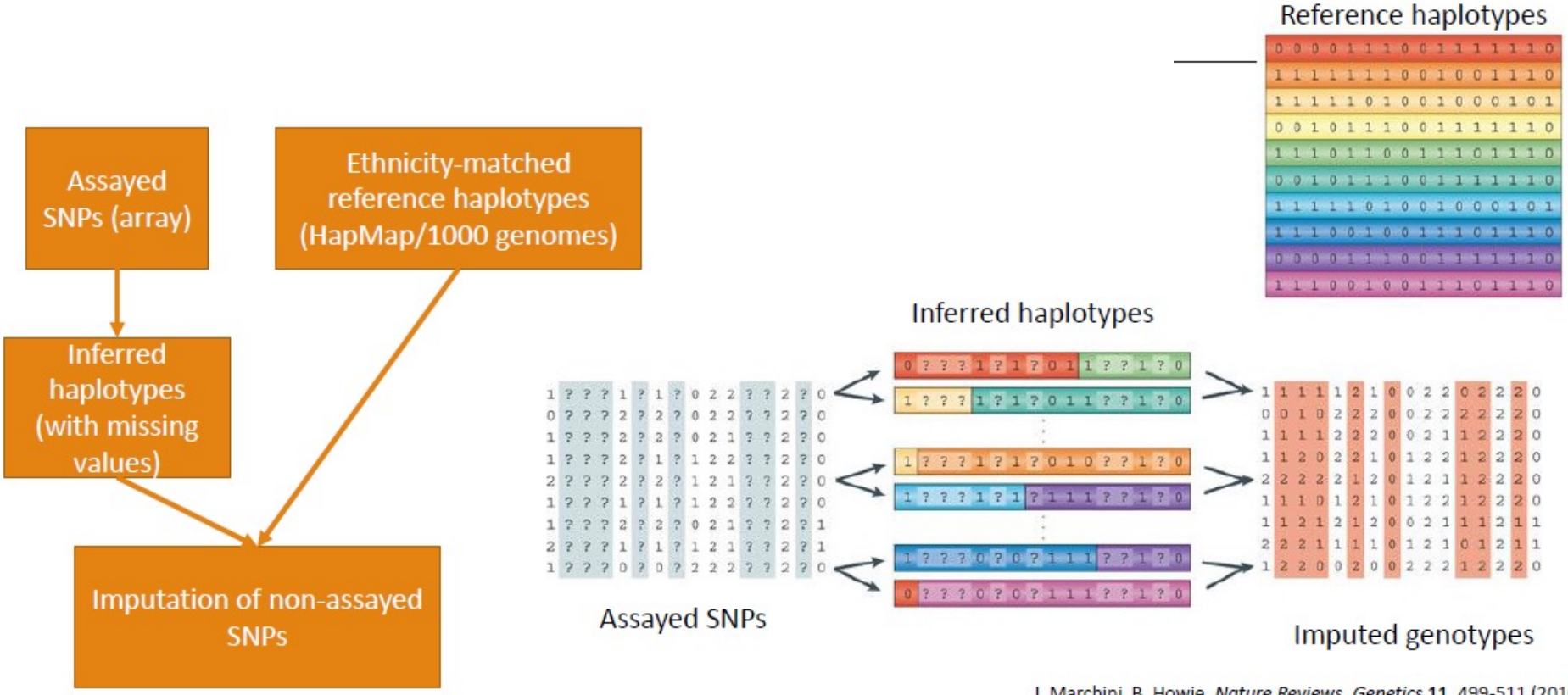
Correct for multiple testing

Divide usual threshold (0.05) by the total number of independent tests

Due to linkage disequilibrium the number of independent SNPs in the genome is estimated at ~1M

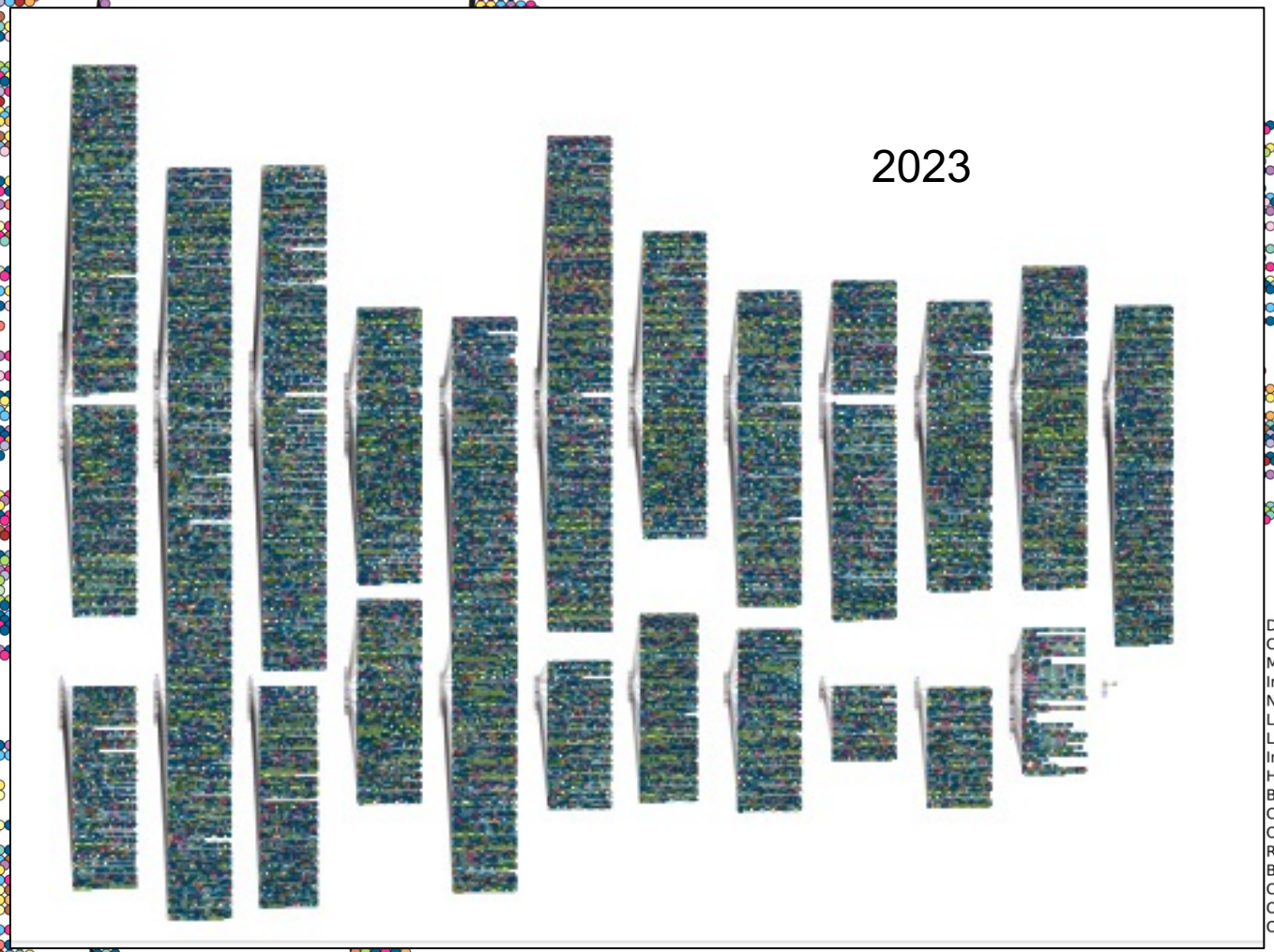
Genome-wide threshold = $5x10^{-8}$

# Imputation




J. Marchini, B. Howie, *Nature Reviews. Genetics* **11**, 499-511 (2010)

# Published Genome-Wide Associations through 12/2013
# $p \leq 5X10^{-8}$ for 17 trait categories



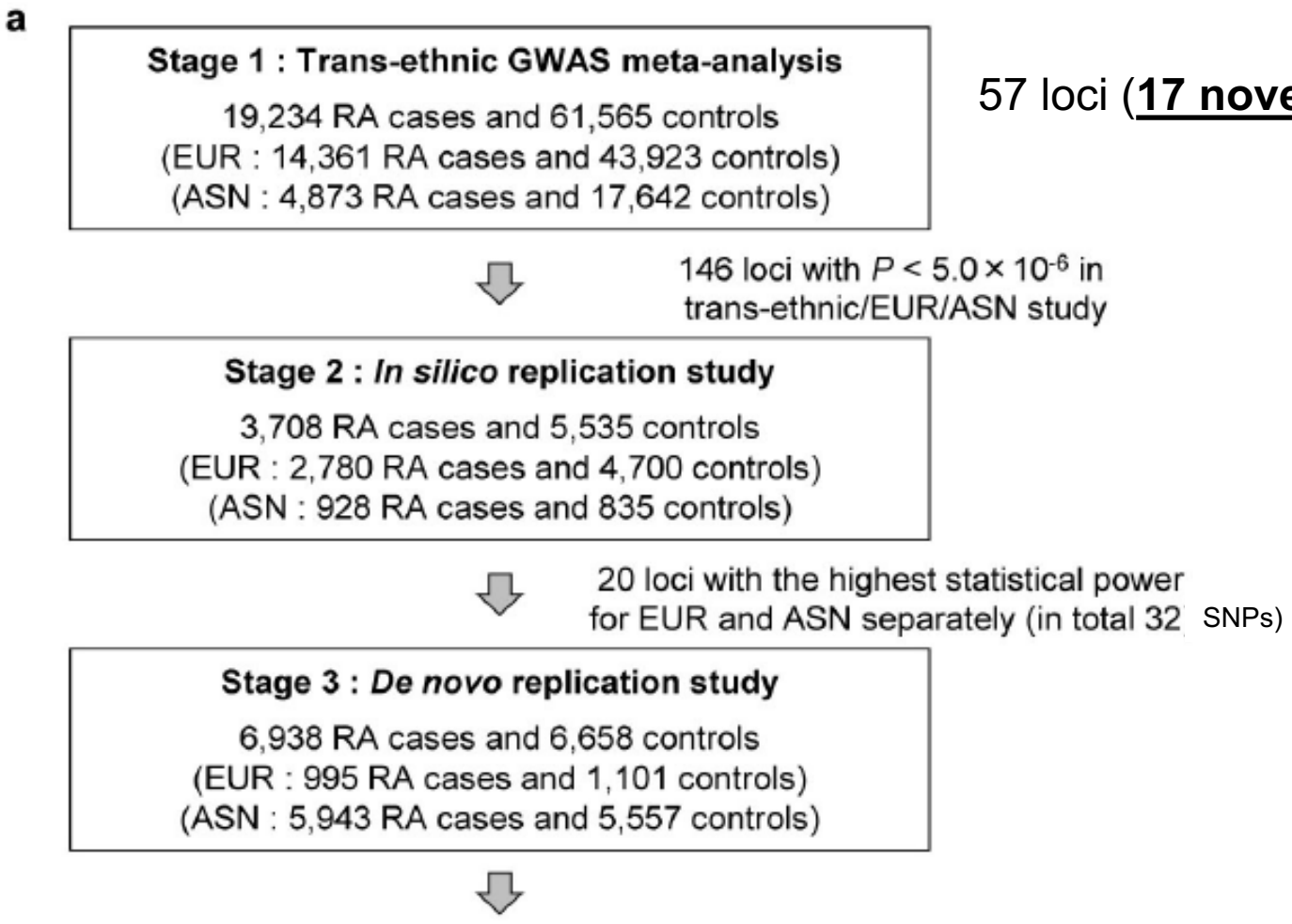

Digestive system disease
Cardiovascular disease
Metabolic disease
Immune system disease
Nervous system disease
Liver enzyme measurement
Lipid or lipoprotein measurement
Inflammatory marker measurement
Hematological measurement
Body measurement
Cardiovascular measurment
Other measurement
Response to drug
Biological process
Cancer
Other disease
Other trait

**www.genome.gov/GWAStudies**
**www.ebi.ac.uk/fgpt/gwas/**

National Human Genome Research Institute

EMBL-EBI

# Study Design

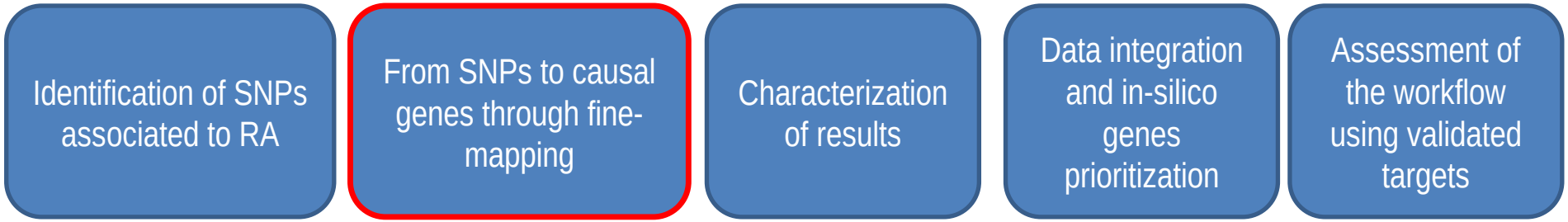



57 loci (**17 novel**) pval<10-8

Combining 1-3: **42** novel loci with $P < 5 \times 10^{-8}$

*Nature. 2014 Feb 20;506(7488):376-81*

**100 Total RA risk loci (58 known + 42 novel), including 377 genes**


Identification of SNPs associated to RA
From SNPs to causal genes through fine-mapping
Characterization of results
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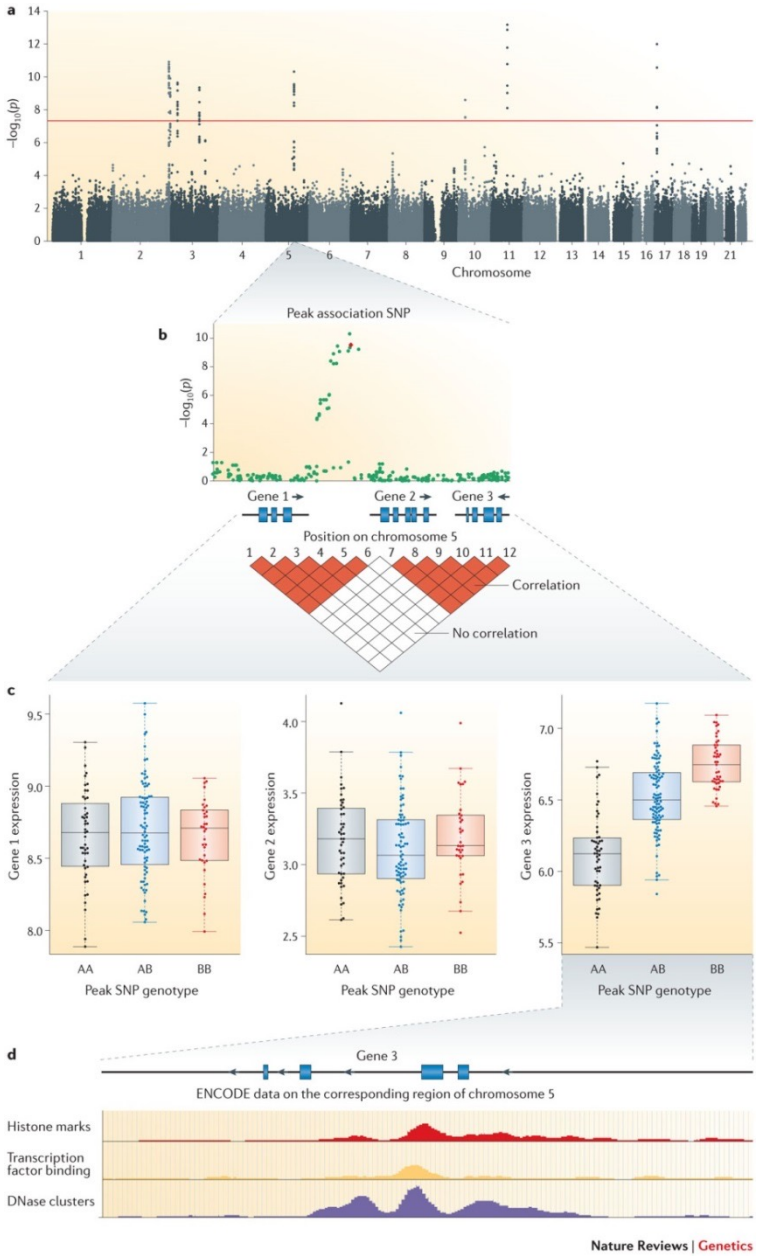
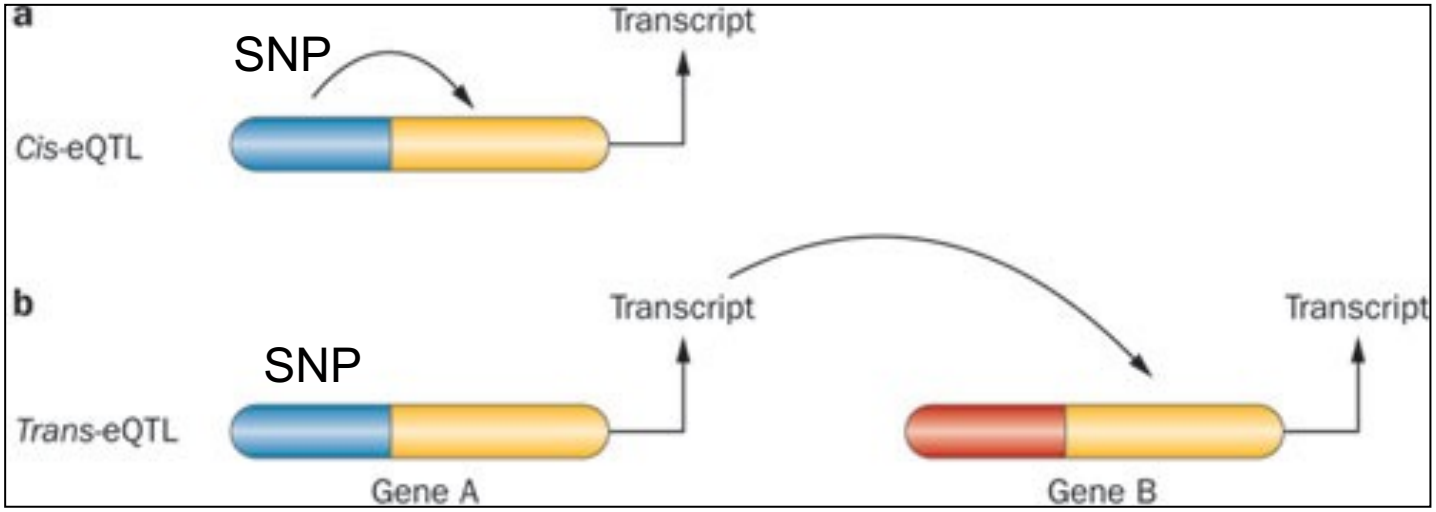
Assessment of the workflow using validated targets

# Fine-Mapping: overlaying different information to identify causal genes

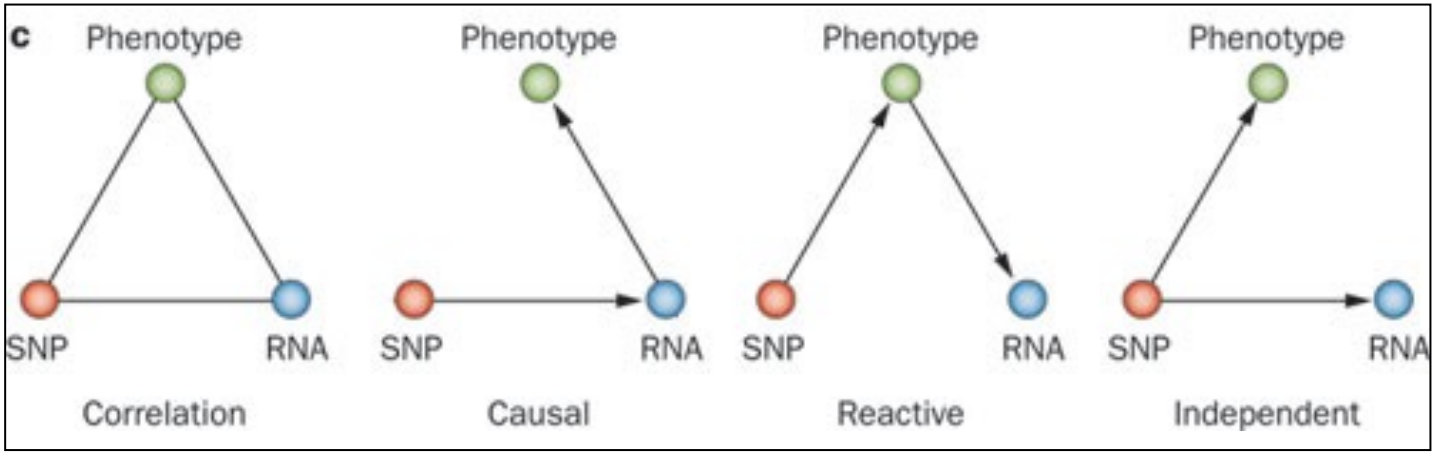
*M. Civelek & A. J. Lusis – Nat. Rev. Genetics (2013)*



# Combining DNA and RNA information – eQTLs and Causal Networks



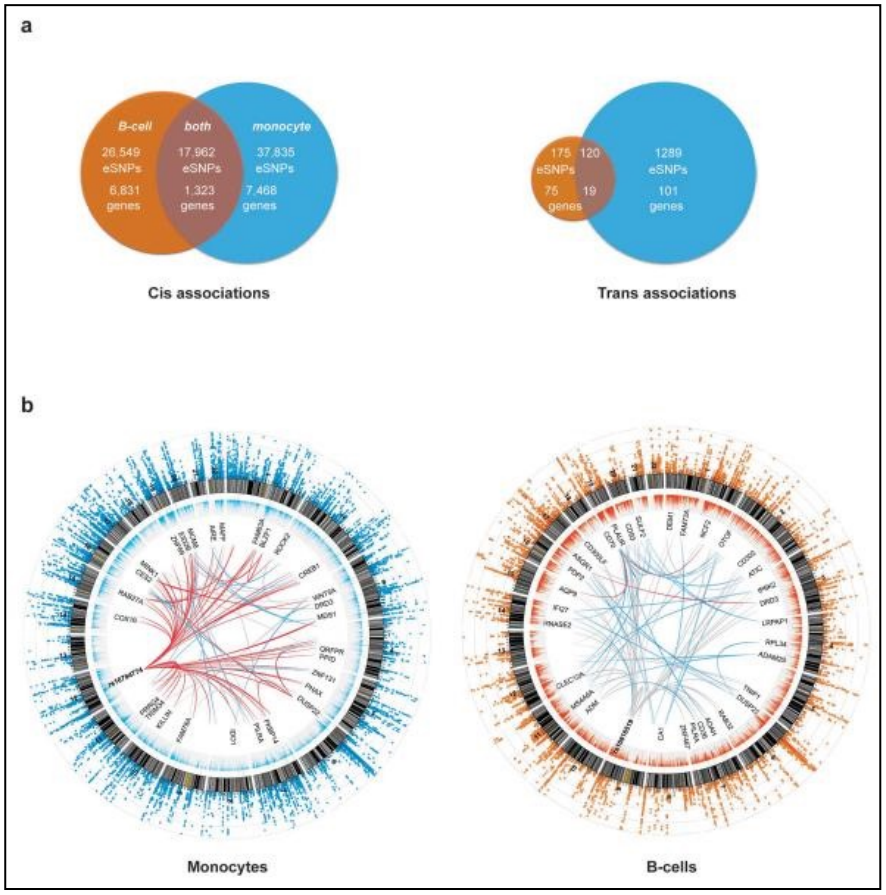

- DNA/RNA combined analysis can highlight eSNPs
- eSNPs exert an effect on genes' transcription

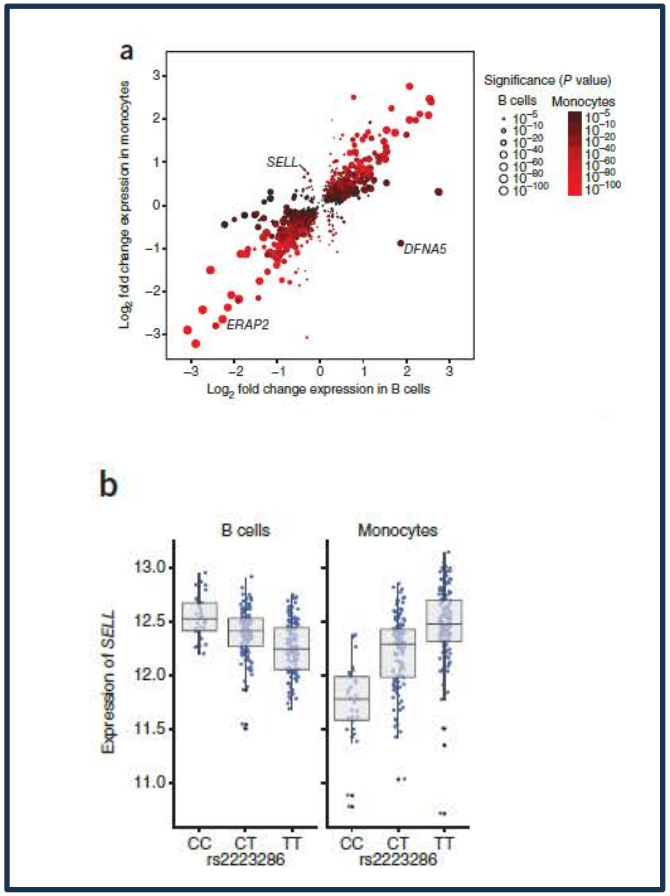



Using the SNP as a 'causal anchor', causal relationships between the three can be modelled: Causal Networks

