Workshop in Systems Immunology

FOCiS ANNUAL MEETING BOSTON · JUNE 20-23 2023

Emanuele de Rinaldis
Magnus Fontes
Giorgio Gaglia
Shameer Khader

June 19th 2023

Today's Plan

8:00-8:10 am	**Course Overview and Objectives** – Emanuele de Rinaldis
8:10-9:00 am	**Introduction to Systems Immunology** – Emanuele de Rinaldis
9:00-9:45 am	**Systems Immunology & Immune Oncology: A Data-Centric View** – Magnus Fontes
9:45-10:00 am	Break
10:00-11:30 am	**Deep Dive Into Selected Scientific Case Studies:** **From Systems Immunology to Novel Therapeutic Insights** – Emanuele de Rinaldis
11:30 am-12:00 pm	**Q/A and Panel Discussion**
12:00-1:00 pm	Break for Lunch
1:00-2:00 pm	**Spatial Biology Methods and Analytics for Immunology & Oncology** – Giorgio Gaglia
2:00-2:15 pm	Break
2:15-3:30 pm	**Artificial Intelligence – A Primer for Immunologists –** Shameer Khader
3:30-3:45 pm	Break
3:45-4:45 pm	**Interactive Data Analysis Session** – Magnus Fontes
4:45-5:00 pm	**Wrap Up Notes & Final Remarks**