MacLellan WR, et al. Nat Rev Cardiol. 2012

# Cell type-specific eQTLs in B-cell and monocytes



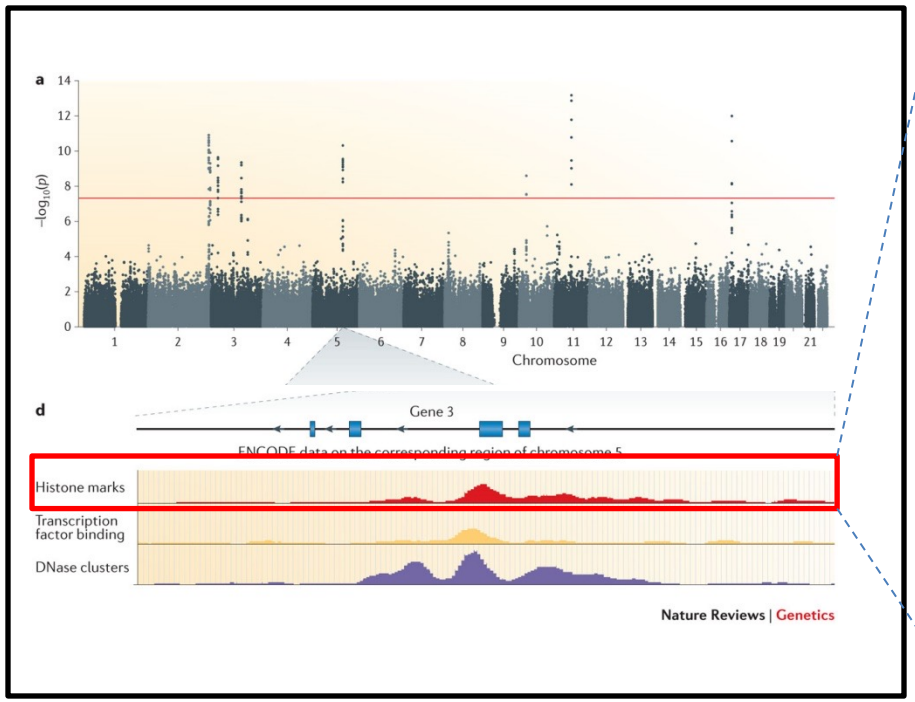




*Circos Plots*

[J. Knight et al. - Nat Genet.](#) *2012 Mar 25;44(5):502-10.*

# Assessment of enrichment of 100 non-MHC RNA risk loci in epigenetic chromatin marks

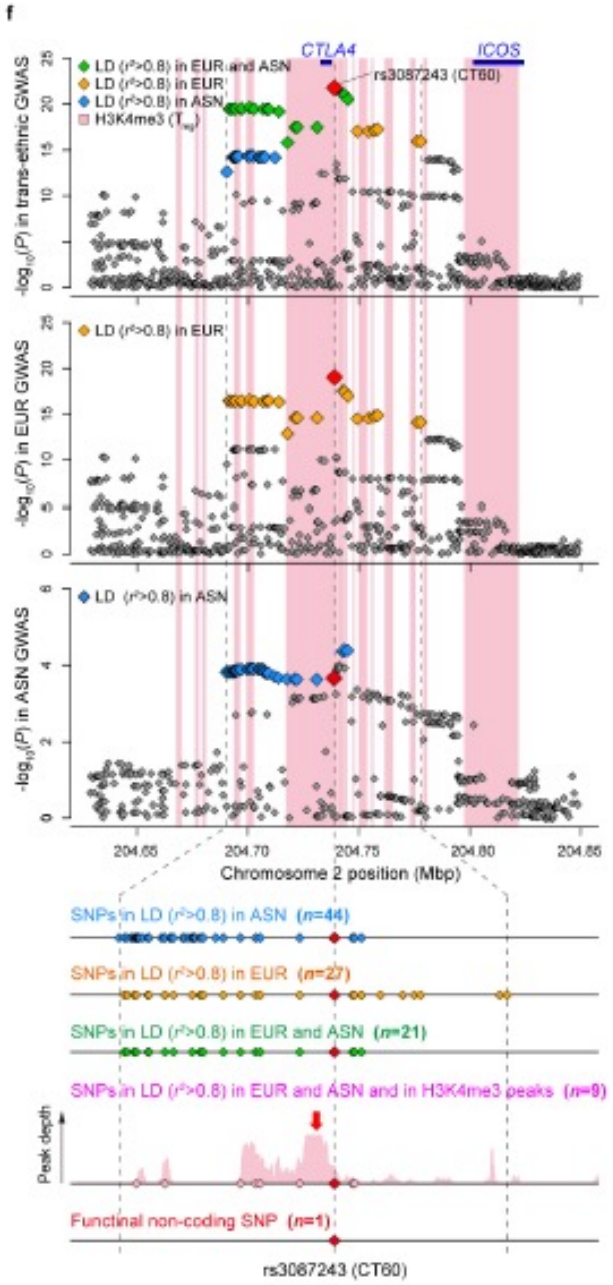
100 RA risk loci




d

| Cell types | *P* for H3K4me3 enrichment |
|---|---|
| $T_{reg}$ primary cells | $\leq 1.0\times10^{-5}$ |
| $CD4^+$ memory primary cells | $3.0\times10^{-5}$ |
| $CD4^+$ naive primary cells | 0.0041 |
| $CD8^+$ memory primary cells | 0.0065 |
| Smooth muscle, rectal | 0.034 |
| Mucosa, colon | 0.038 |
| $CD8^+$ naive primary cells | 0.12 |
| Mucosa, stomach | 0.13 |
| $CD34^+$ primary cells | 0.18 |
| $CD34^+$ cultured cells | 0.19 |
| Mobilized $CD34^+$ primary cells | 0.19 |
| $CD19^+$ primary cells | 0.24 |
| $CD3^+$ primary cells | 0.30 |
| Mucosa, duodenum | 0.40 |
| Muscle satellite cultured cells | 0.46 |
| Cingulate gyrus (brain) | 0.53 |
| Skeletal muscle | 0.77 |
| Mucosa, rectal | 0.77 |
| Smooth muscle, colon | 0.79 |
| Mesenchymal stem cells (adipose) | 0.81 |
| Adipose nuclei | 0.84 |
| Smooth muscle, duodenum | 0.85 |
| Mid frontal lobe (brain) | 0.86 |
| Hippocampus middle (brain) | 0.91 |
| Mesenchymal stem cells (bone marrow) | 0.91 |
| Pancreatic islets | 0.93 |
| Inferior temporal lobe (brain) | 0.93 |
| Substantia nigra (brain) | 0.93 |
| Adult kidney | 0.94 |
| Adult liver | 0.95 |
| Mesenchymal stem cells (adipocyte) | 0.98 |
| Mesenchymal stem cells (chondrocytes) | 0.99 |
| Anterior caudate (brain) | 0.99 |
| Smooth muscle, stomach | 0.99 |

# Example: Fine Mapping of CTLA4

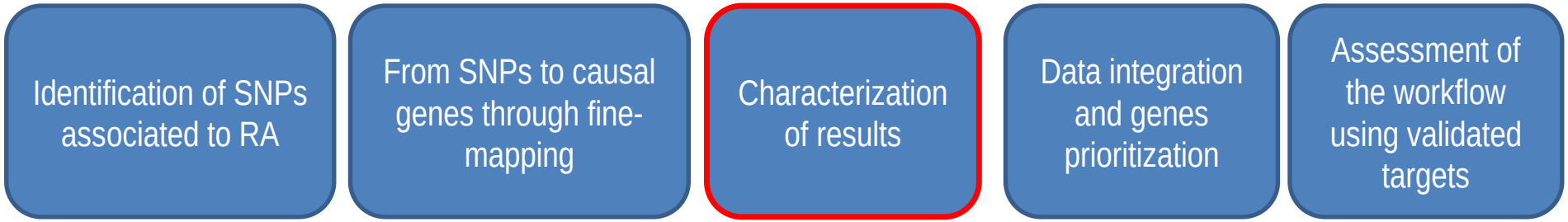



Regional (trans-ethnic, European, Asian) SNP associations of the CTLA4 locus in stage 1 GWAS meta-analysis

1. functional non-coding variant of CT60 (rs3087243) showed the most significant association with RA.

2. Trans-ethnic fine mapping of candidate causal variants decreased the number of candidate variants from 44 (LD in Asians) and 27 (LD in Europeans) to 21 (LD in both populations).

3. **Selected the 9 candidate variants included in Treg H3K4me3 peaks, including CT60 (close to H3K4me3 summit)**


Identification of SNPs associated to RA
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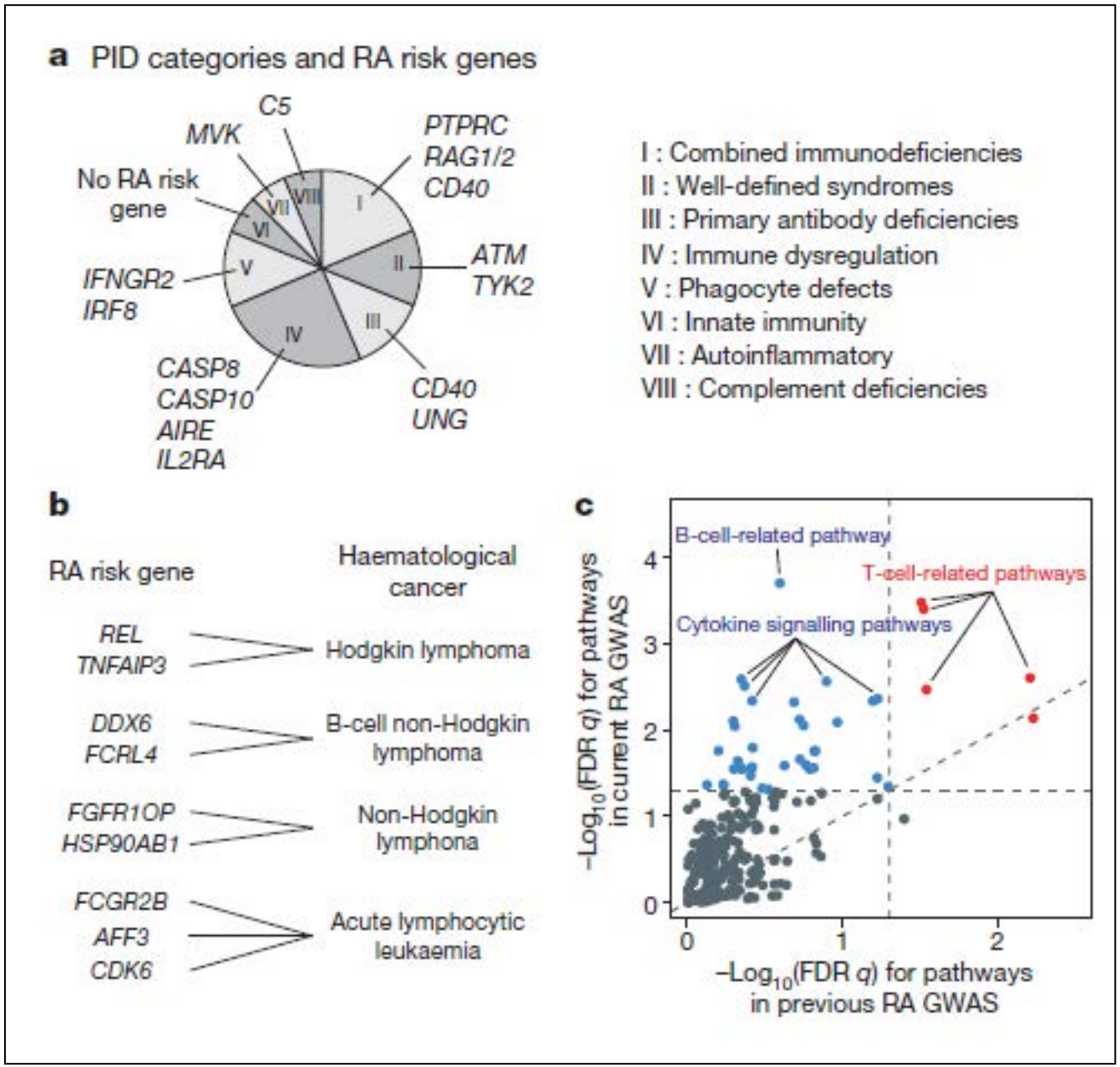
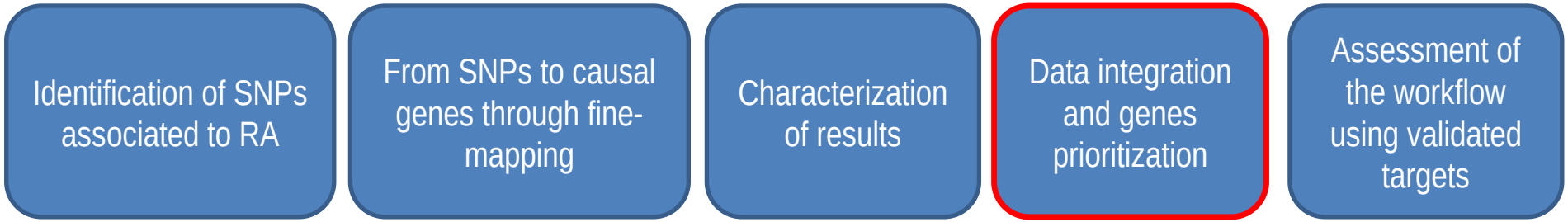
# Characterization of Results (100 loci, 377 genes)




Identification of SNPs associated to RA
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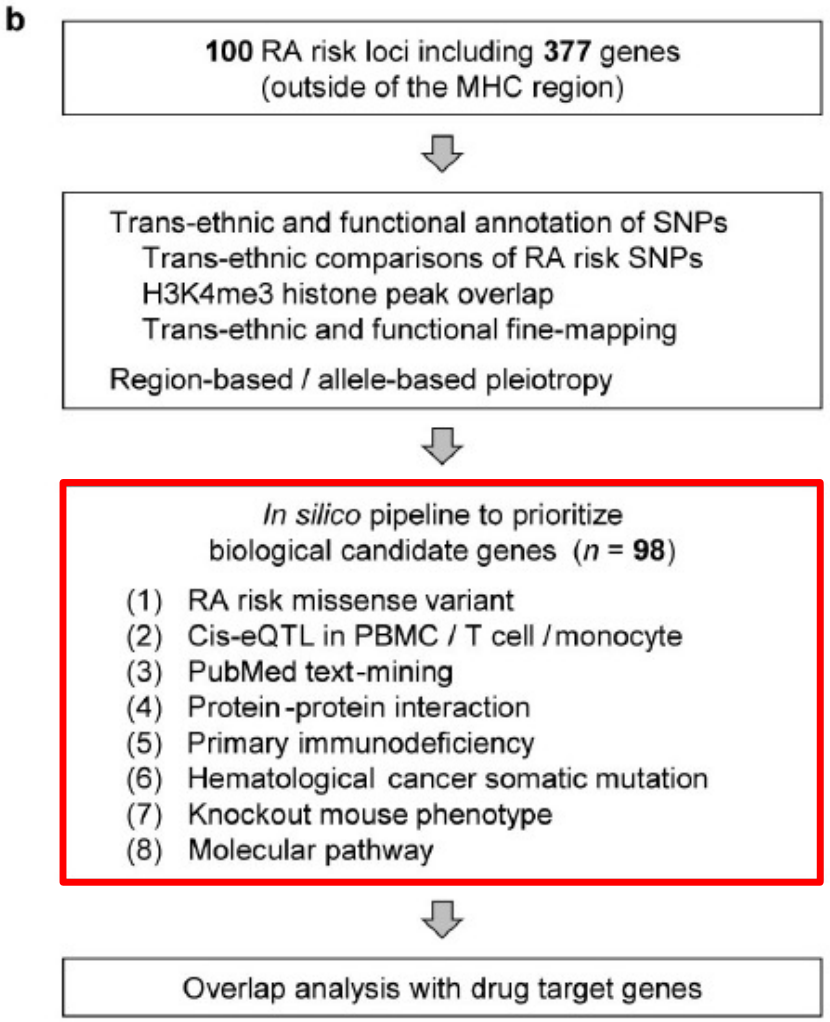
# *In-silico* genes prioritization




*Nature. 2014 Feb 20;506(7488):376-81*

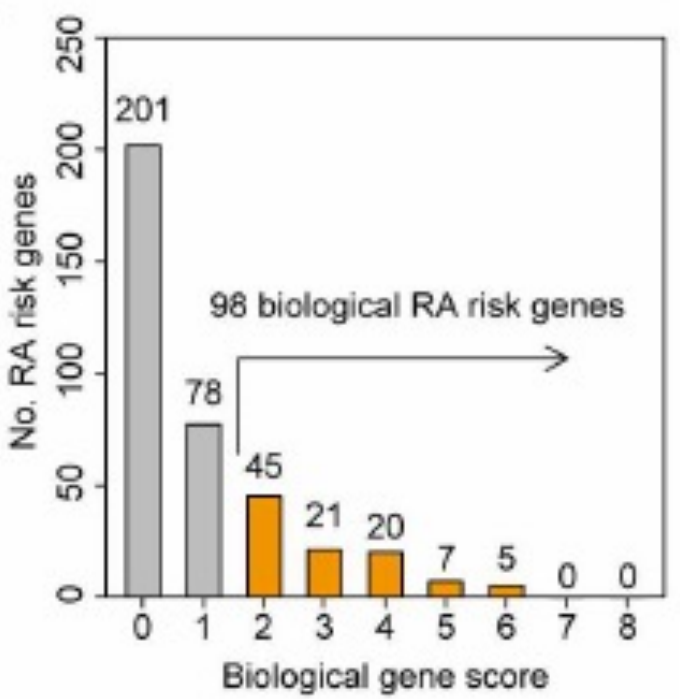
# Prioritization of biological candidate genes from RA risk loci

a

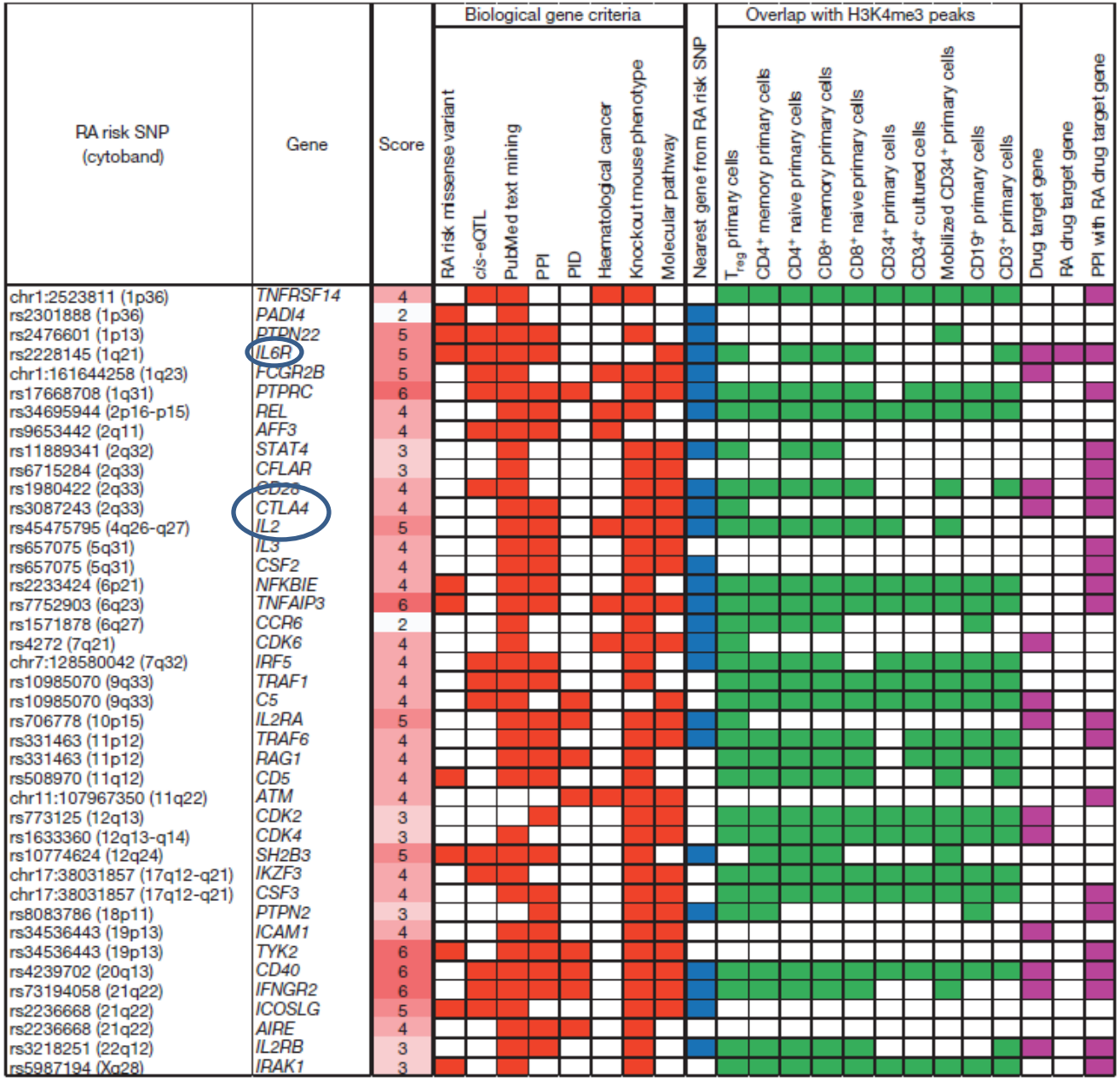
**Biological RA risk gene prioritization criteria**

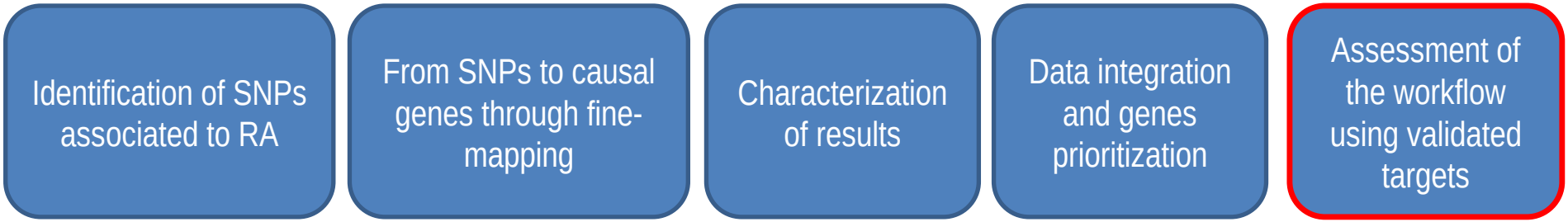
(1) RA risk missense variant ($n$ = 19)
(2) Cis-eQTL ($n$ = 51)
(3) PubMed text-mining ($n$ = 90)
(4) Protein-protein interaction ($n$ = 63)
(5) Primary immunodeficiency ($n$ = 15)
(6) Hematological cancer ($n$ = 17)
(7) Knockout mouse phenotype ($n$ = 86)
(8) Molecular pathway ($n$ = 35)

b



# Data Integration and Gene Prioritization




Identification of SNPs associated to RA
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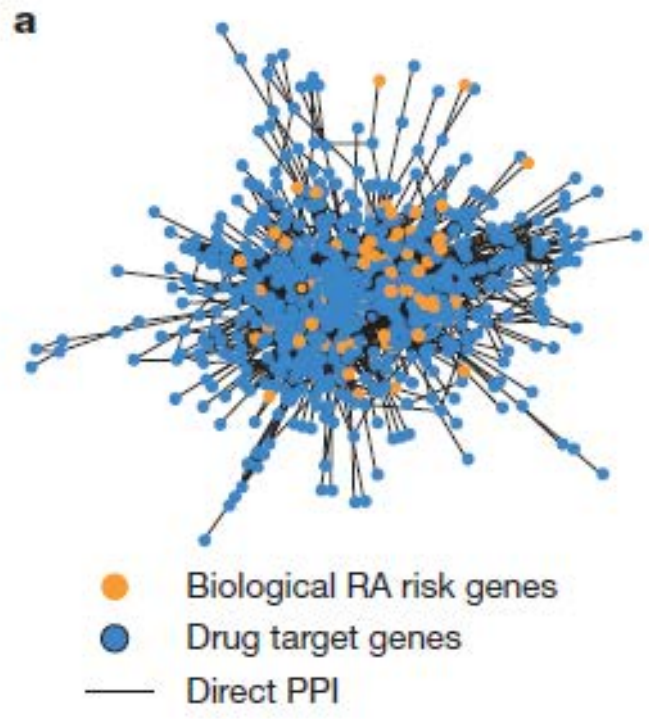
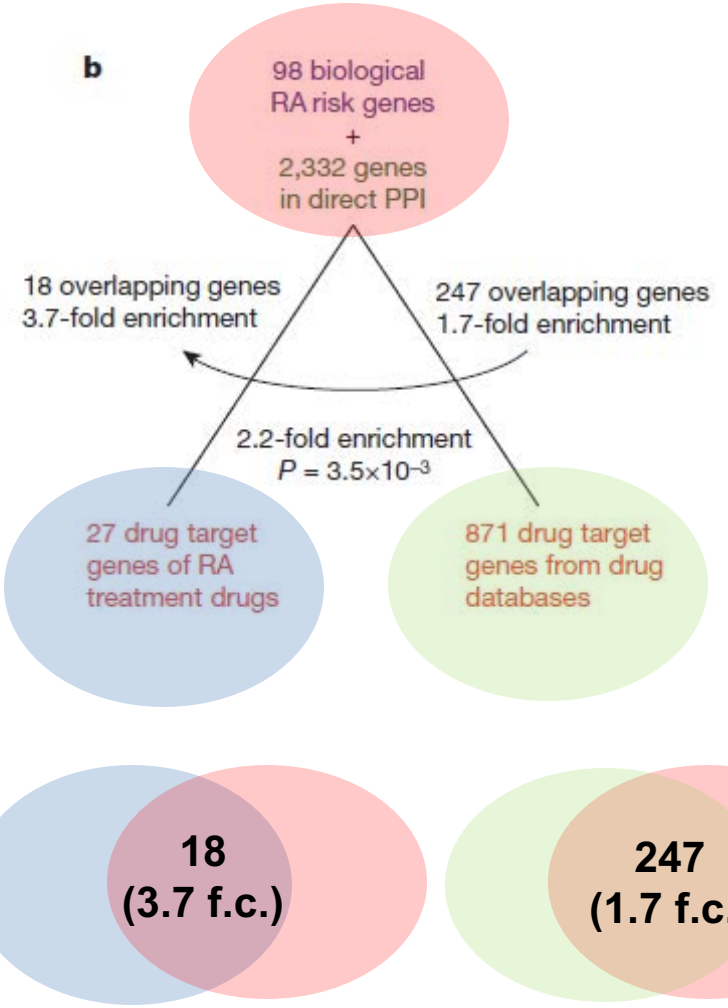
# Assessment of the workflow using validated targets

- 98 biological RA risk genes (score >=2)
- +2.322 genes in PPI

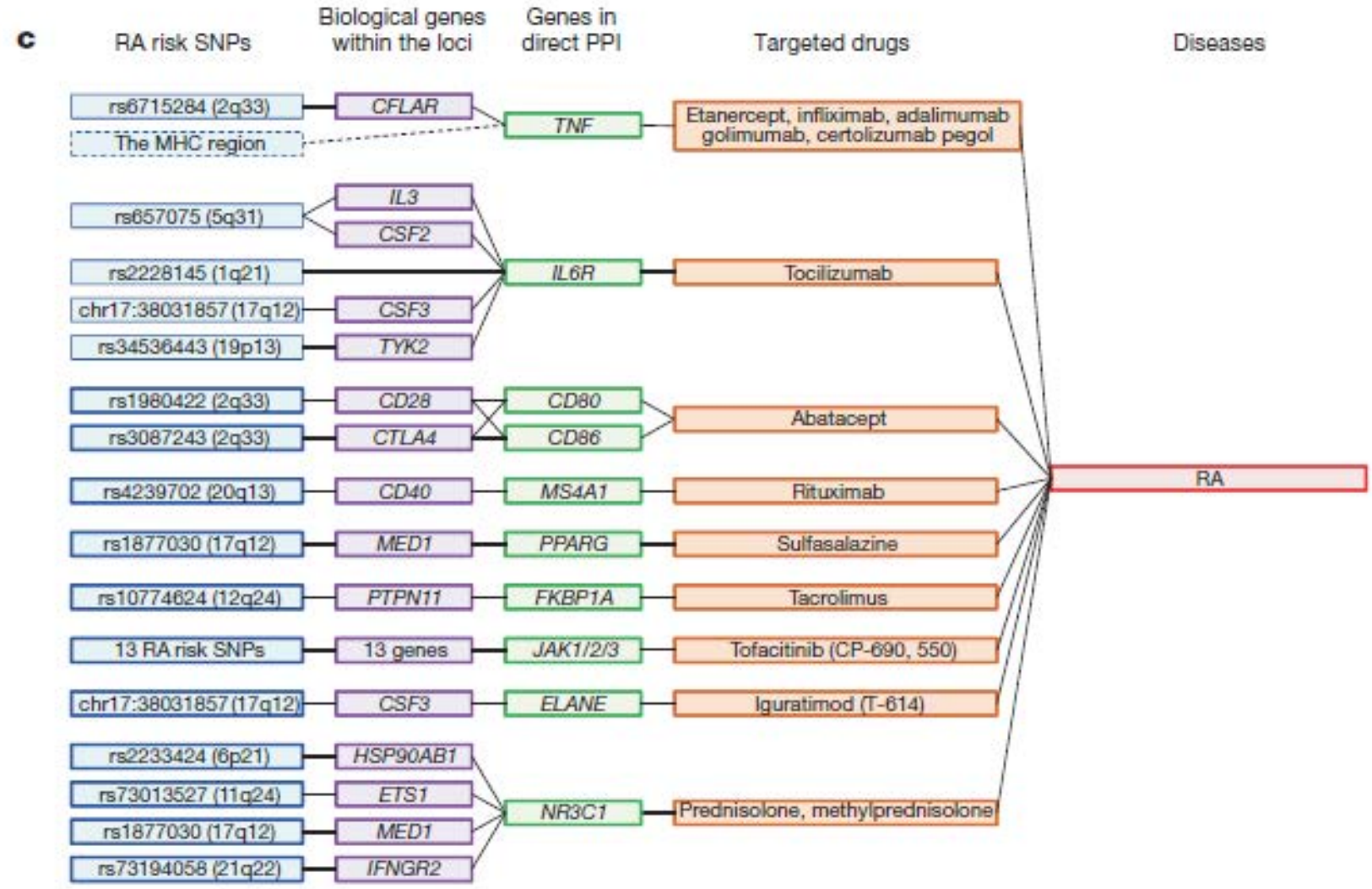



➔ Strong enrichment for RA approved drugs (extracted from DrugBank TherapeuticTargets Database - TTD)

# Mapping RA risk SNPs to drug targets

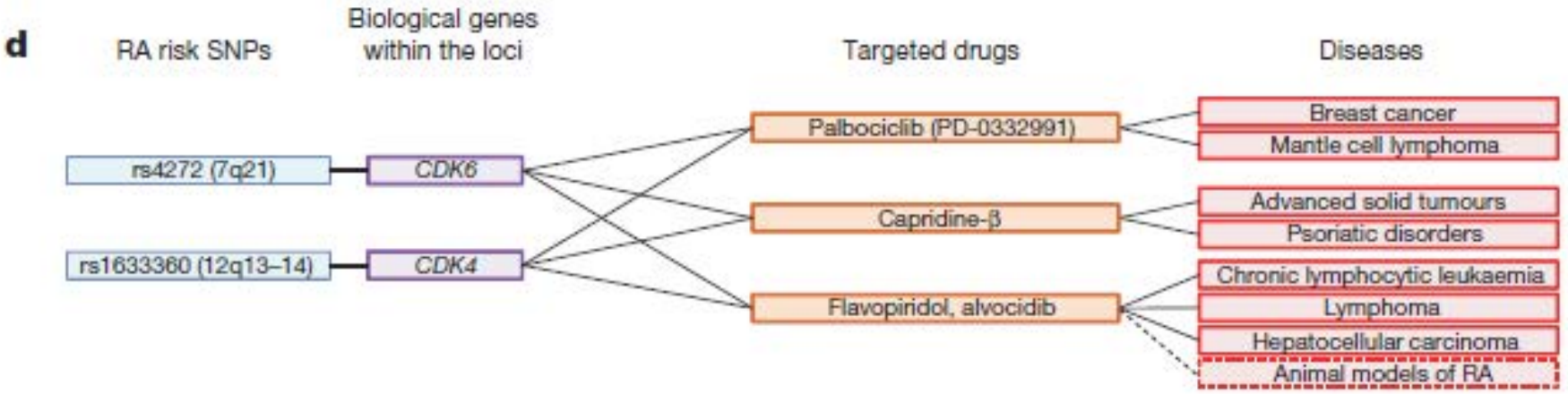
Example: PPI link to known RA drugs




- 98 biological RA risk genes (score >=2)

# Drug Repurposing

Connections between RA genes and drugs indicated for other diseases



# Study Summary

- Comprehensive genetic study with.100,000 subjects:
  - identified 42 novel RA risk loci
  - provided novel insights into RA pathogenesis.
  - demonstrated role of genetics for drug discovery

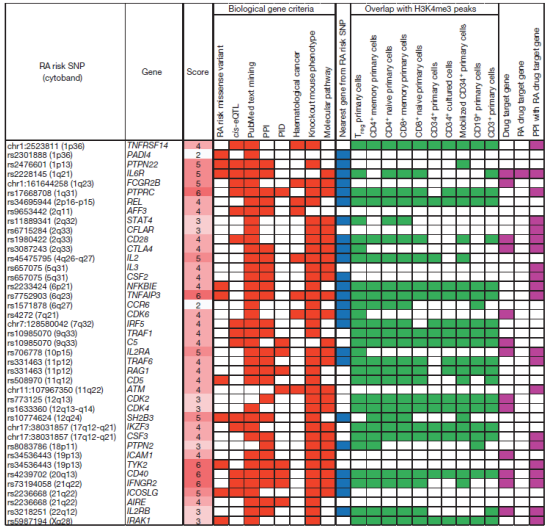
- Systematic approach to derive disease biological insights and novel drug candidates by integrating human genetic data with different layers of orthogonal information

# Take Home Messages

- GWAS can be used to identify candidate regions and genes associated with risk of RA
- Testing millions of hypotheses implies hunting for very low p-values
- Using a series of strategies and additional data to understand the functions of associated genes to disease and prioritize them as possible targets
  - Epigenetics
  - PPI Network analysis
  - Link genetics to intermediate phenotypes (e.g. eQTLs)
- Enrichments for existing RA and other drugs supports the pipeline
- If new candidate RA genes can be targeted by existing drugs, drug repositioning opportunities can be evaluated

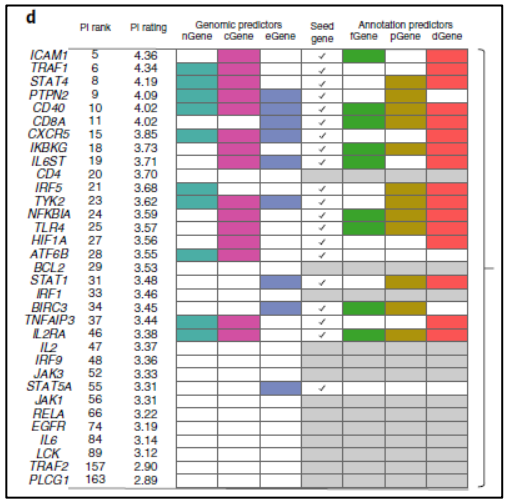
# Similar Approaches to Data Integration

**Genetics, functional genomics, network connectivity. RA**




*R. Plenge Nature 2014*

**Genetics, functional genomics, immune-related annotations, network connectivity. 30 immune traits**




*J.Knight Nat Genetics 2019*

**Genetics, animal models, text-mining, druggability, animal models, text mining, pathways. All diseases**


*Open Targets*

https://www.targetvalidation.org/

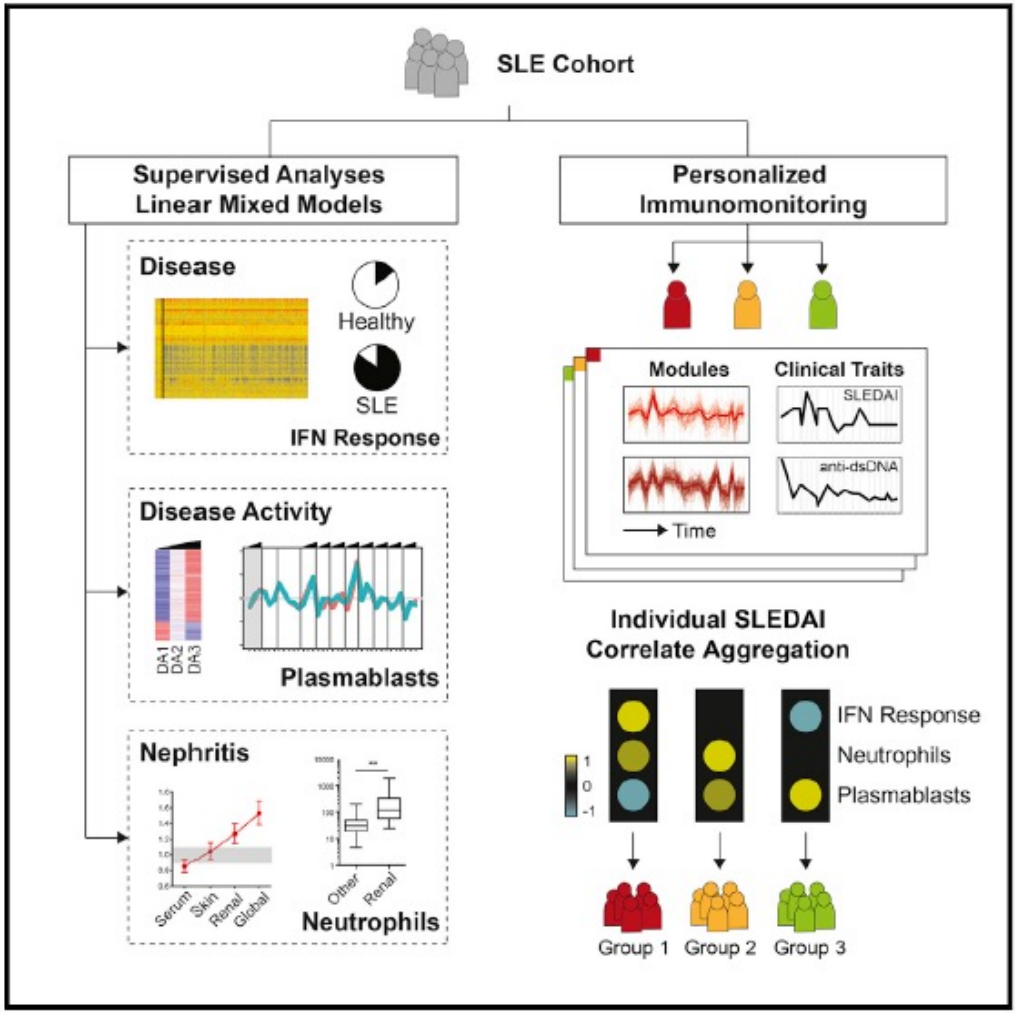
# SLE Molecular Immune Monitoring – Study Workflow

## Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients

Romain Banchereau,[1,7] Seunghee Hong,[1,7] Brandi Cantarel,[1] Nicole Baldwin,[1] Jeanine Baisch,[1] Michelle Edens,[1] Alma-Martina Cepika,[1] Peter Acs,[1] Jacob Turner,[1] Esperanza Anguiano,[1] Parvathi Vinod,[1] Shaheen Khan,[2] Gerlinde Obermoser,[1] Derek Blankenship,[1] Edward Wakeland,[2] Lorien Nassi,[2,3] Alisa Gotte,[2,3,4] Marilynn Punaro,[2,3] Yong-Jun Liu,[1,5] Jacques Banchereau,[6] Jose Rossello-Urgell,[1] Tracey Wright,[2,3] and Virginia Pascual[1,3,*]

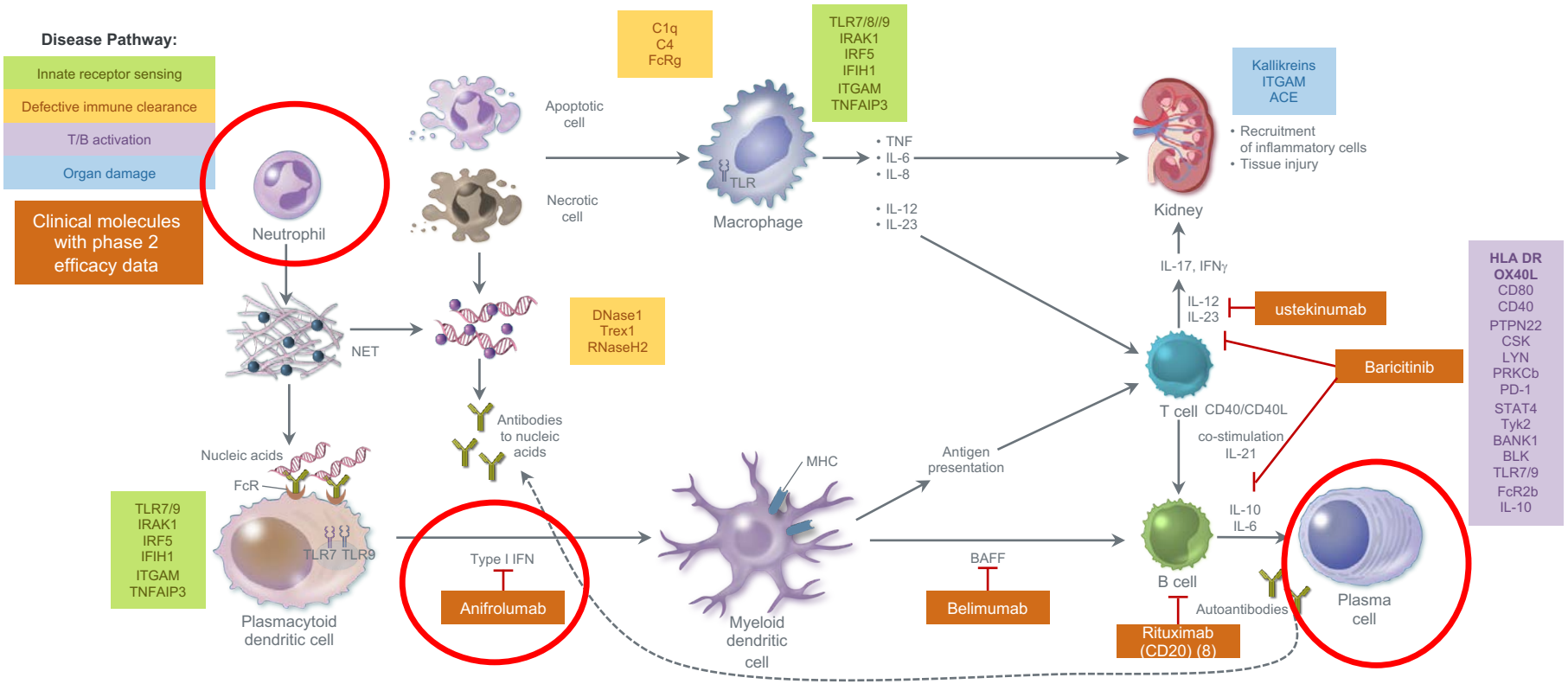
[1]Baylor Institute for Immunology Research, Dallas, TX 75204, USA
[2]UT Southwestern Medical Center, Dallas, TX 75235, USA
[3]Texas Scottish Rite Hospital for Children, Dallas, TX 75219, USA
[4]Vanderbilt University School of Medicine, Nashville, TN 37232, USA
[5]MedImmune, Gaithersburg, MD 20878, USA
[6]The Jackson Laboratory for Genomic Medicine, Farmington, CT 06030, USA
[7]Co-first author
*Correspondence: virginia.pascual@bswhealth.org
http://dx.doi.org/10.1016/j.cell.2016.03.008

Clinical and transcriptional profiling of 158 lupus pediatric patients, up to a period of 4 years




Cell. 2016 Apr 21;165(3):551-65

# Cells and Pathways Driving SLE

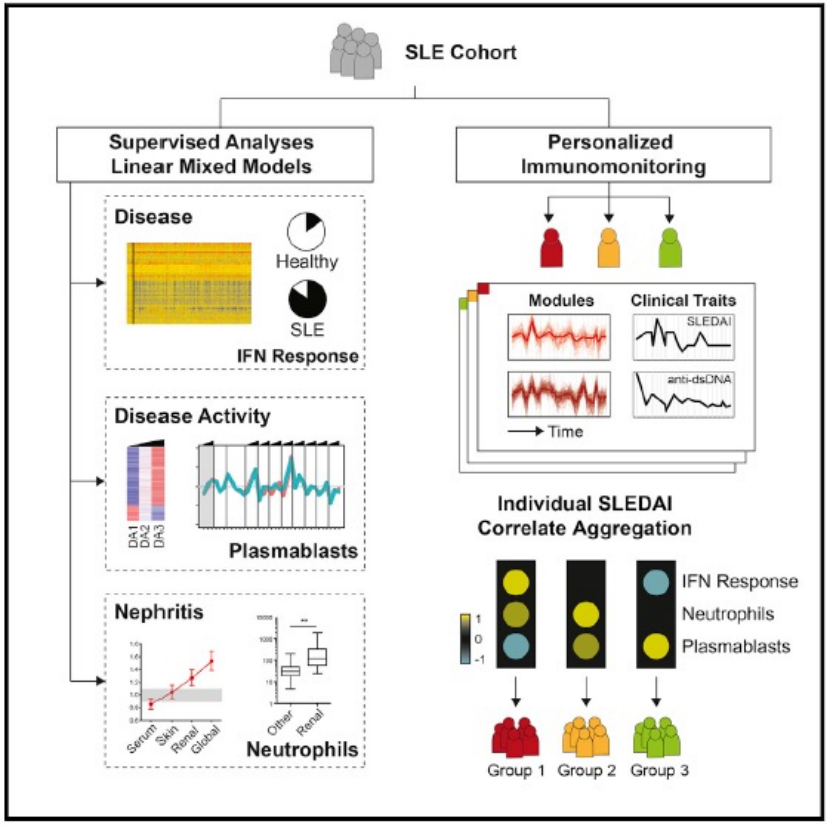





Lih-Ling Lin - Adapted from Tsokos et al., *Nat Rev Rheumatol*. 2016

SANOFI

# Analytical Study Workflow




Clinical and transcriptional profiling of 158 lupus pediatric patients, up to a period of 4 years

Genes associated to SLE, DA, Race, Treatment, disease subtypes

From genes to blood modules

WGCNA modules for each patient and correlation with SLEDAI

Linking WGCNA to blood modules

Stratification of Patients into Groups

# Toolbox

- **Gene Expression Analysis**
  - Multivariate linear regression modelling
  - Heat maps and Hierarchical Clustering
  - Aggregating gene expression through modules
    - WGCNA modules
    - Blood modules
  - Gene Set Enrichment Analysis

# SLE Blood Transcriptional Fingerprint

Genes associated to SLE, DA, Race, Treatment, disease subtypes

From genes to ”blood modules”

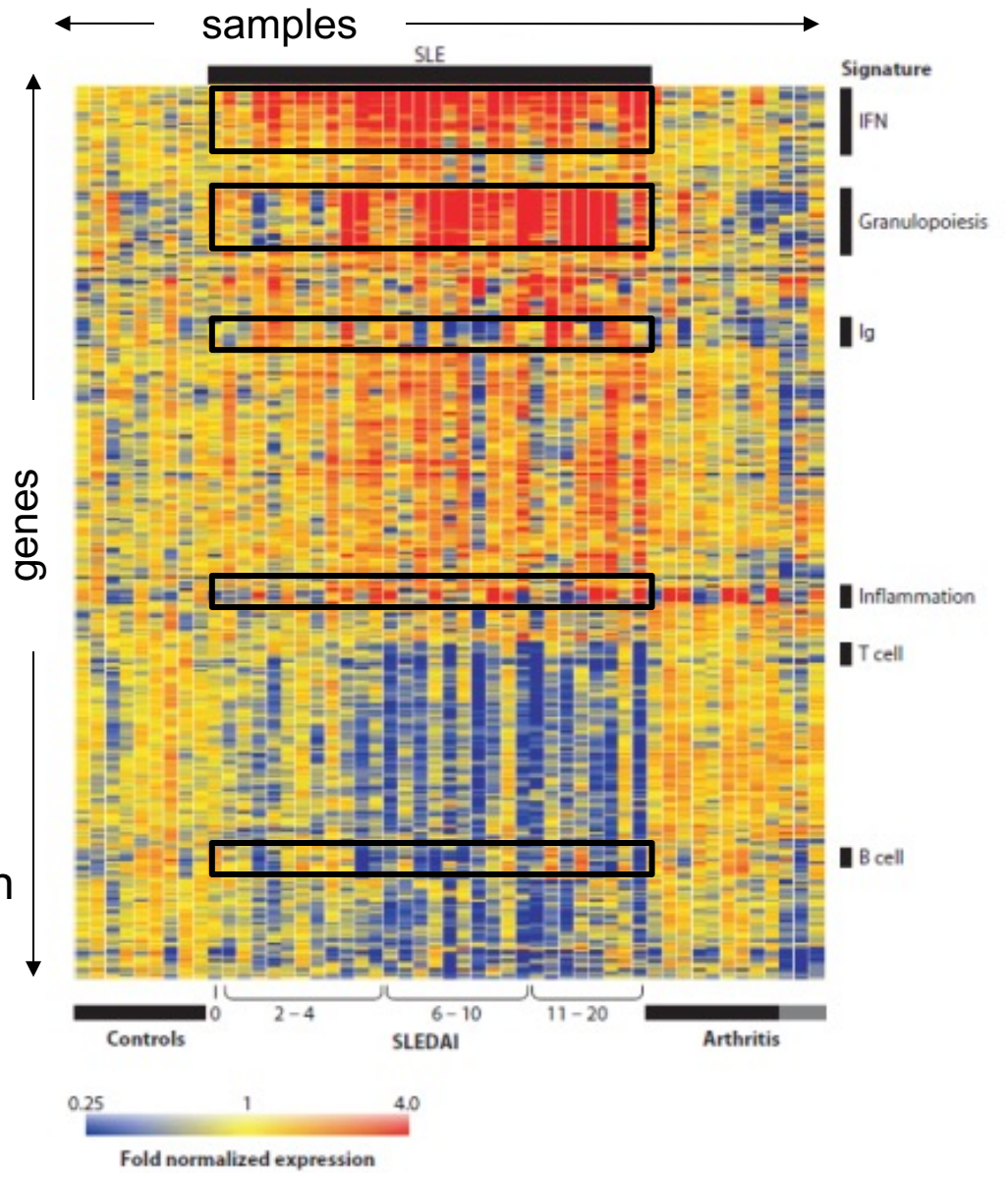
WGCNA modules for each patient and correlation with SLEDAI

Linking WGCNA to blood modules for inference of biological function

Stratification of patients into groups

# Gene Signatures

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
|---|---|---|---|---|---|---|
| Gene 1 | 1235 | 546 | 943 | 263 | 136 | 314 |
| Gene 2 | 1266 | 32 | 556 | 435 | 687 | 2718 |
| Gene 3 | 947 | 2829 | 389 | 3820 | 2039 | 1414 |
| Gene 4 | 392 | 2398 | 84 | 829 | 4392 | 512 |
| ... | ... | ... | ... | ... | ... | ... |




- Group of genes whose expression values, altogether, are associated with a given feature.
- Genes in signatures often show coordinated expression levels although this is not a requirement.