Introduction to Systems Immunology

Technologies, Methods and Applications

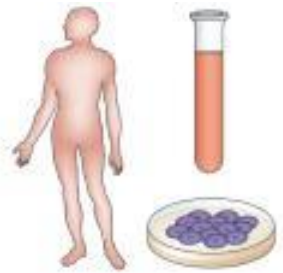
Emanuele de Rinaldis

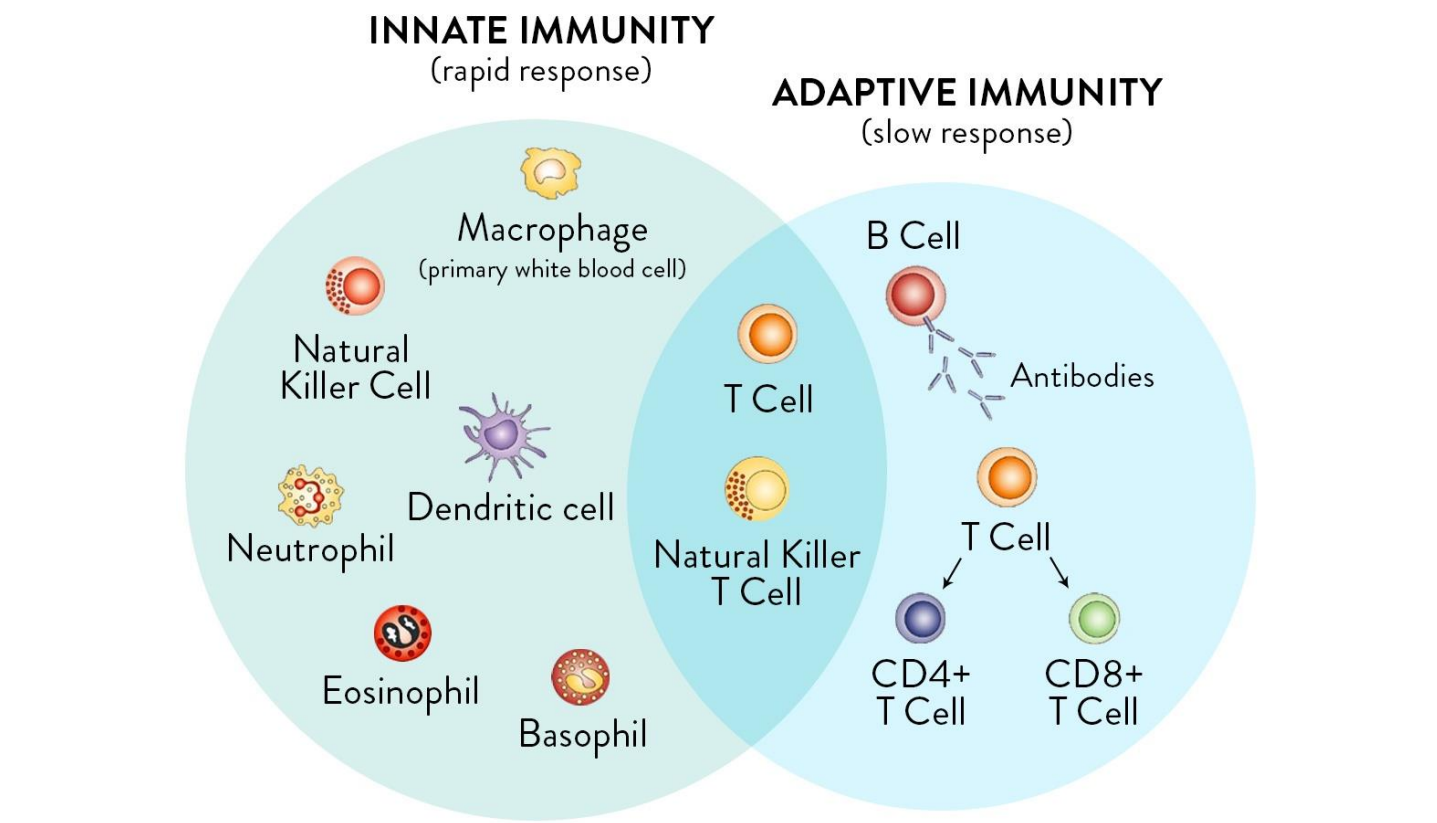
FOCIS - June 19th, 2023

Outline

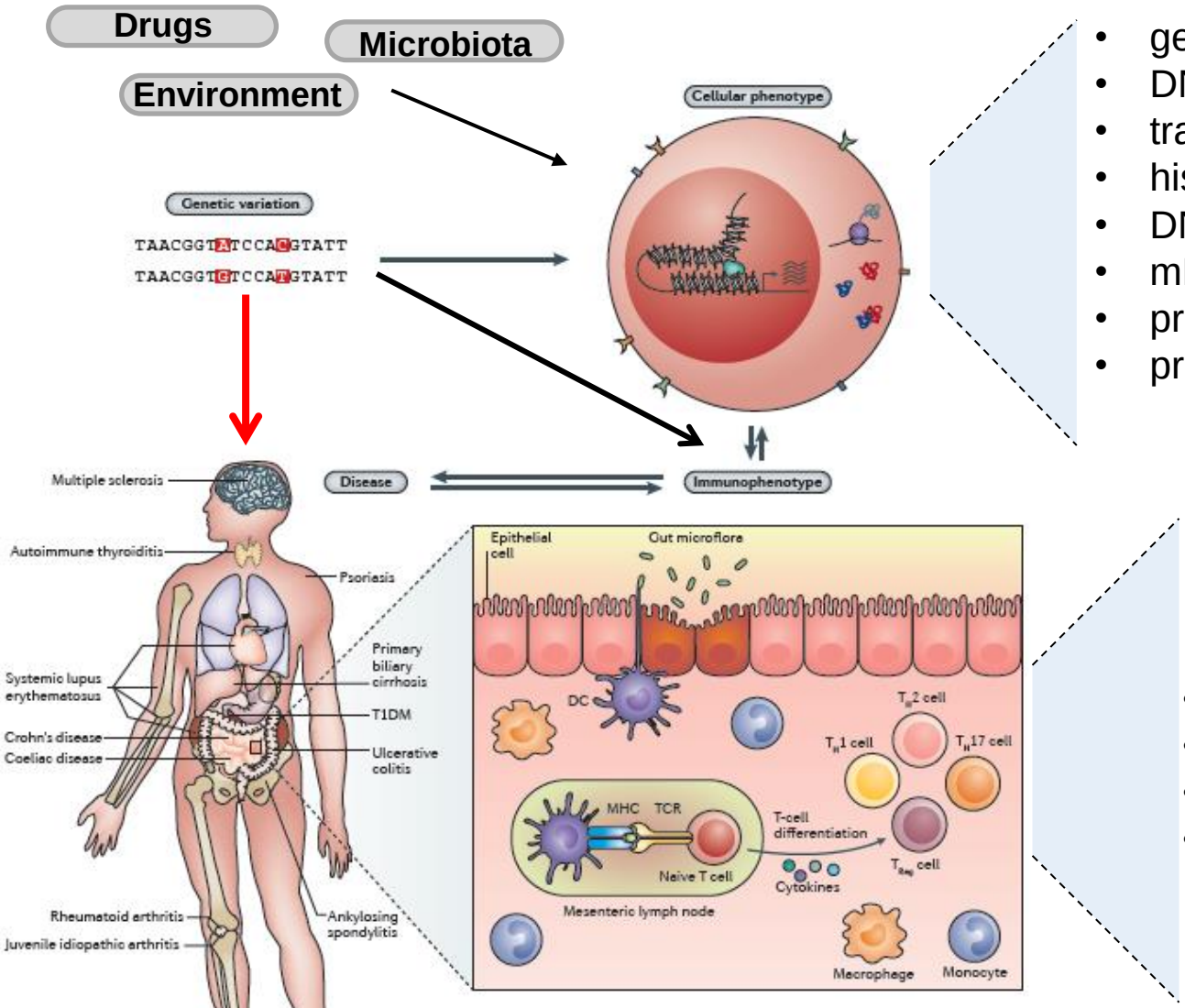
- Introduction to Systems Immunology Concepts
- Brief view/recap on immune profiling technologies and applications
  - Sequencing
  - Bulk vs Single-Cell
  - Application of Single-Cell sequencing
- Main techniques and rationales for data aggregation and visualization