*Annu. Rev. Immunol. 2010. 28:535–71*

# Differential Expressed Genes: Multivariate Linear Model

Example: 2 groups – SLE and Healthy Controls

$$y_{gi} = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \ldots + \beta_p x_{pi} + \varepsilon_g$$

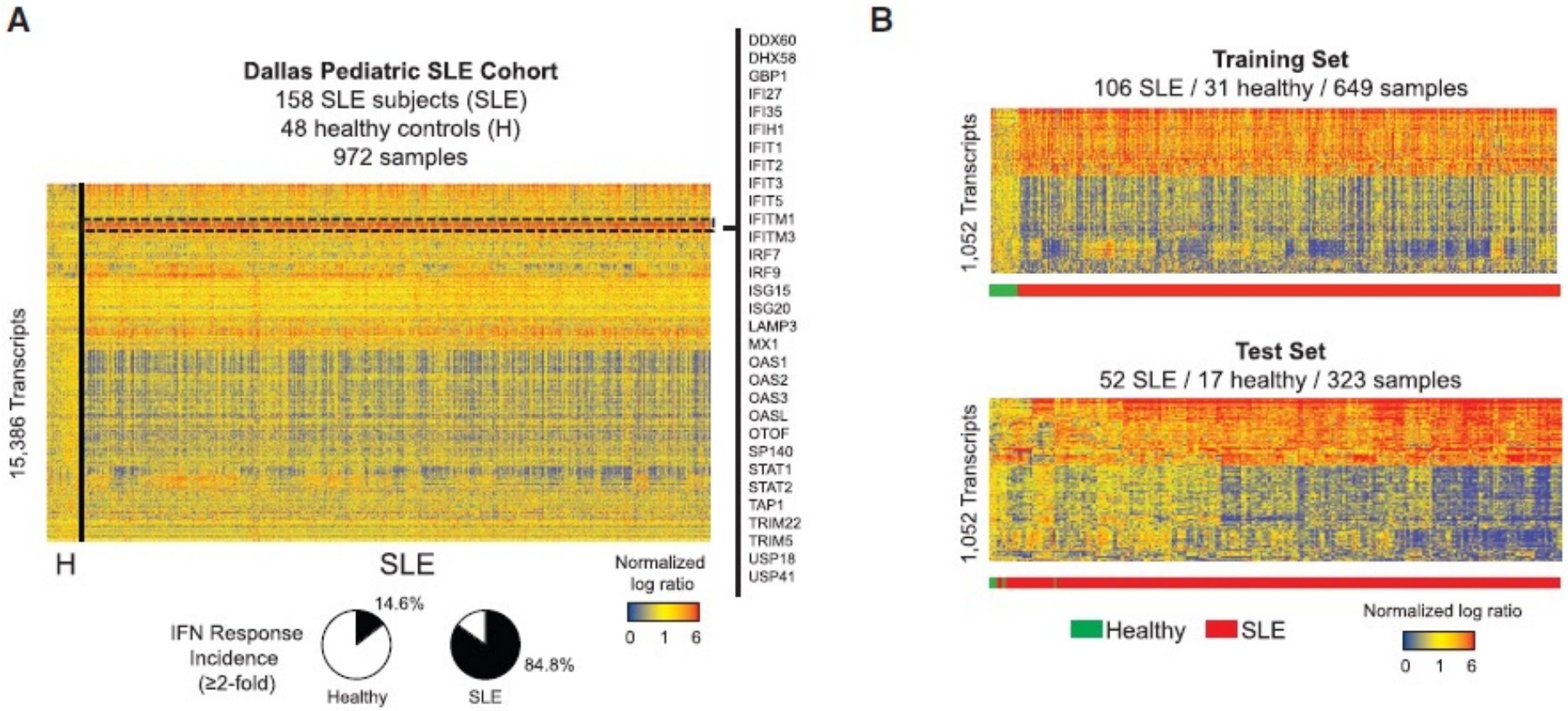
Explanatory Variables (e.g. SLE/Healty, Disease Activity, Treatment etc.)

Expression of gene A

- Values of B coefficients
- P-value of B coefficients being different than 0

# Genes Associated with SLE

Interferon response

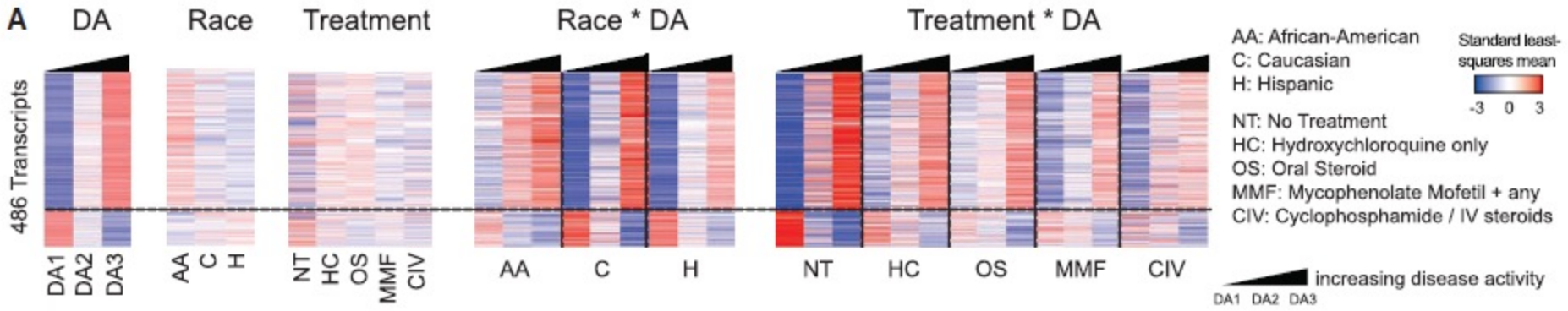



Cell. 2016 Apr 21;165(3):551-65



➔ 1.052 genes differentially expressed in SLE vs Healthy

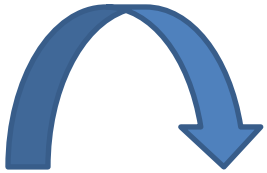
# Genes Associated With SLE Disease Activity




- 486 Transcripts Differentially Expressed between DA1 (SLEDAI: 0-2) and DA3 (SLEDAI >7)
- Results stratified by Race, Treatment

# SLE Blood Transcriptional Fingerprint

- % of genes up/down
- QuSage fold-change



Genes associated to SLE, DA, Race, Treatment, disease subtypes

From genes to “Blood Modules”

WGCNA modules for each patient and correlation with SLEDAI

Linking WGCNA to blood modules for inference of biological function

Stratification of patients into groups

# Building Transcriptional Blood Modules


Blood Samples (239)

Blood Module 1

Blood Module 2

Blood Module 3

239 Blood Samples:
- systemic juvenile idiopathic arthritis (n = 47)
- systemic lupus erythematosus (n = 40)
- type I diabetes (n = 20)
- metastatic melanoma (n = 39)
- acute infections (Escherichiacoli [n = 22]
- Staphylococcus aureus [n = 18], Influenza A [n = 16])
- liver-transplant recipients undergoing immunosuppressive therapy (n = 37).

260 blood modules identified

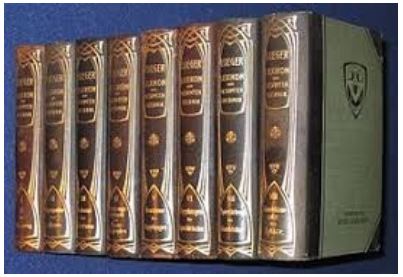


**Table 1. Functional Interpretation of Transcriptional Modules**

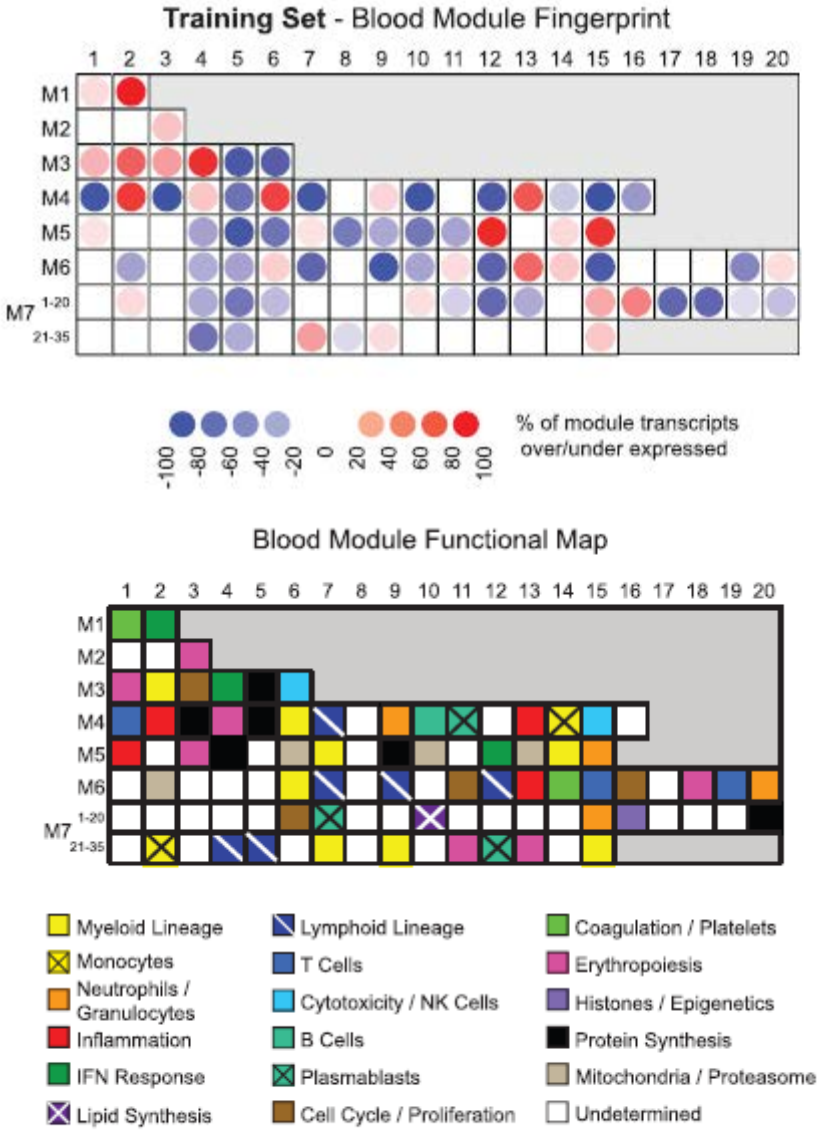
| Module I.D. | Number of Probe Sets | Keyword Selection | Interpretation |
|---|---|---|---|
| M 1.1 | 76 | Ig, Immunoglobulin, Bone, Marrow, PreB, IgM,Mu. | Plasma cells. Includes genes coding for Immunoglobulin chains (e.g., IGHM, IGJ, IGLL1, IGKC, IGHD) and the plasma cell marker CD38. |
| M 1.2 | 130 | Platelet, Adhesion, Aggregation, Endothelial, Vascular | Platelets. Includes genes coding for platelet glycoproteins (ITGA2B, ITGB3, GP6, GP1A/B) and platelet-derived immune mediators such as PPPB (pro-platelet basic protein) and PF4 (platelet factor 4). |

Immunity. 2008 Jul 18;29(1):150-64

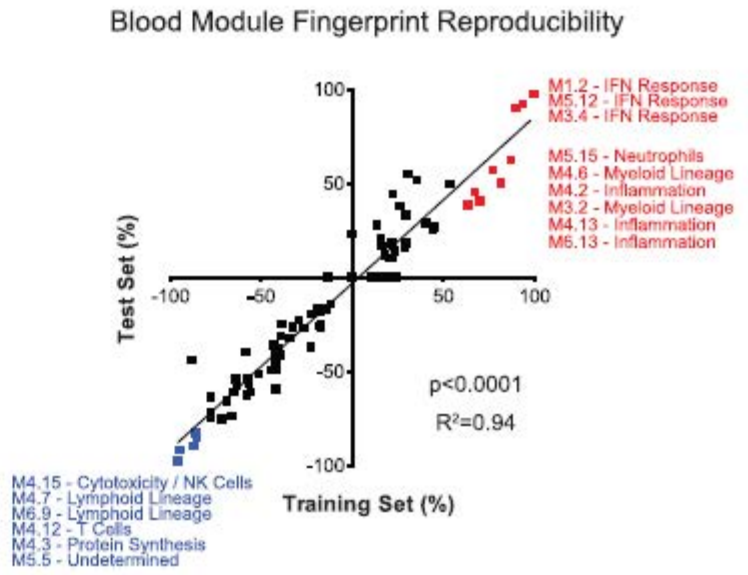
# SLE vs Healthy: From Genes to Modules

C

## Modules perturbed in SLE vs Healthy



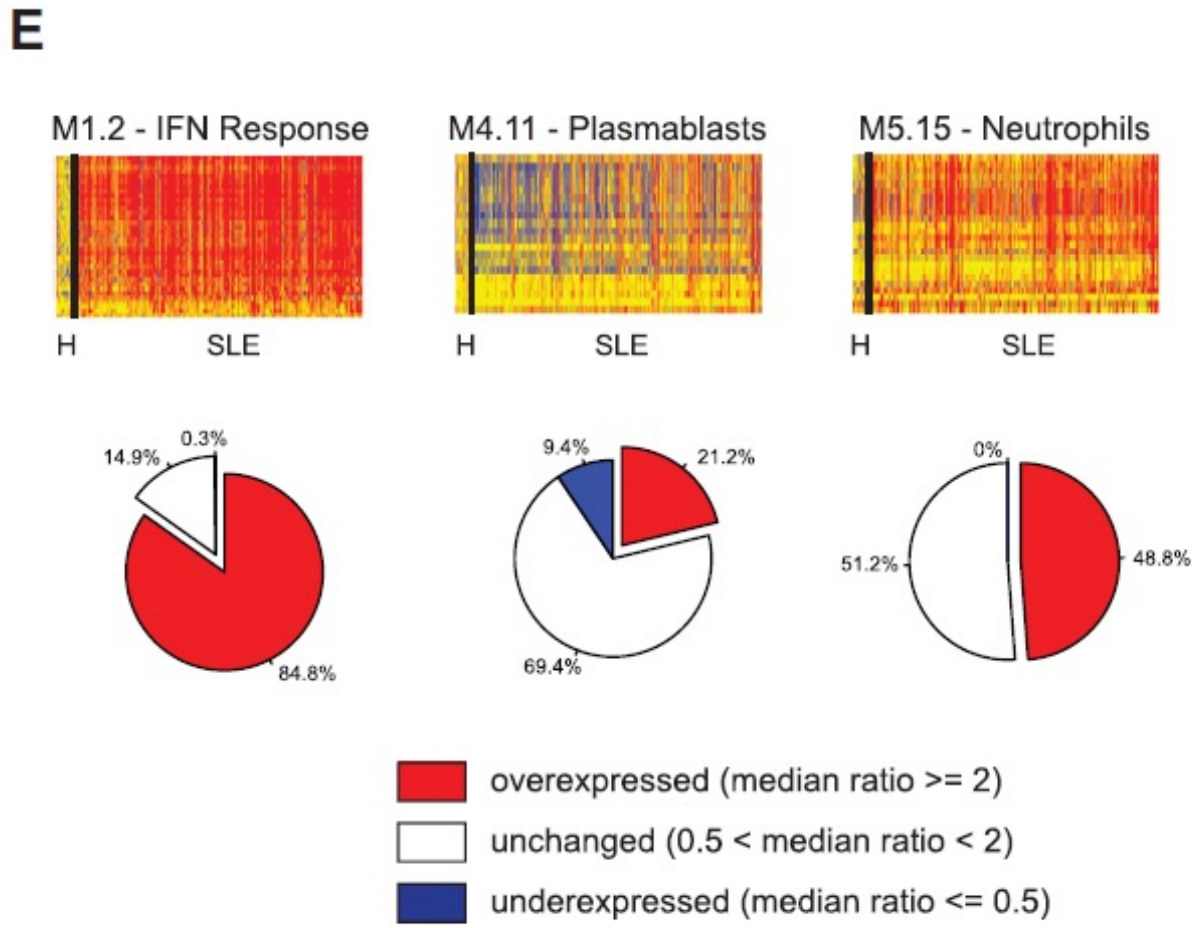

D




- **Over-expression of IFN response, neutrophil, inflammation, cell cycle, erythropoiesis, and histone modules.**
- **Down-regulation of NK cell/cytotoxicity, lymphoid lineage, B cells, T cells, and protein synthesis**

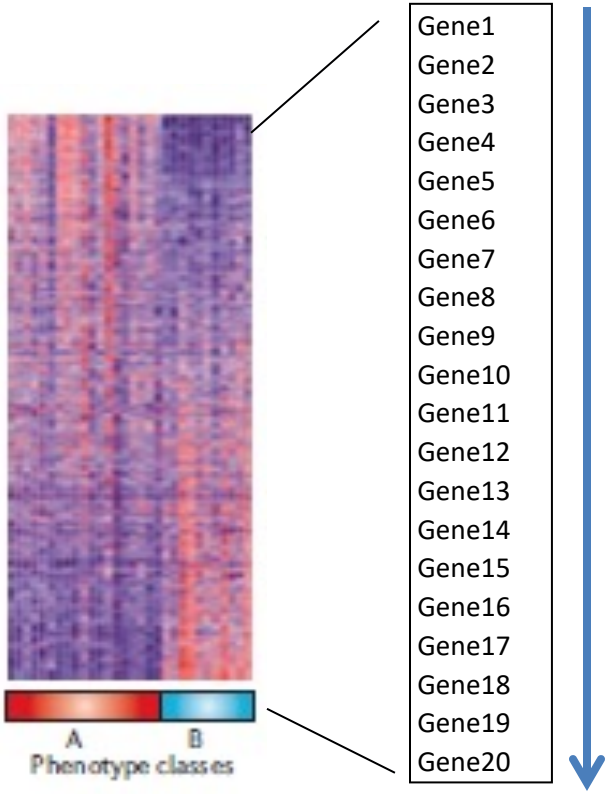
Cell. 2016 Apr 21;165(3):551-65

# Over-expression of IFN, plasmablast and neutrophil module genes

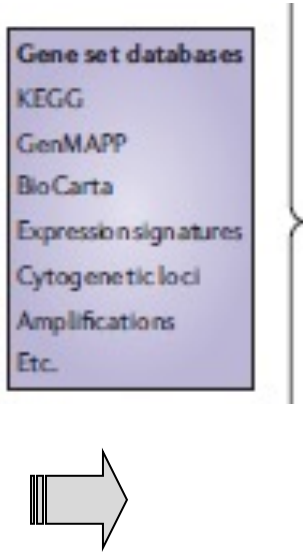


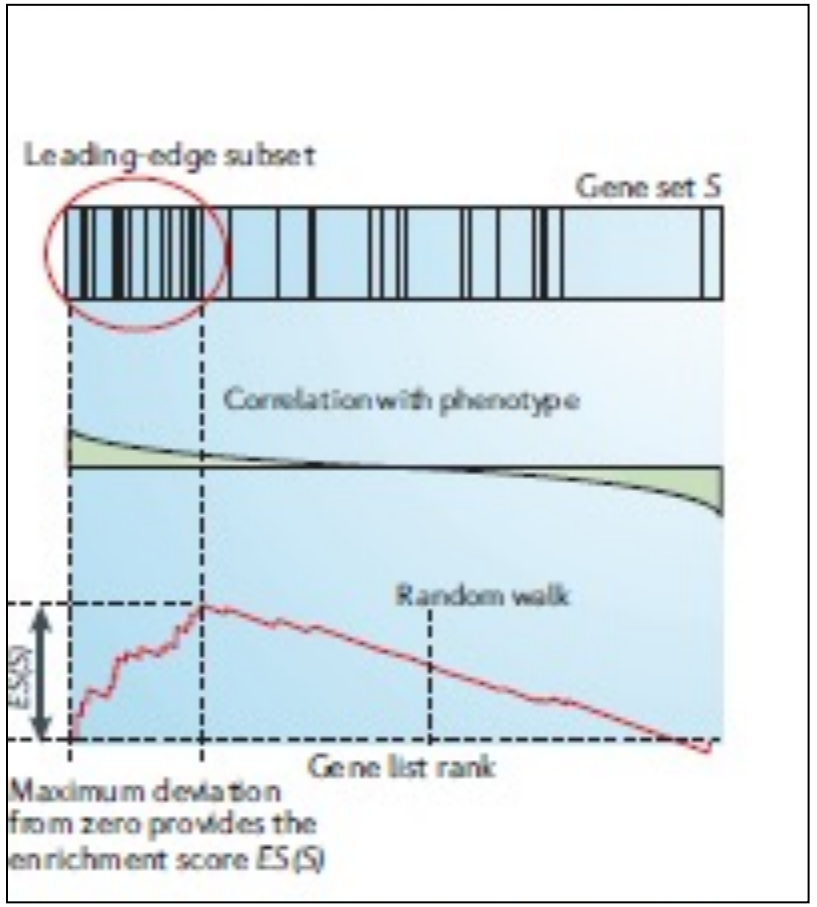

Cell. 2016 Apr 21;165(3):551-65

# Gene Set Enrichment Analysis (GSEA)




*Genes are ranked according to their fold-change*

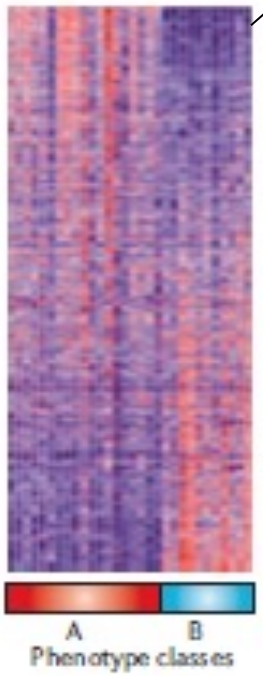



*An enrichment score is calculated for each pathway, taking into account the directionality of the input list*

*Aravind Subramanian et al, PNAS 2005*

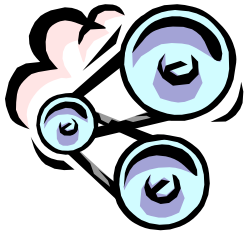
# From Gene Expression To Gene Set Scores




Gene1
Gene2
Gene3
Gene4
Gene5
Gene6
Gene7
Gene8
Gene9
Gene10
Gene11
Gene12
Gene13
Gene14
Gene15
Gene16
Gene17
Gene18
Gene19
Gene20

***List of perturbed genes (differentially expressed between class A and B)***

**Gene set databases**
KEGG
GenMAPP
BioCarta
Expression signatures
Cytogenetic loci
Amplifications
Etc.

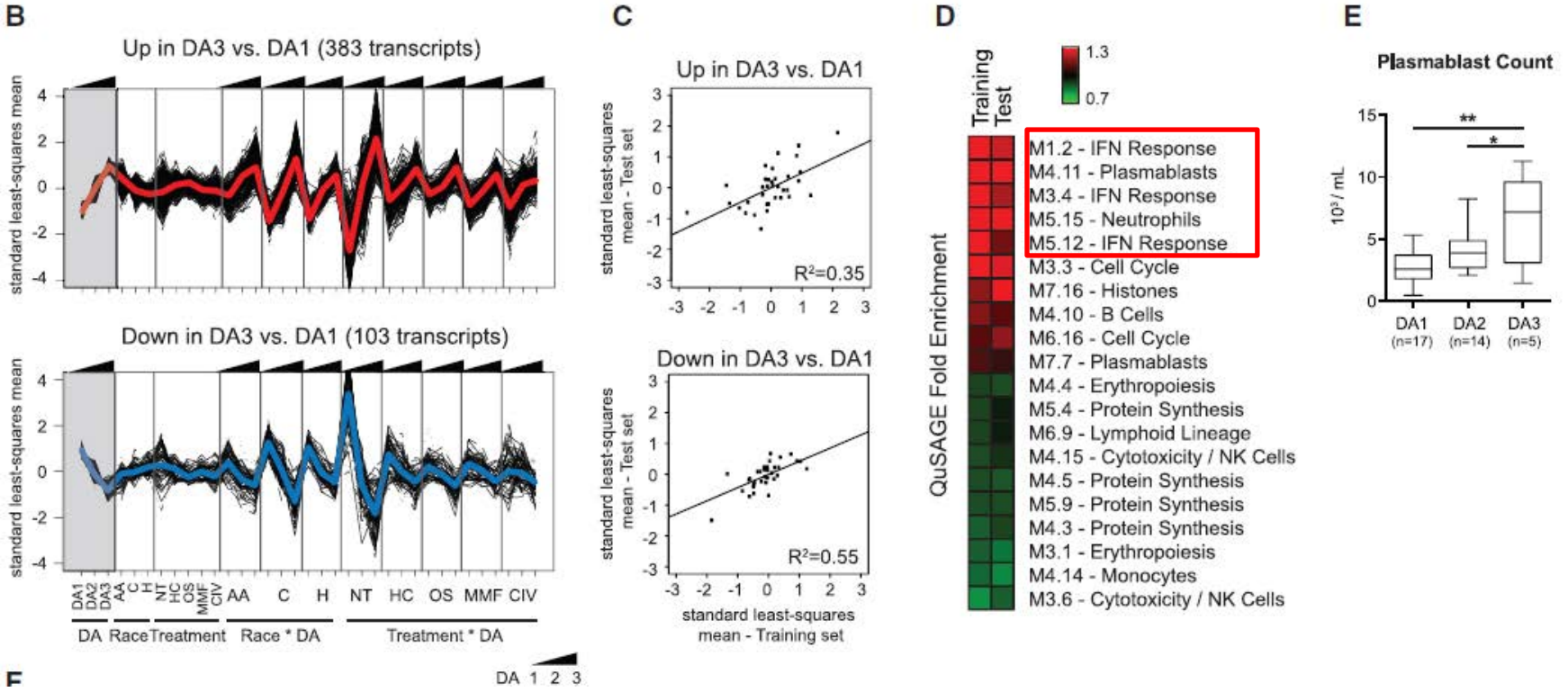


***Bioinformatics analysis using pathway DBs***

- **Apoptosis** (p-val=0.001)
- **Cell Cycle**(p-val=0.00004)

***List of perturbed pathways***

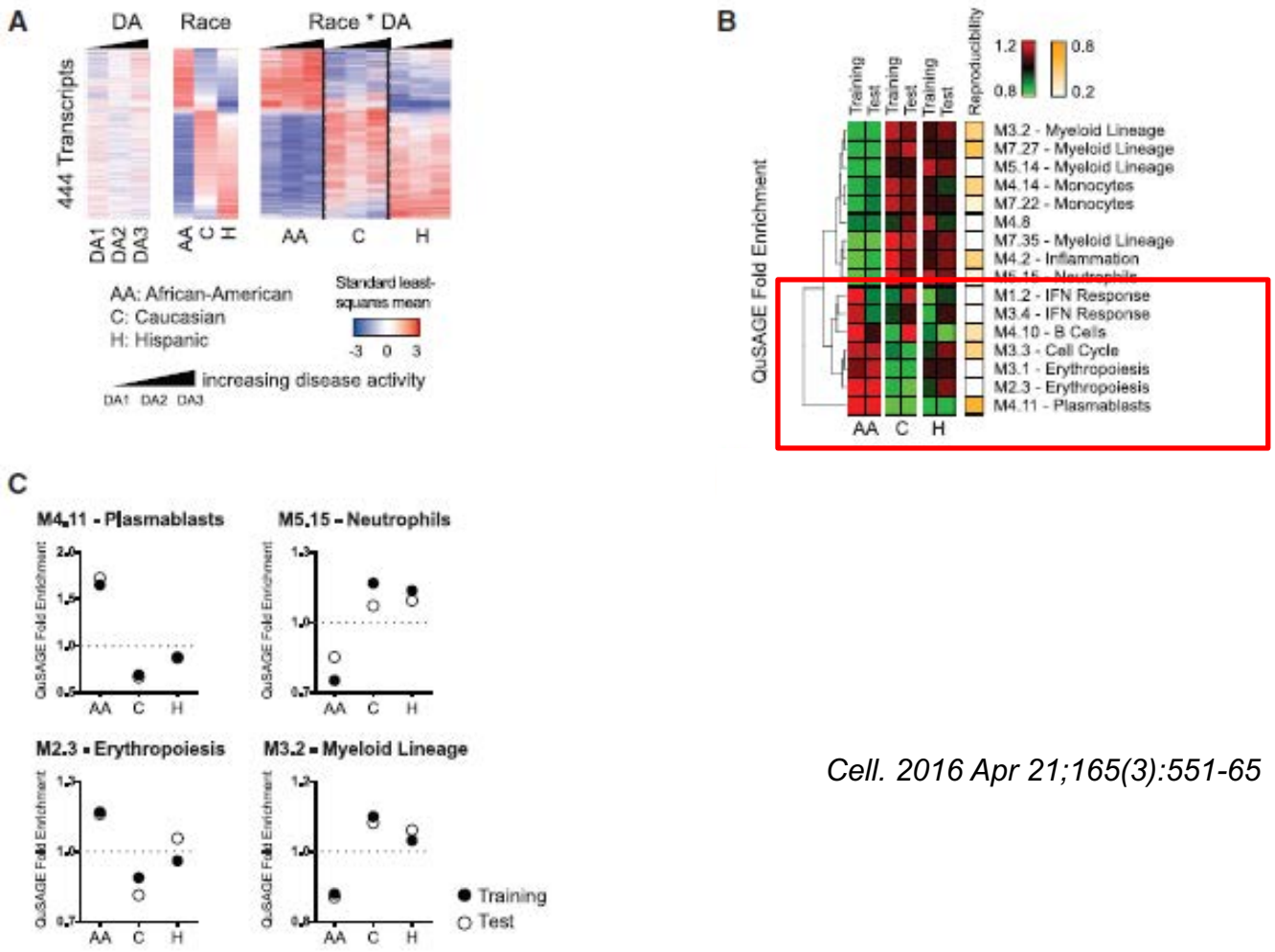
# From Genes to Modules Associated with Disease Activity




➔ Genes associated with DA are enriched for **IFN and Plasmablast modules**

*Cell. 2016 Apr 21;165(3):551-65*

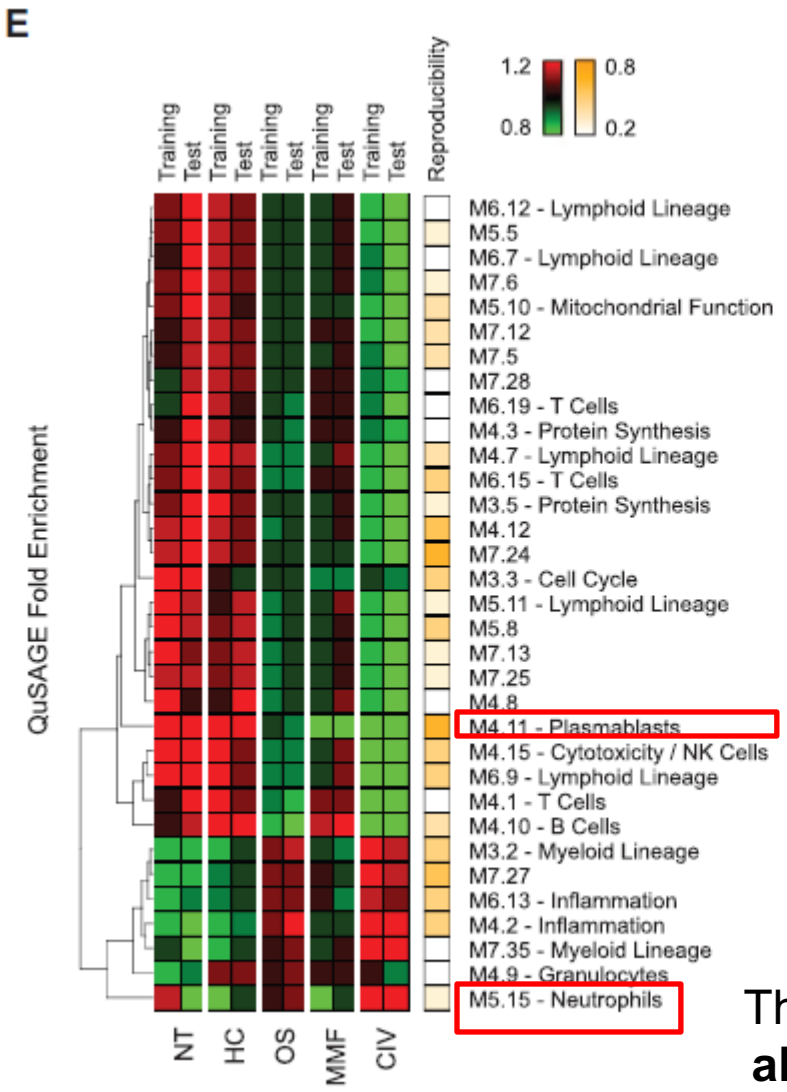
# Genes and Modules Associated With Race

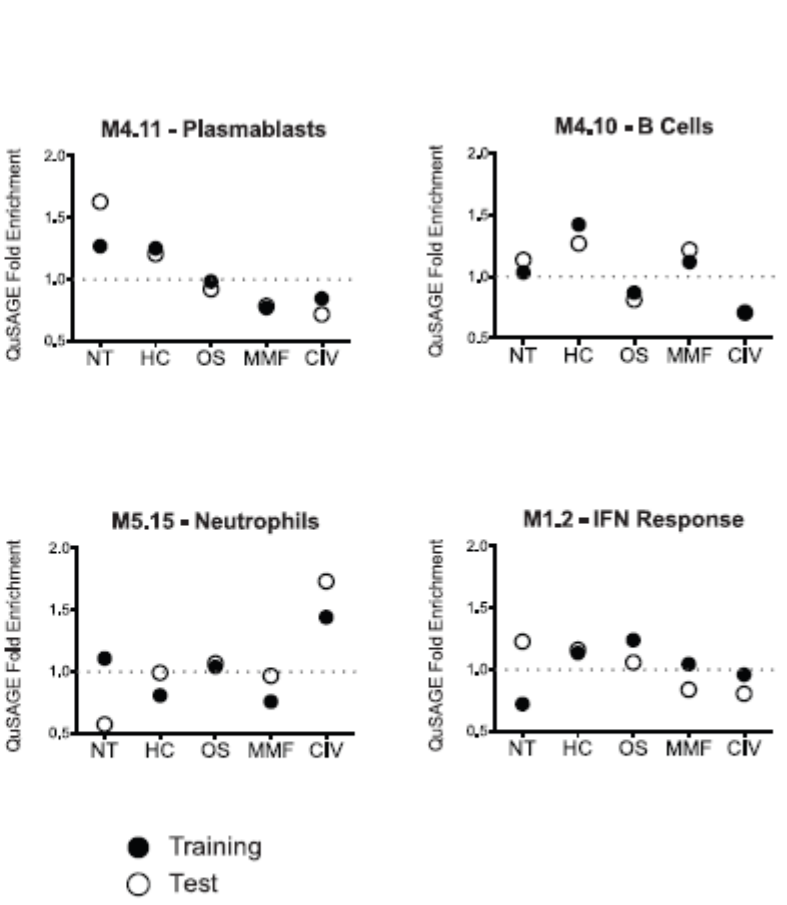



*Cell. 2016 Apr 21;165(3):551-65*

**Increased Plasmablast Responses in African-American Patients**

# Modules Associated With Treatment



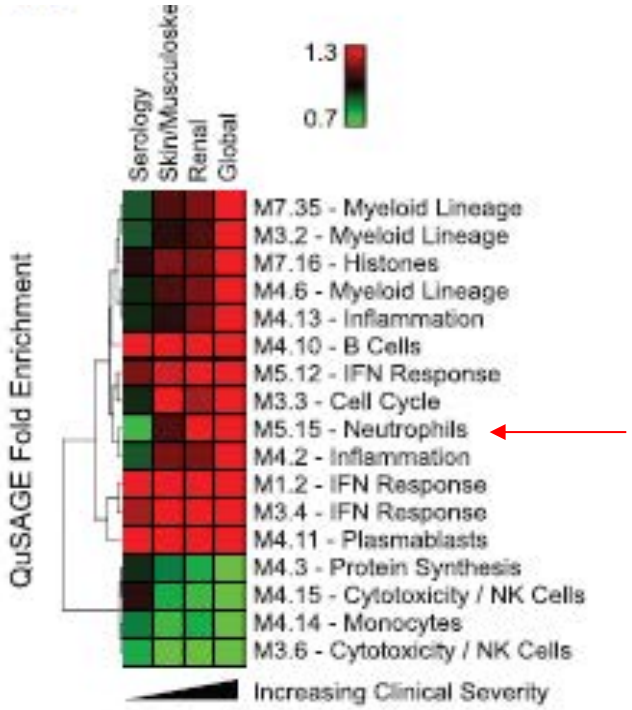



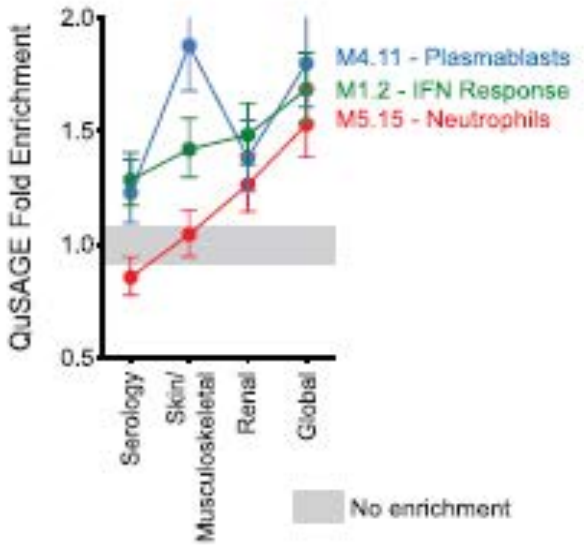

NT: No Treatment
HC: Hydroxychloroquine only
OS: Oral Steroid
MMF: Mycophenolate Mofetil + any
CIV: Cyclophosphamide / IV steroids

The **plasmablast signature was decreased by all treatments compared to no treatment**, but most strongly by (MMF) and CIV, two cytostatic drugs that suppress activated lymphocytes

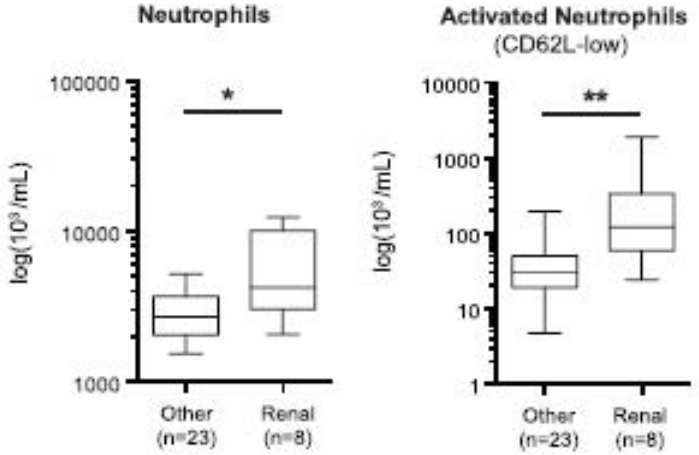
*Cell. 2016 Apr 21;165(3):551-65*

# Modules associated with Disease Types




**Neutrophil module is mostly associated with Lupus Nephritis**

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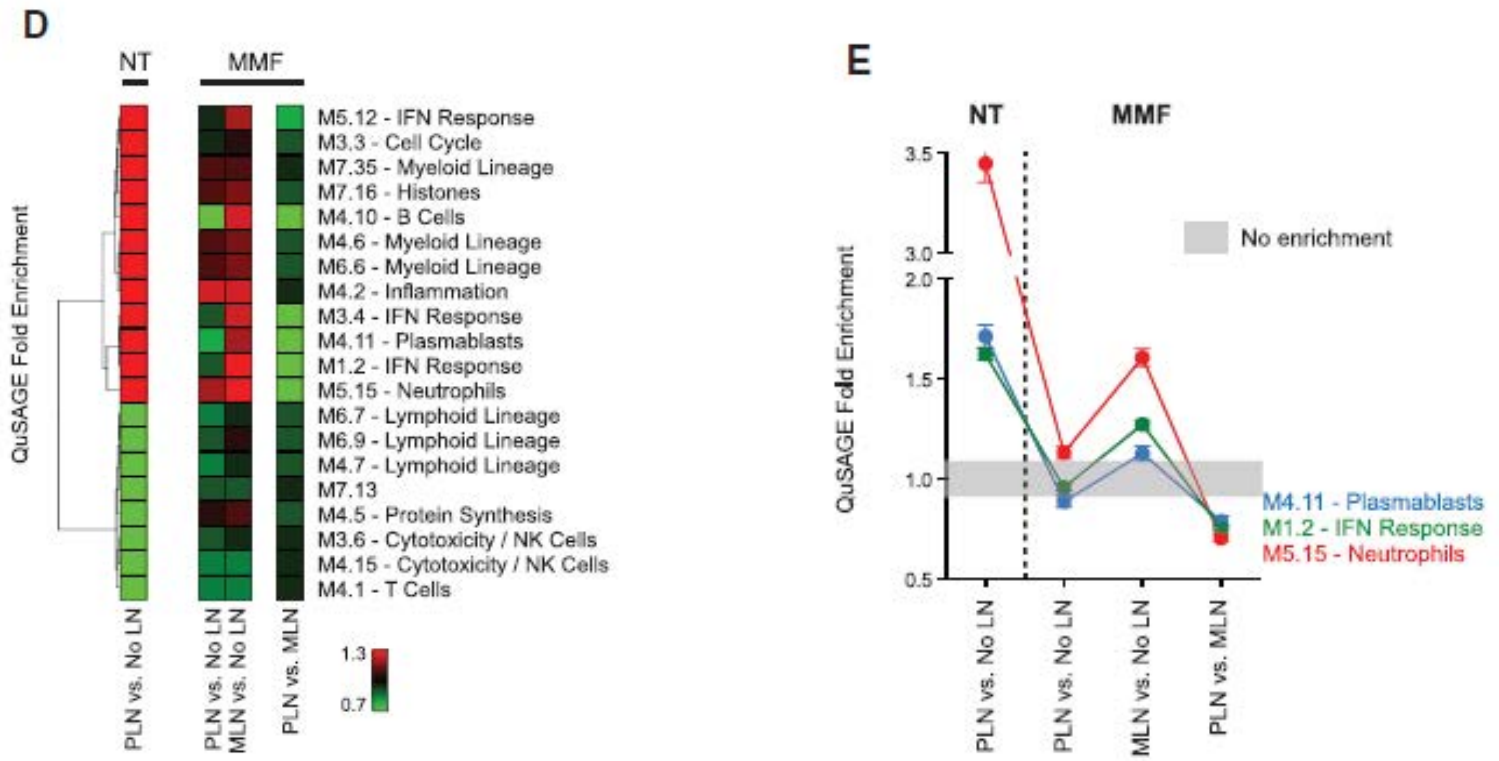
# Modules associated with treatment in different nephritis sub-classes




Distinct signatures **in response to treatment in different nephritis subclasses**: PLN (proliferative nephritis ) vs MLN (membranous nephritis) , treated with MMF (mycophenolate mofetil)

*Cell. 2016 Apr 21;165(3):551-65*

# SLE Blood Transcriptional Fingerprint

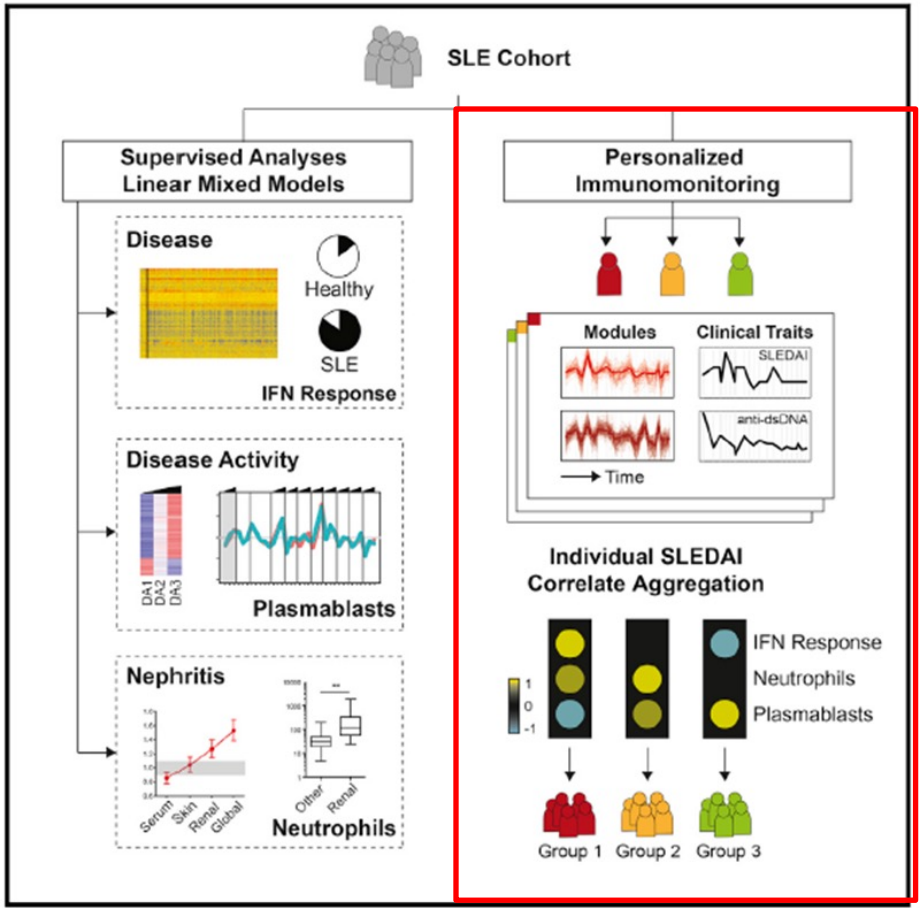
Genes associated to SLE, DA, Race, Treatment, disease subtypes

From genes to "blood" modules

WGCNA modules for each patient and correlation with SLEDAI

Linking WGCNA to blood modules for inference of biological function

Stratification of patients into groups

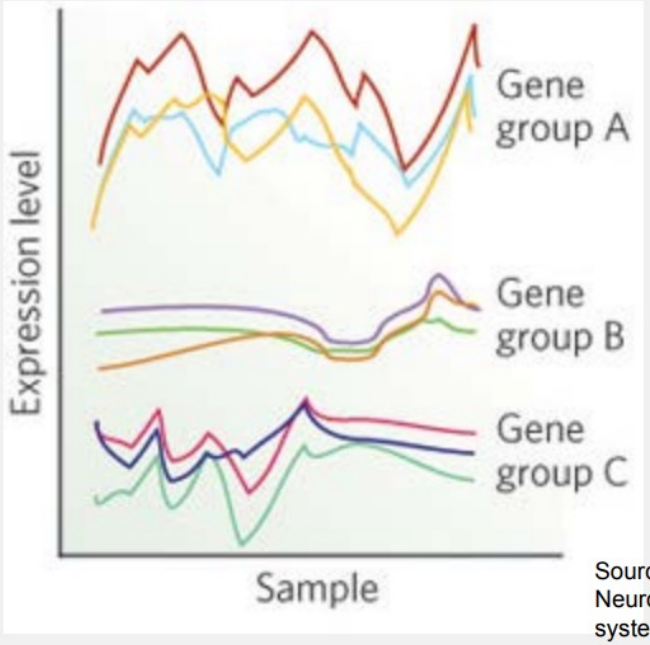


# WGCNA – Weighted Gene Correlation Network

Aim of WGCNA: summarizing individual genes into modules, based on correlation

**Modules found in WGCNA:**

Groups of co-expressed genes (with similar expression profiles over a large group of individuals)



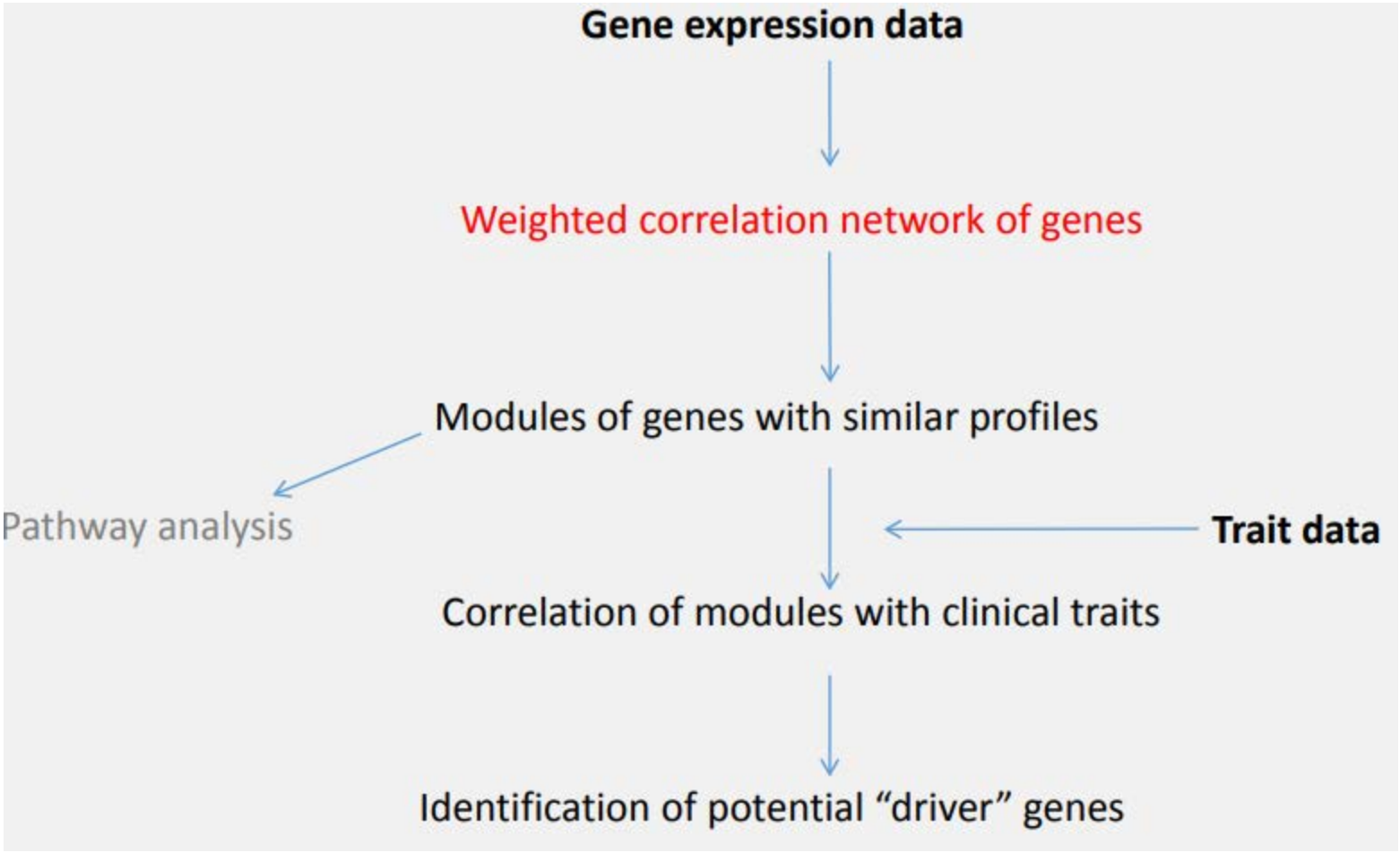

Source: Daniel H. Geschwind & Genevieve Konopka. Neuroscience in the era of functional genomics and systems biology, Nature 461, 908-915

Central Hypothesis:

Genes with **similar expression patterns** are of interest because they may be

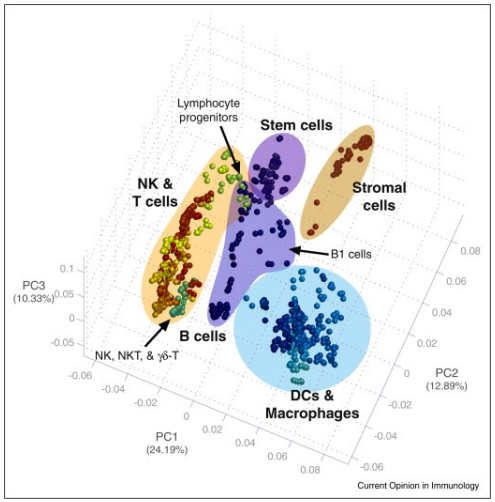
- tightly co-regulated
- functionally related
- members of the same pathway

# WGCNA – Workflow



# Measuring Modules with One Metrics: Eigengenes

| | WGCNA MODULE X | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Gene 1 | Gene 2 | Gene 3 | Gene 4 | Gene 5 | Gene 6 | Gene 7 | Eigengene Value |
| sample 1 | 17 | 55 | 80 | 41 | 3 | 70 | 70 | A |
| sample 2 | 43 | 100 | 56 | 91 | 72 | 22 | 2 | B |

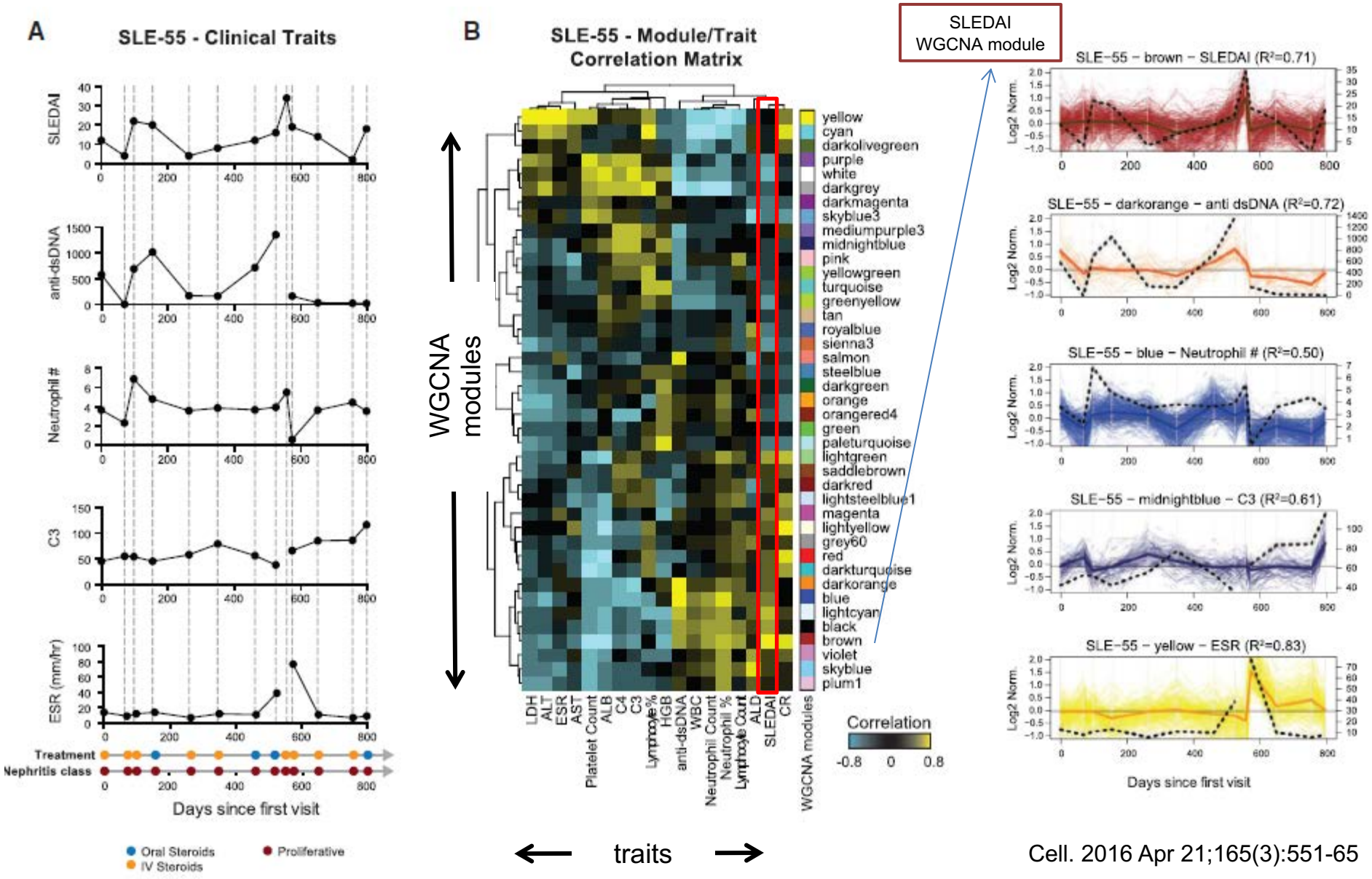



*Kim and Lanier – Curr Opin Immun 2013*

- Module's "eigengene" is the first principal component of the expression matrix of the corresponding module
- It can be used as summary score for a vector of genes in a sample

# Patient 55:Linking WGCNA modules to clinical traits by correlation

Patient- specific modules of co-expressed transcripts over time are identified by WGCNA.
Analysis run on WGCNA modules (eigengenes) correlated with continuous clinical traits

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# Patient 55: Linking WGCNA Modules to Blood Modules

Genes associated to SLE, DA, Race, Treatment, disease subtypes

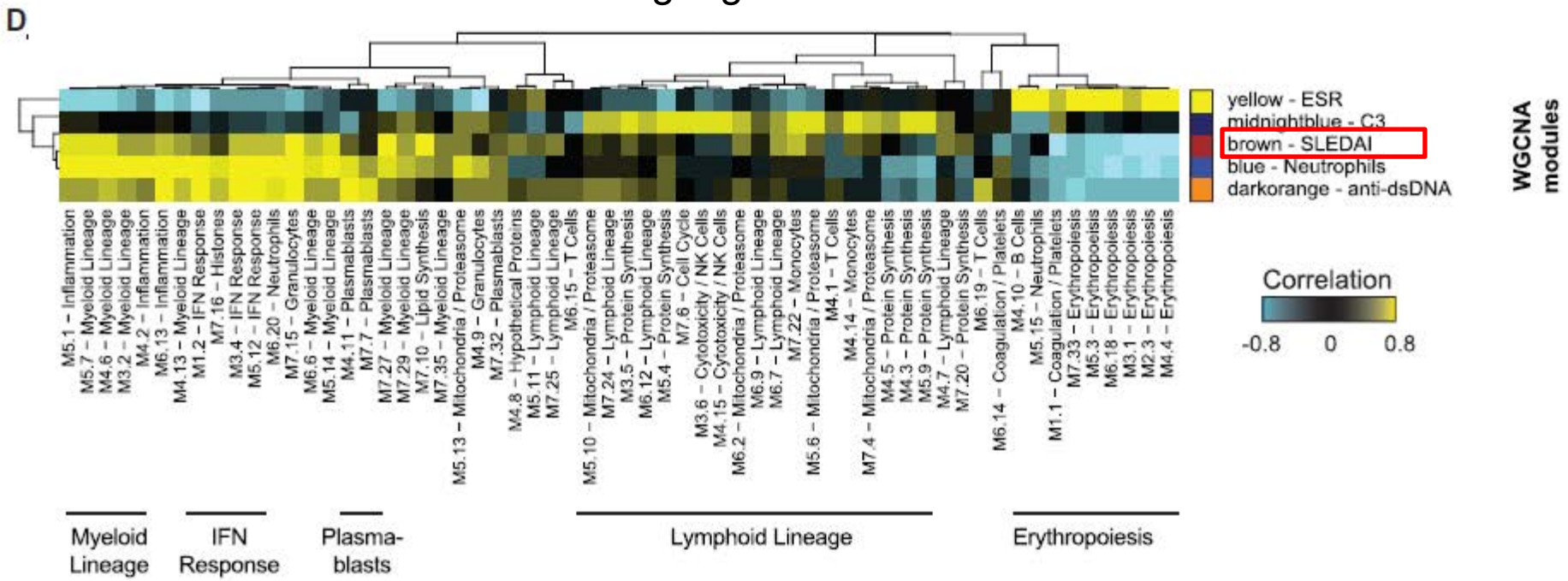
From genes to "blood modules"

Extract WGCNA modules for each patient and correlation with SLEDAI

Linking WGCNA to blood modules for inference of biological function

Stratification of patients into groups

## Correlation between patient SLE-55 WGCNA and blood modules using eigengenes

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# SLE Blood Transcriptional Fingerprint

Genes associated to SLE, DA, Race, Treatment, disease subtypes

From genes to “blood” modules

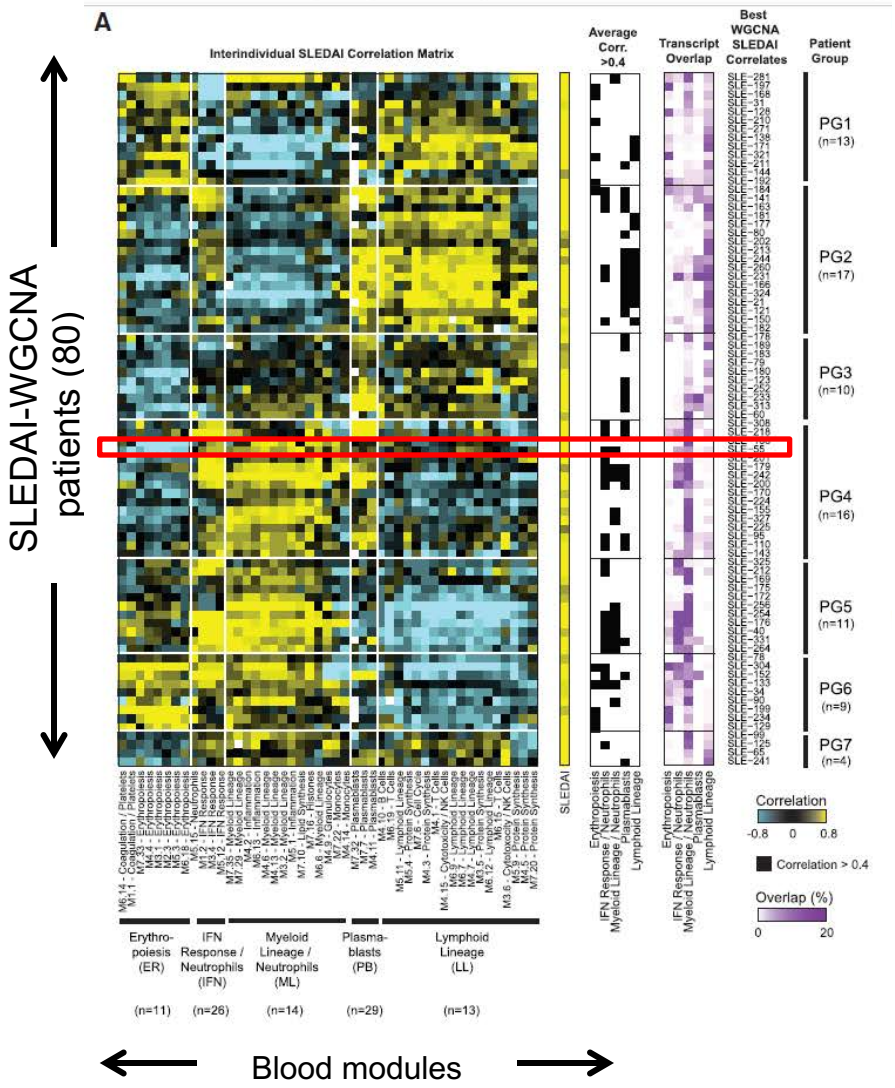
WGCNA modules for each patient and correlation with SLEDAI

Linking WGCNA to blood modules for inference of biological function

Stratification of patients into groups

# Stratification of SLE Patients Based on Transcriptional Correlates of SLEDAI

matrix of correlation between the SLEDAI WGCNA and blood modules, for all patients




- 7 patient groups identified based on the combination of blood immune signatures that best correlate with the SLEDAI-WGCNA.

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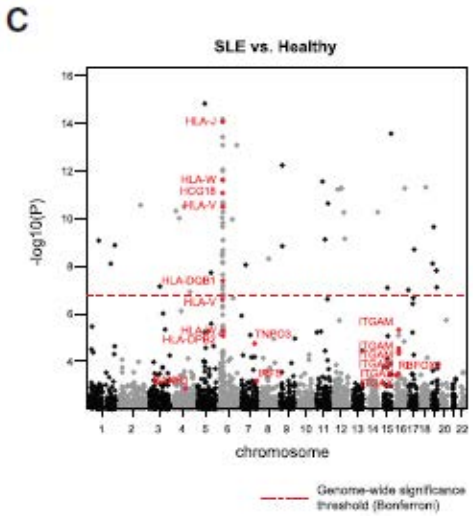
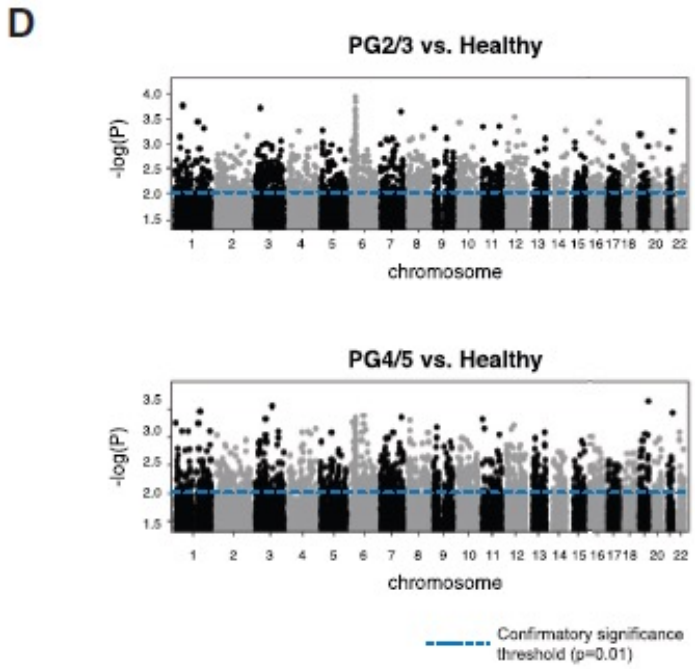
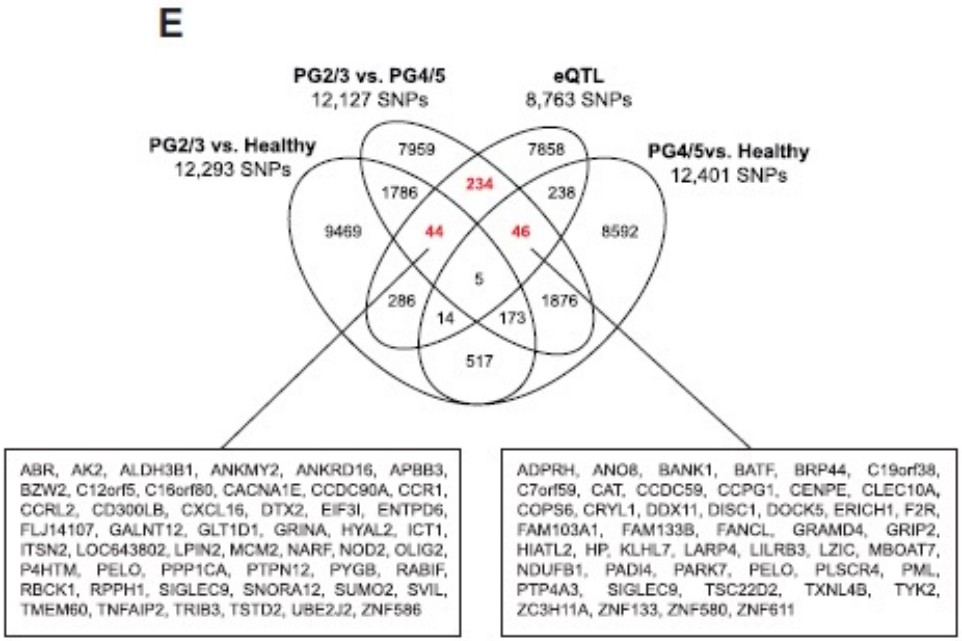
# Genetic Analysis of Patients Groups




- To find a genetic basis for these clusters, SNP analysis was conducted (135 patients + HCs)
- SNPs differentiating between PG2/3 and PG/4/5 were found
- Intersection with eQTL SNPs (SNPs associated with DA genes expression) points to IFN-inducible genes



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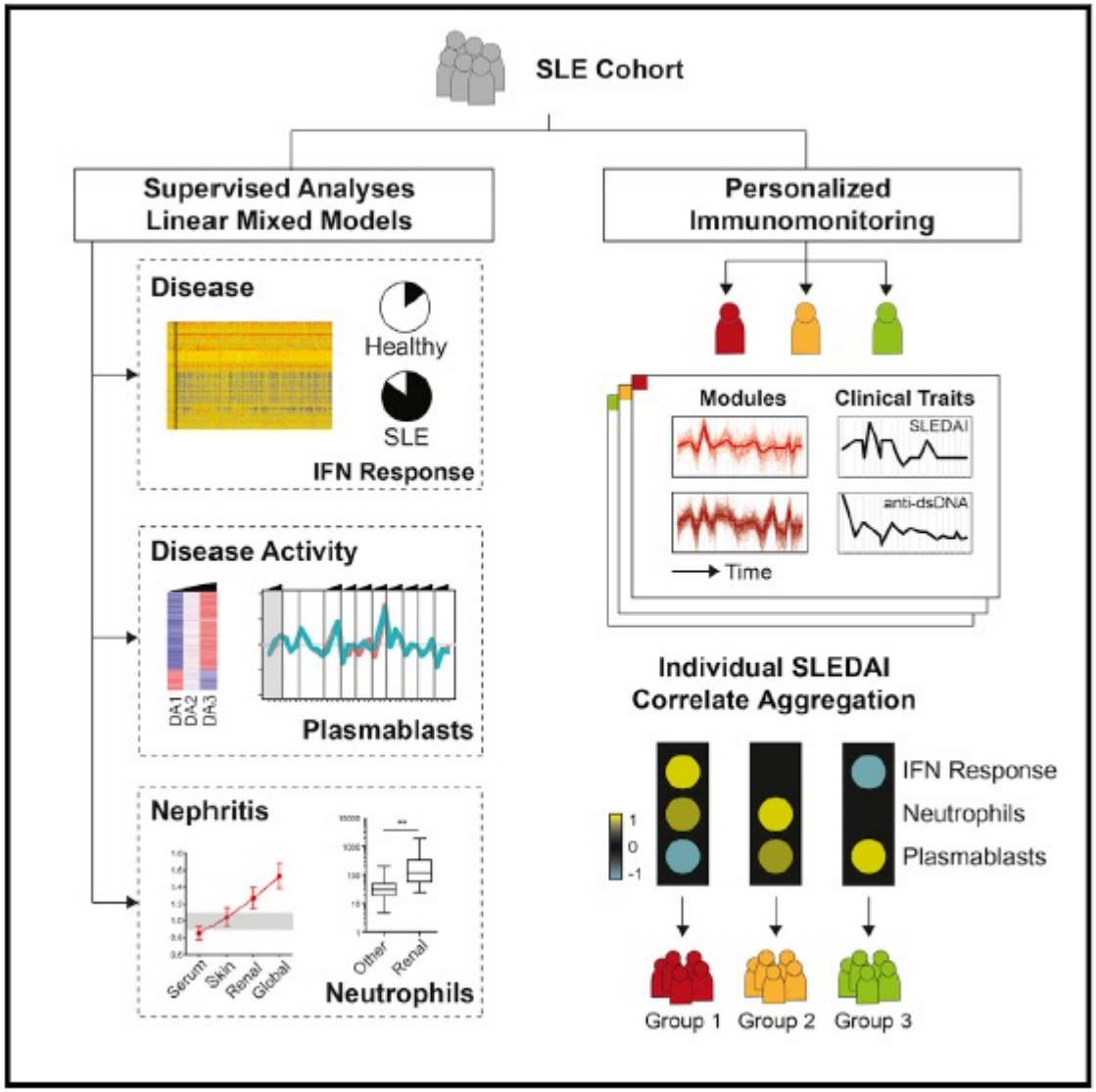
# Let's Recap

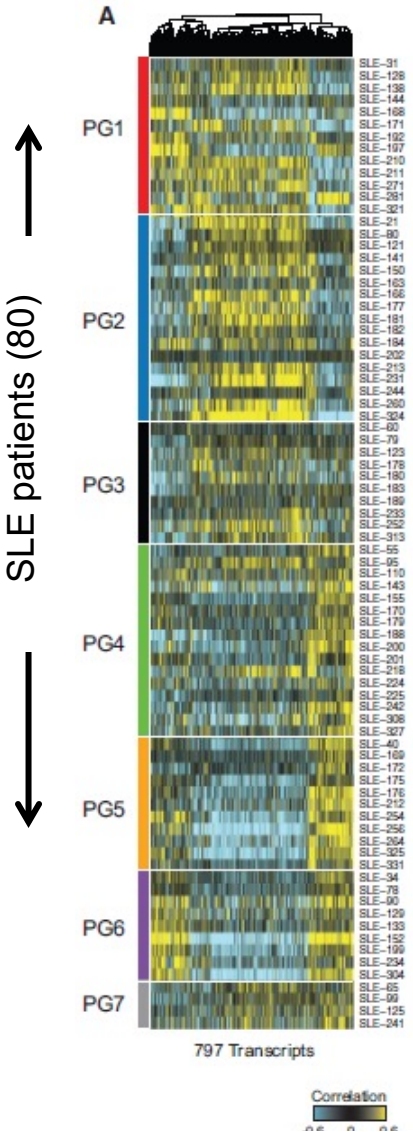



- Supervised analysis, linear models and blood module analyses led to identification of: i) IFN response module in SLE ii) Plasmablasts associated to DA iii) neutrophils associated to nephritis
- Different immune signatures correlating with SLEDAI (7 groups)
- Groups supported by different genotypes
- Supports the development of customized treatment strategies.

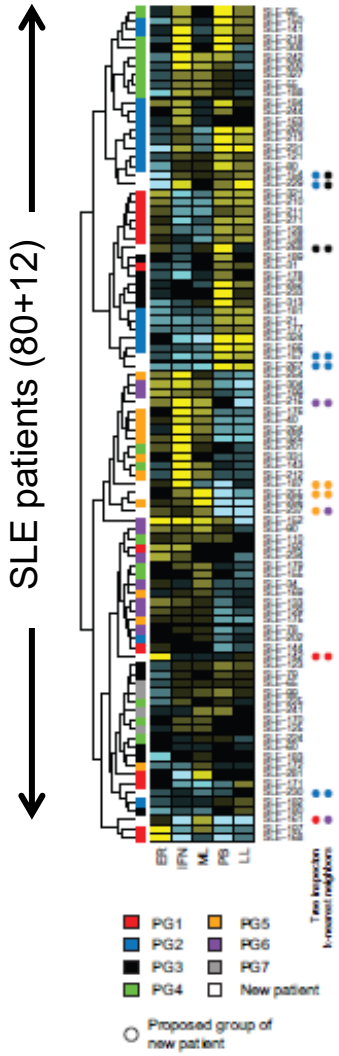
# A Targeted Panel for SLE Patient Stratification

## Can we find a gene panel which allows direct patients stratification ?

- Hierarchical clustering of the 797 transcripts differentially correlating with SLEDAI between the seven patient groups




- Assignment of novel 12 patients to each of the groups “guilt-by-association”

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# Study Summary

- Clinical and transcriptional profiling of 158 lupus patients up to a period of 4 years
- IFN, Plasmablast and Netrophil signatures driving SLE
- Neutrophil-related signatures associate with progression to active nephritis
- Molecular correlates of disease activity stratify patients into seven major groups
- Molecular stratification may improve the outcome of clinical trials in SLE

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# Take Home Messages

- Interpreting biological meaning of ~25K genes in a heterogeneous sample such as blood – starting from bulk data – is extremely complex!

- One way to address this is to aggregate this information into functional building blocks (i.e. dimensionality reduction):
  - "blood modules" : of general use, obtained from previous analysis of 239 disease blood samples
  - "WGCNA modules": patient specific and obtained from correlation analysis of the SLE study data set
- Blood modules can be (tentatively) assigned a biological meaning looking at their gene content

- Patient-specific WGCNA modules can be associated to clinical phenotypes based on longitudinal correlation

- For each sample, each module can be measured with one number (e.g. % of genes up- or down-regulated in the module, or eigenvalue). This approach has the statistical advantage of reducing number of tests

- Patients' SLEDAI can be associated with different modules. On this basis patients can be stratified into classes, presumably having different underlying biology and needing different treatments

# Single-cell RNA sequencing of human tissue supports successful drug targets

Emma Dann[*1,2], Erin Teeple[*3], Rasa Elmentaite[2], Kerstin B Meyer[1], Giorgio Gaglia[3], Frank Nestle[4], Virginia Savova[3], Emanuele de Rinaldis[3+], Sarah A Teichmann[1,2,5]

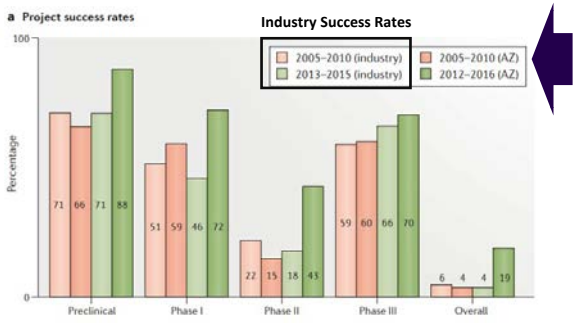
1. Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge, UK
2. Ensocell Therapeutics, BioData Innovation Centre, Wellcome Genome Campus, Hinxton, Cambridge, UK
3. Precision Medicine & Computational Biology, Sanofi Research US, Cambridge, MA, USA
4. Research & Development, Sanofi, Cambridge, MA, USA
5. Theory of Condensed Matter, Cavendish Laboratory/Dept Physics, University of Cambridge, JJ Thomson Ave, Cambridge, UK

*Authors contributed equally +Co-Corresponding and senior authors

June 17 2024

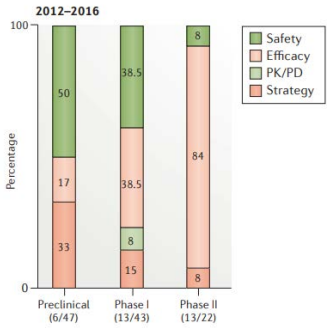
FOCIS Workshop

# Motivation: how can single cell data support decision making in target discovery?




AstraZeneca (AZ) performance improvement after instituting a new drug development framework focusing on 5 priority areas: target, tissue, safety, patient, potential (5Rs). Note that Phase II success rates remain below half of projects.

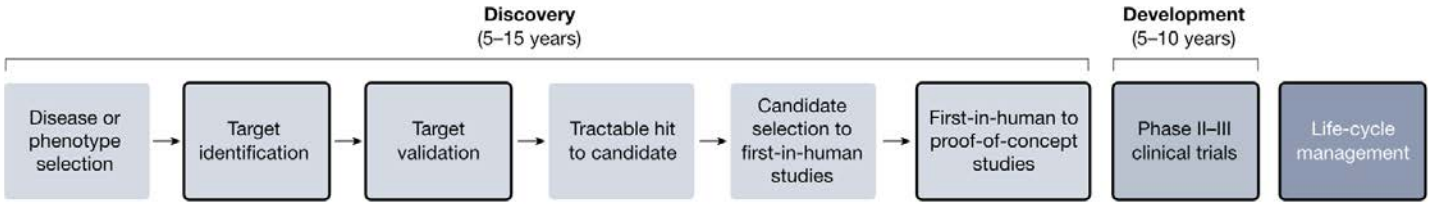
Morgan et al. *Nat Rev Drug Discov* 2018




2012-2016 reasons for failure by project phase. There exists a persistent need for new information to inform drug development work.

Morgan et al. *Nat Rev Drug Discov* 2018

90 % of drug development failures stem from inadequate target selection for a disease

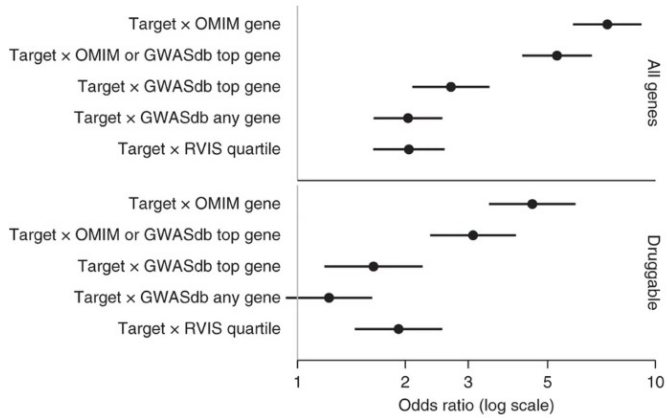



*How can single-cell RNA-seq boost the selection of promising drug targets ?*

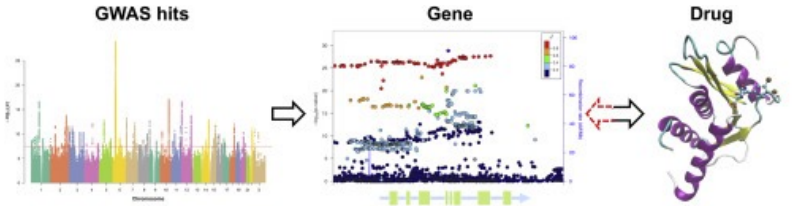
sanofi

# Lessons Learnt from Human Genetics

**Figure 2: Enrichment of target genes for drugs approved in the United States or the European Union.**




Nelson et al. (2015) *Nature Genetics*




| Trait | Gene with GWAS hits | Known or candidate drug |
|---|---|---|
| Type 2 Diabetes | *SLC30A8/KCNJ11* | ZnT-8 antagonists/Glyburide |
| Rheumatoid Arthritis | *PADI4/IL6R* | BB-Cl-amidine/Tocilizumab |
| Ankylosing Spondylitis(AS) | *TNFR1/PTGER4/TYK2* | TNF-inhibitors/NSAIDs/fostamatinib |
| Psoriasis(Ps) | *IL23A* | Risankizumab |
| Osteoporosis | *RANKL/ESR1* | Denosumab/Raloxifene and HRT |
| Schizophrenia | *DRD2* | Anti-psychotics |
| LDL cholesterol | *HMGCR* | Pravastatin |
| AS, Ps, Psoriatic Arthritis | *IL12B* | Ustekinumab |

Visschner et al. (2017) 10 Years of GWAS Discovery: Biology, Function, and Translation. *AJHG*

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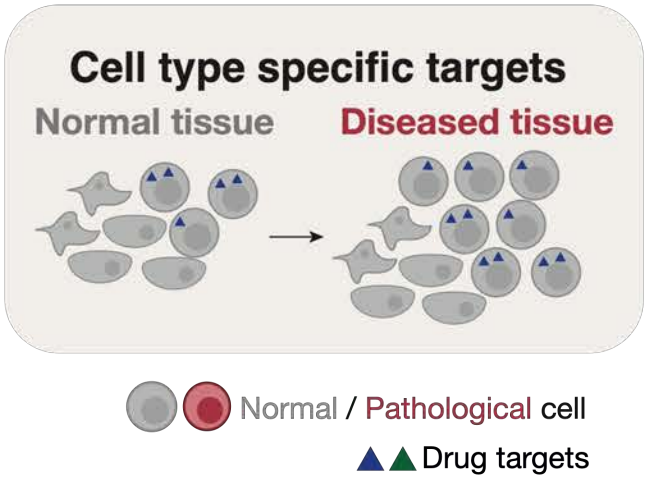
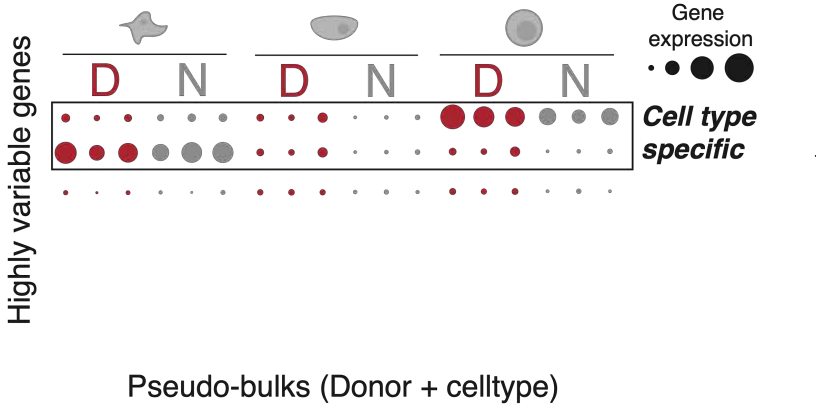
# scRNAseq support: cell type-specific expression in disease-relevant tissue




Cell type specific support defined as differential expression across cell types in disease.

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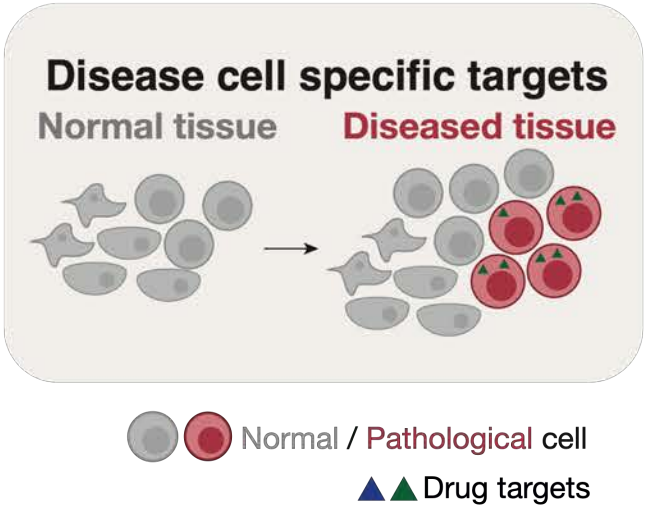
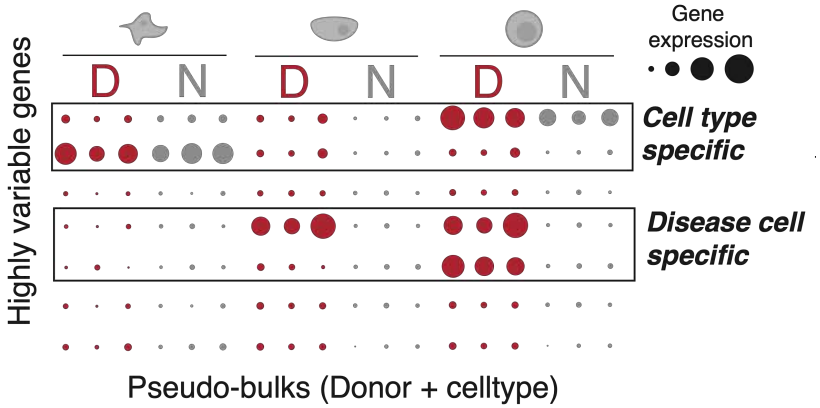
# scRNAseq support: cell type-specific expression in Disease (D) versus Normal (N)




Disease cell specific support defined as differential expression in cell types in disease.

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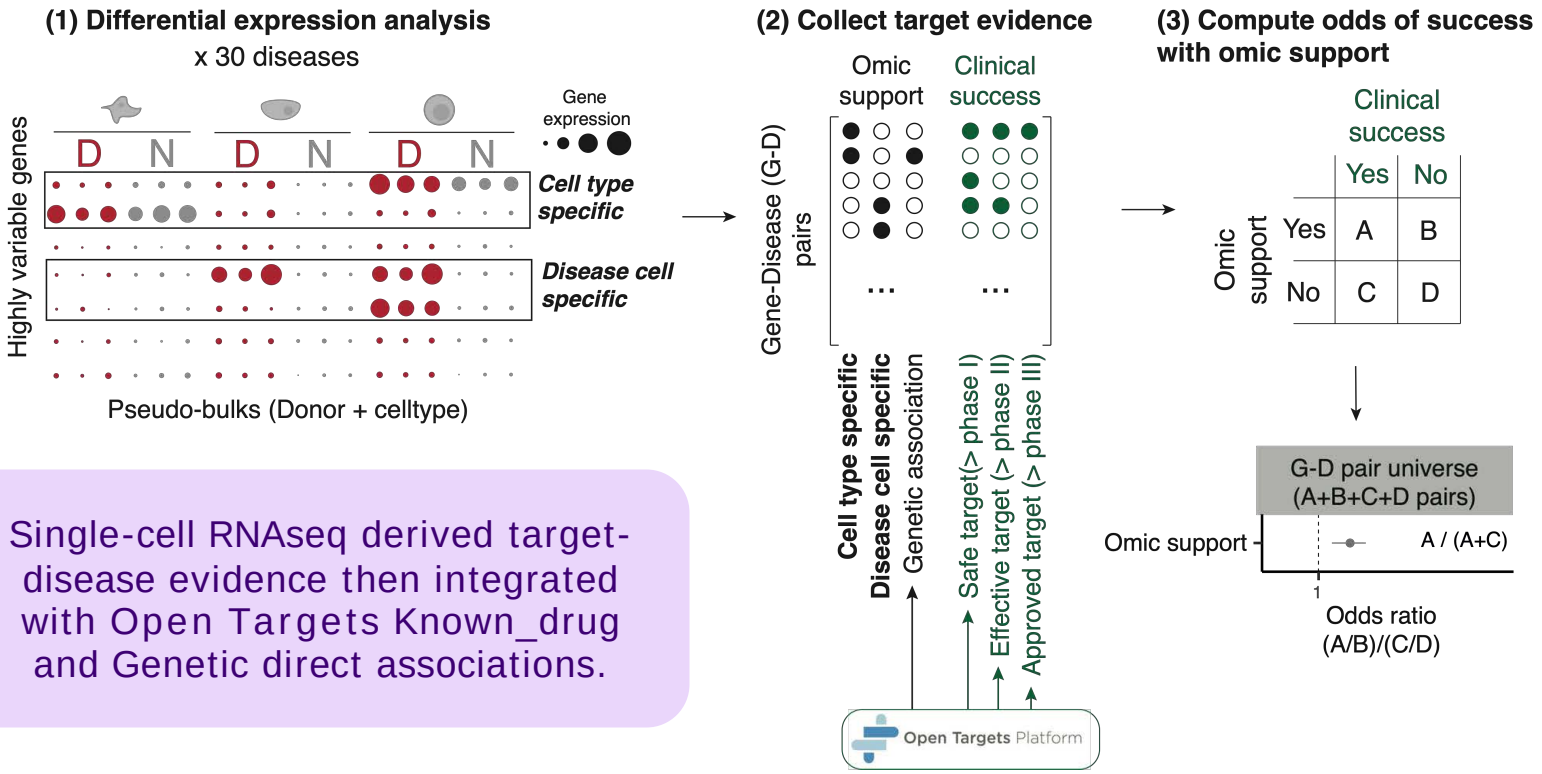
# Approach: Evaluating scRNA-seq support for known drug targets




Single-cell RNAseq derived target-disease evidence then integrated with Open Targets Known_drug and Genetic direct associations.

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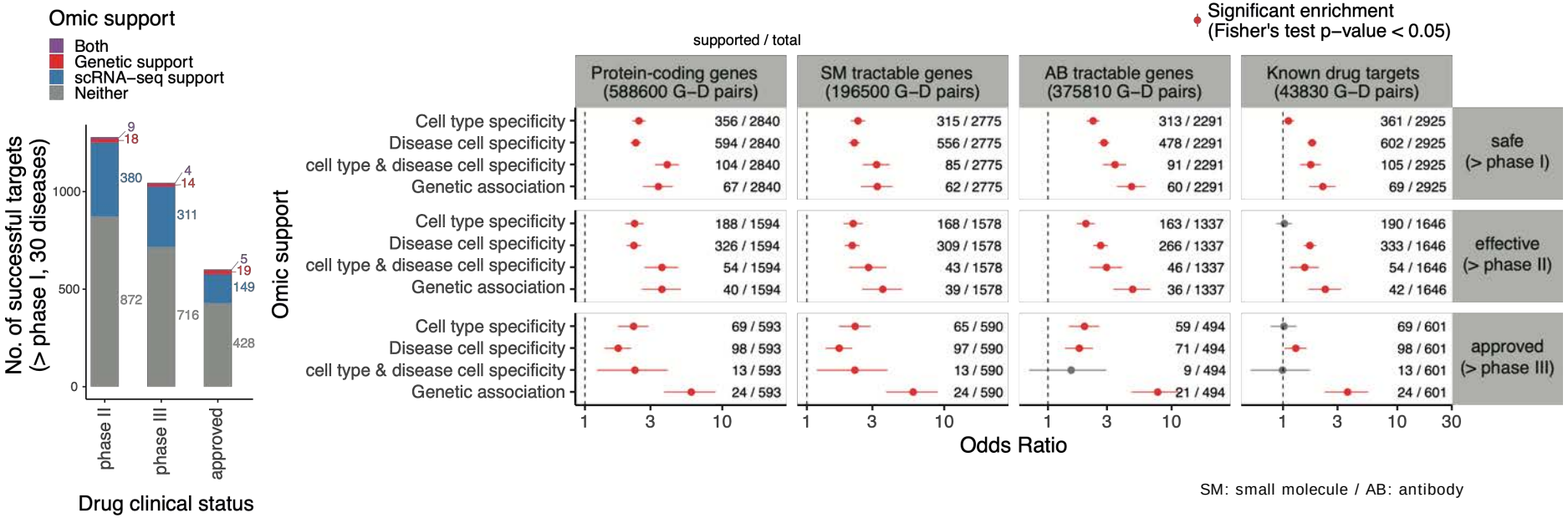
# Targets with single-cell support have 3x higher odds of clinical success

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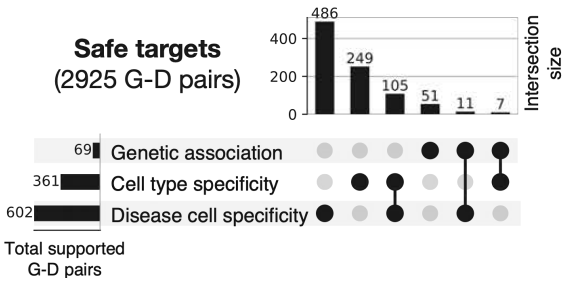
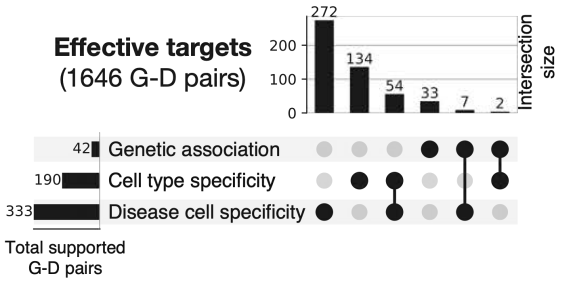
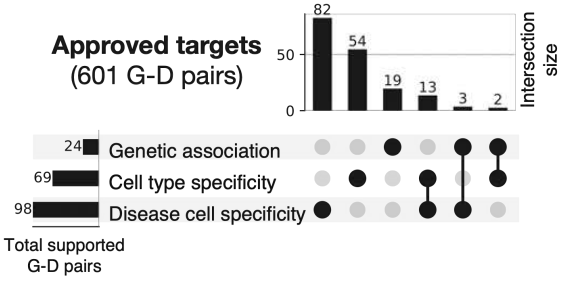
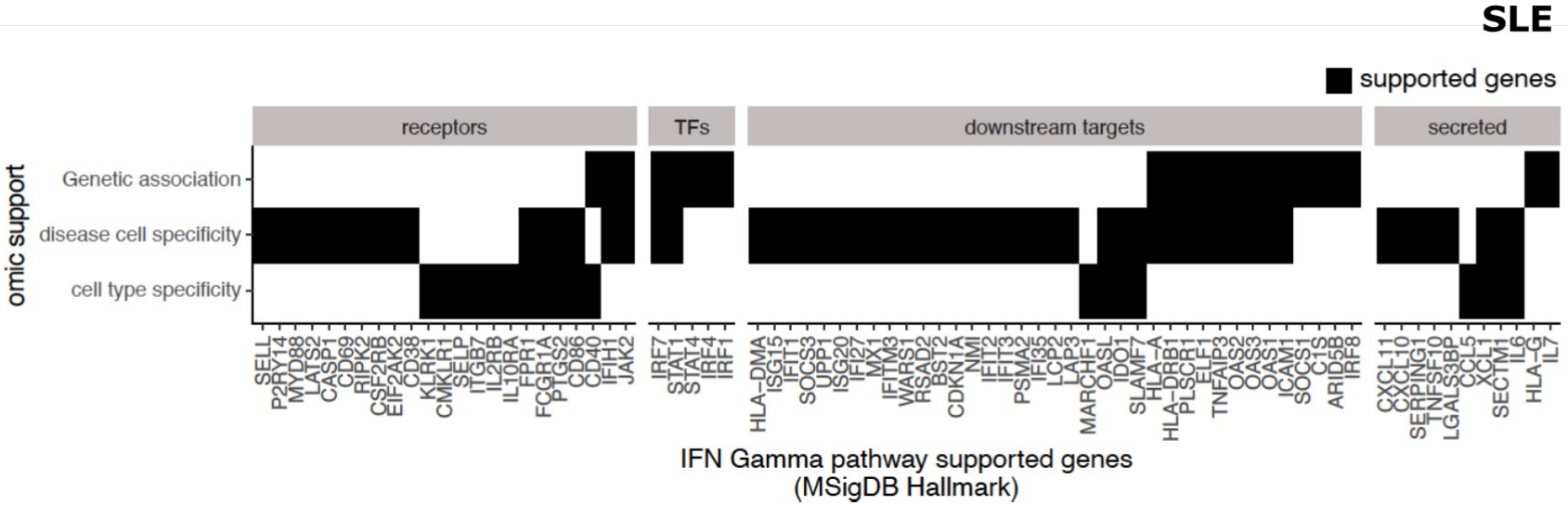
# Single-cell support identifies a target space complementary to direct genetic evidence

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# Summary

- Retrospective analysis of association between cell type-specific expression and target success.
- scRNA-seq support is found to be associated with greater odds of clinical trial success
- scRNA-seq support identifies a target space complementary to genetics, with distinctive molecular and druggability characteristics.

- **Next steps:**
  - Expand the number of diseases included in the analysis by matching to disease-relevant tissue (not all will have disease versus control evidence analysis)
  - scRNAseq is a rich data source – additional association evidence approaches can be considered

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# Q & A


FOCIS 2024
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# My Best Reference for Systems Immunology: ImmunoScope

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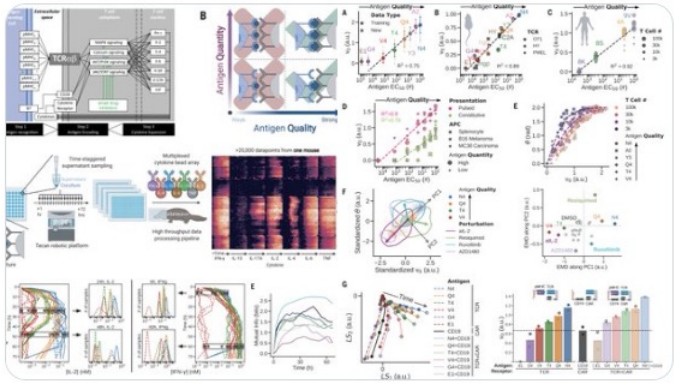

4  36

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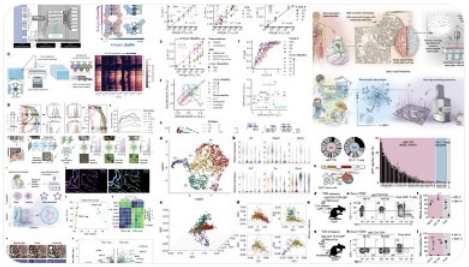

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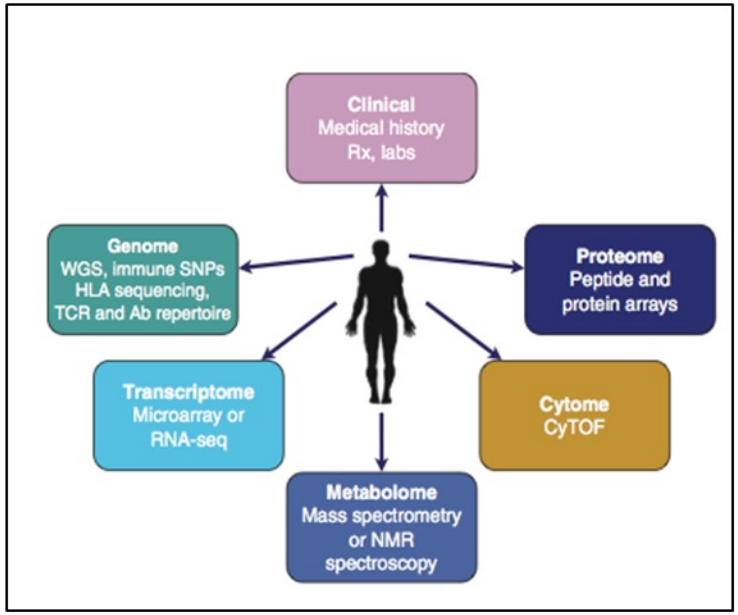
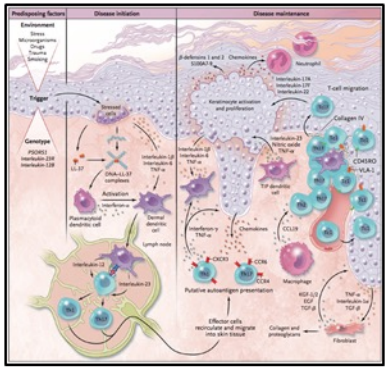
# Computational Immunology




*Kidd et al. Nat. Immunology 2014*



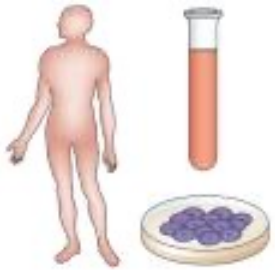
*Understanding Molecular Aetiology*

**P~ f(m)**

*Explaining clinical phenotype as function of molecular data. Finding biomarkers*

*Identifying Novel therapeutic targets*

# Immune Phenotypes

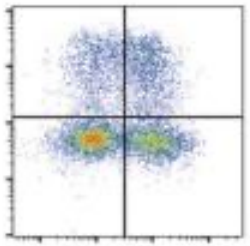


**Cell state or material**

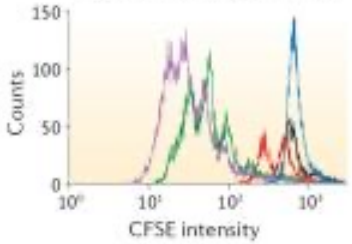
Resting | Cytokine stimulation | Non-antigenic stimulation | Antigenic stimulation | Blood plasma

**Technique**

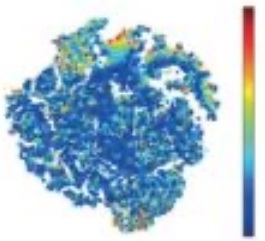
**Flow cytometry**



**CFSE**
(fluorescent cell-staining dye)

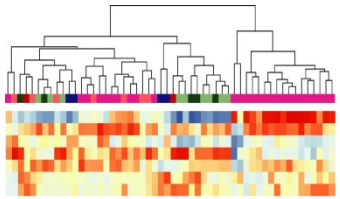



**Mass cytometry**



**Luminex**

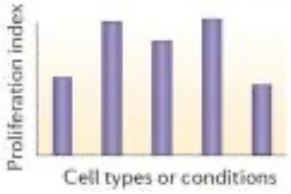
RNA-Seq
Microarrays



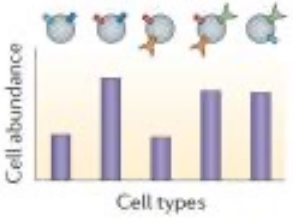
**Measurement**

**Signalling response**

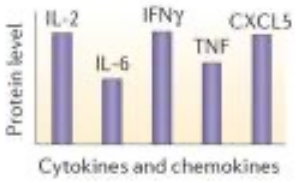

**Cell proliferation**




**Cell frequencies**




**Serum protein levels**




- RNA-levels
- Cell frequencies
- Cell clusters

# The last two decades of single-cell

CellPress

Immunity
Perspective

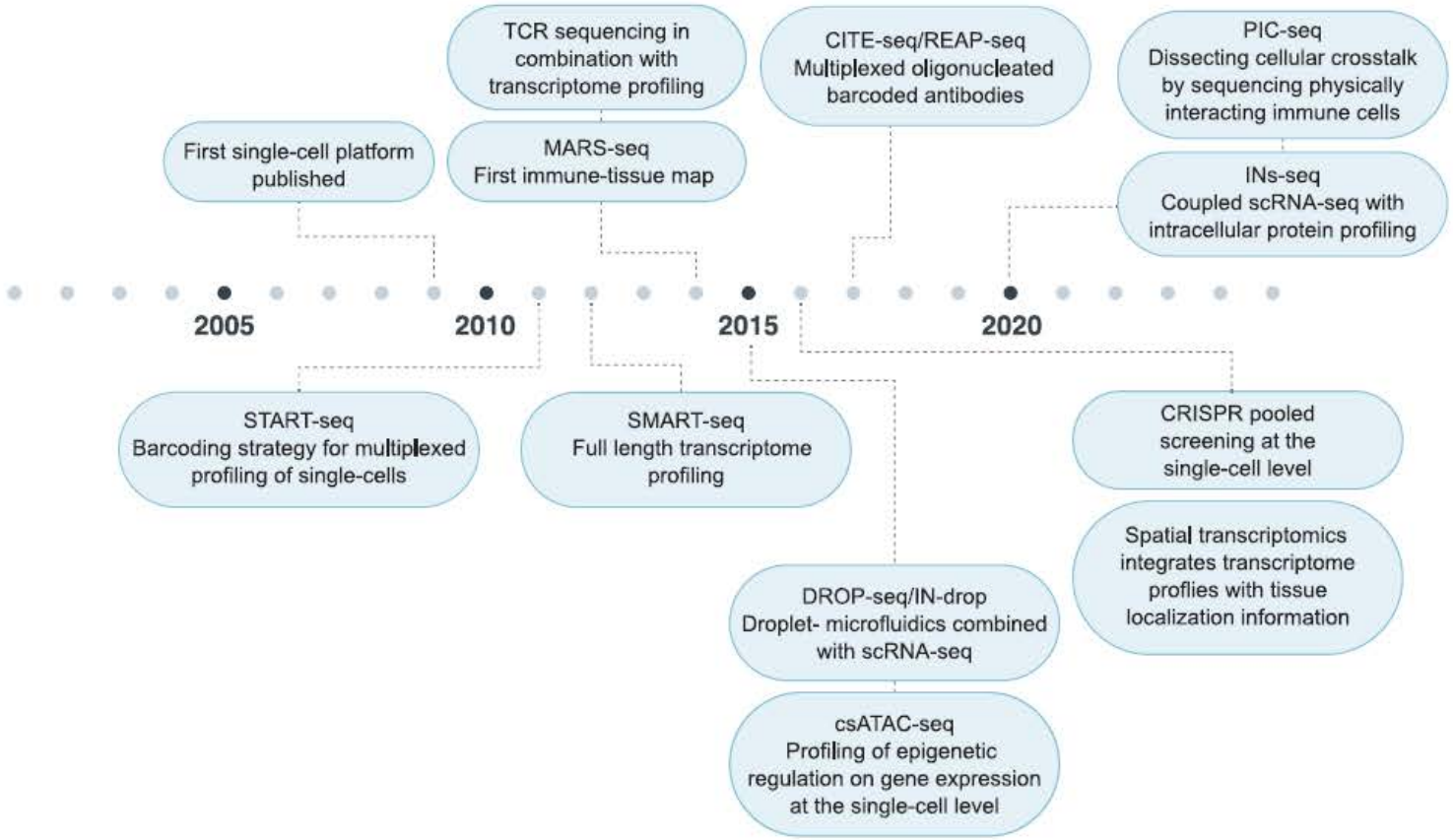
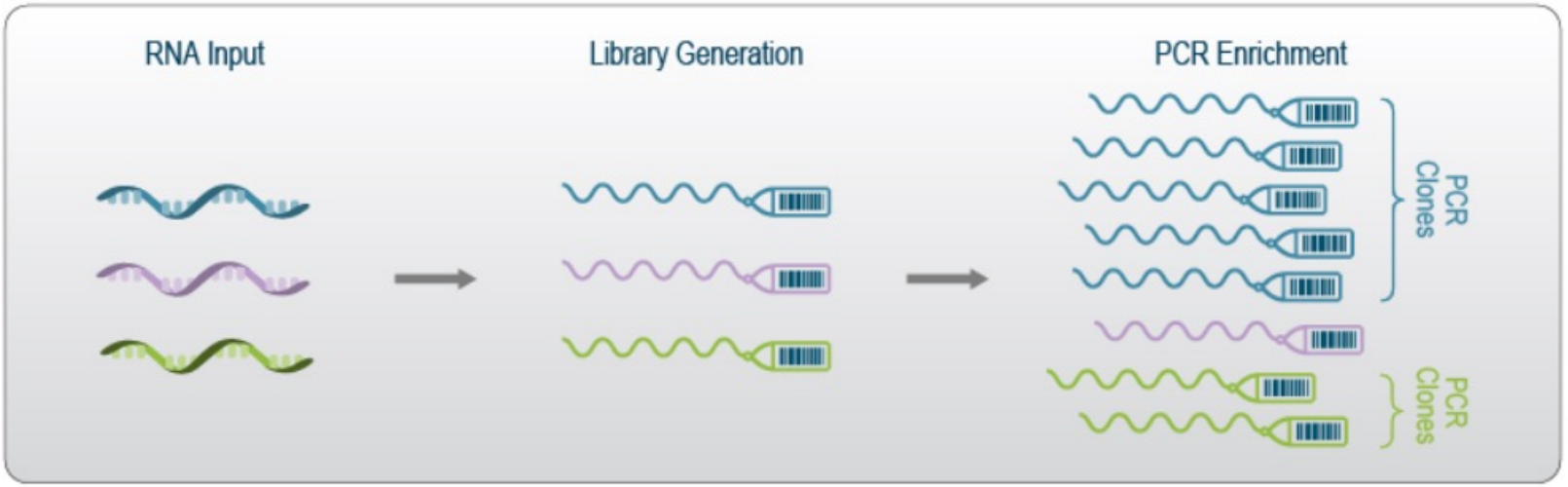
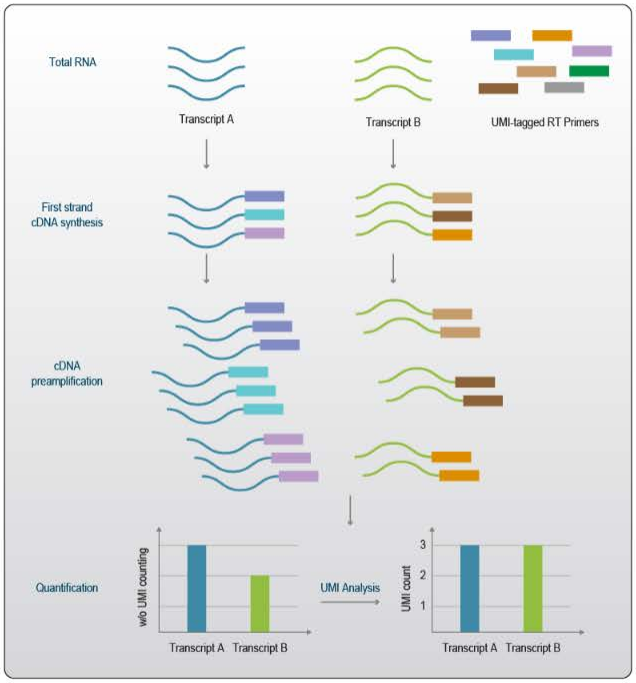



**Figure 2. Timeline describing emerging single-cell technologies and their application in immunology research**

Ginhoux F, Yalin A, Dutertre CA, Amit I. Immunity. 2022

# What are Unique Molecular Identifiers (UMIs) and Why do We Need Them?

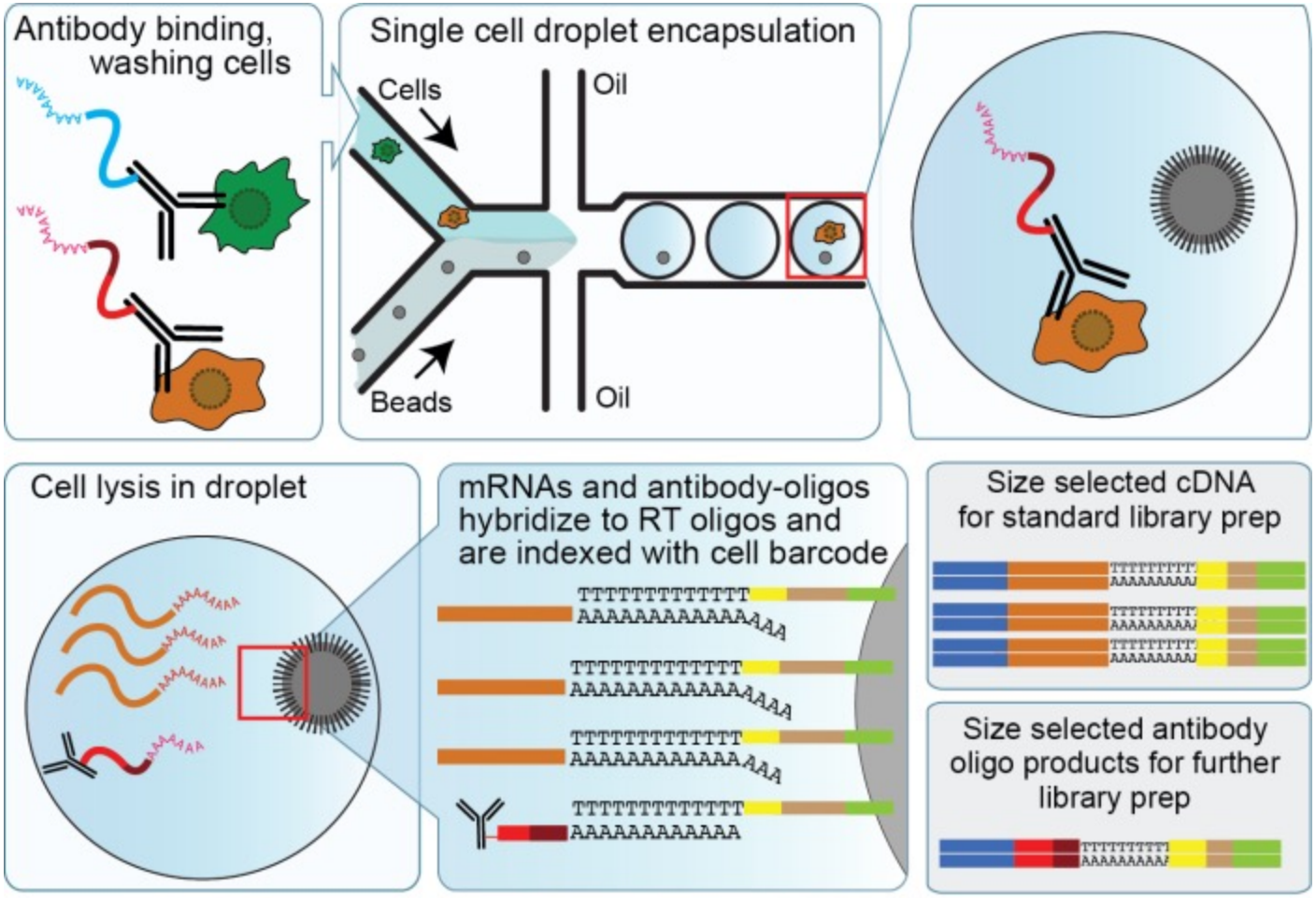



*Transcript level quantification with UMIs. Transcripts or cDNAs are tagged with UMIs in an early step of library generation. The UMI sequences can then be used for quantification of the number of molecules that were originally present in the sample. UMIs can thus control for amplification biases associated with PCR-based sample preparation*

Kolodziejczyk, A.A., and Lönnberg, T. (2018) Global and targeted approaches to single-cell transcriptome characterization, *Briefings in Functional Genomics*,17: 209- 219, doi: 10.1093/bfgp/elx025.

# CITE-Seq


Antibody binding, washing cells
Single cell droplet encapsulation
Cells
Oil
Beads
Oil
Cell lysis in droplet
mRNAs and antibody-oligos hybridize to RT oligos and are indexed with cell barcode
TTTTTTTTTTTTT
AAAAAAAAAAAAAAA
Size selected cDNA for standard library prep
Size selected antibody oligo products for further library prep

# Experimental methods for unimodal and multimodal single-cell measurements

Table 1 | **Current experimental methods for unimodal and multimodal single-cell measurements**

| Data types | Method name | Feature throughput | Cell throughput | Refs |
|---|---|---|---|---|
| *Unimodal* | | | | |
| mRNA | Drop-seq | Whole transcriptome | 1,000–10,000 | 4 |
| | InDrop | Whole transcriptome | 1,000–10,000 | 5 |
| | 10X Genomics | Whole transcriptome | 1,000–10,000 | 6 |
| | Smart-seq2 | Whole transcriptome | 100–300 | 98 |
| | MARS-seq | Whole transcriptome | 100–300 | 7 |
| | CEL-seq | Whole transcriptome | 100–300 | 1 |
| | SPLiT-seq | Whole transcriptome | ≥50,000 | 8 |
| | sci-RNA-seq | Whole transcriptome | ≥50,000 | 7 |
| Genome sequence | SNS | Whole genome | 10–100 | 9 |
| | SCI-seq | Whole genome | 10,000–20,000 | 10 |
| Chromatin accessibility | scATAC-seq | Whole genome | 1,000–2,000 | 13 |
| | sciATAC-seq | Whole genome | 10,000–20,000 | 14 |
| | scTHS-seq | Whole genome | 10,000–20,000 | 15 |
| DNA methylation | scBS-seq | Whole genome | 5–20 | 17 |
| | snmC-seq | Whole genome | 1,000–5,000 | 18 |
| | sci-MET | Whole genome | 1,000–5,000 | 19 |
| | scRRBS | Reduced representation genome | 1–10 | 20 |
| Histone modifications | scChIP-seq | Whole genome + single modification | 1,000–10,000 | 24 |
| Chromosome conformation | scHi-C-seq | Whole genome | 1–10 | 25 |
| *Multimodal* | | | | |
| Histone modifications + spatial | NA | Single locus + single modification | 10–100 | 28 |
| mRNA + lineage | scGESTALT | Whole transcriptome | 1,000–10,000 | 31 |
| | ScarTrace | Whole transcriptome | 1,000–10,000 | 32 |
| | LINNAEUS | Whole transcriptome | 1,000–10,000 | 33 |
| Lineage + spatial | MEMOIR | NA | 10–100 | 27 |
| mRNA + spatial | osmFISH | 10–50 RNAs | 1,000–5,000 | 35 |
| | STARmap | 20–1,000 RNAs | 100–30,000 | 31 |
| | MERFISH | 100–1,000 RNAs | 100–40,000 | 100 |
| | seqFish | 125–250 RNAs | 100–20,000 | 26 |
| mRNA + cell surface protein | CITE-seq | Whole transcriptome + proteins | 1,000–10,000 | 30 |
| | REAP-seq | Whole transcriptome + proteins | 1,000–10,000 | 29 |
| mRNA + chromatin accessibility | sci-CAR | Whole transcriptome + whole genome | 1,000–20,000 | 40 |
| mRNA + DNA methylation | scM&T-seq | Whole genome | 50–100 | 46 |
| mRNA + genomic DNA | G&T-seq | Whole genome + whole transcriptome | 50–200 | 44 |
| mRNA + intracellular protein | NA | 96 mRNAs + 38 proteins | 50–100 | 50 |
| | | 82 mRNAs + 75 proteins | 50–200 | 49 |
| DNA methylation + chromatin accessibility | scNOMe-seq | Whole genome | 10–20 | 41 |

**Stuart T**, Satija R.Nat Rev Genet. 2019