The Immune System





Systems Immunology

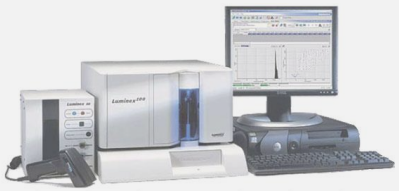


- gene transcription
- DNA–DNA interactions
- transcription factor binding
- histone modifications
- DNA methylation
- mRNA stability and translation
- protein levels
- protein–protein interactions

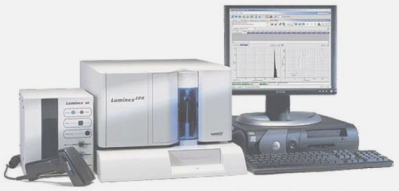
- signalling response
- cell-type abundances
- RNA/protein levels
- cytokine production

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

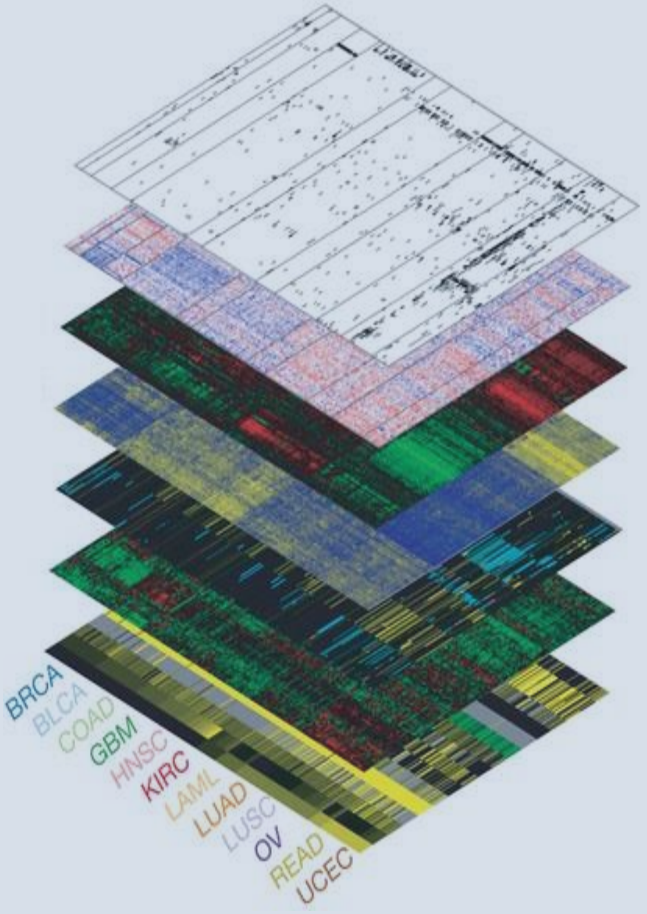
Technologies



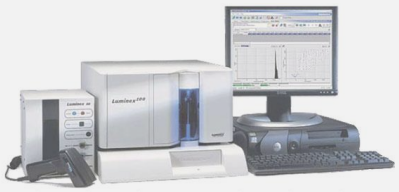
Technologies



Data



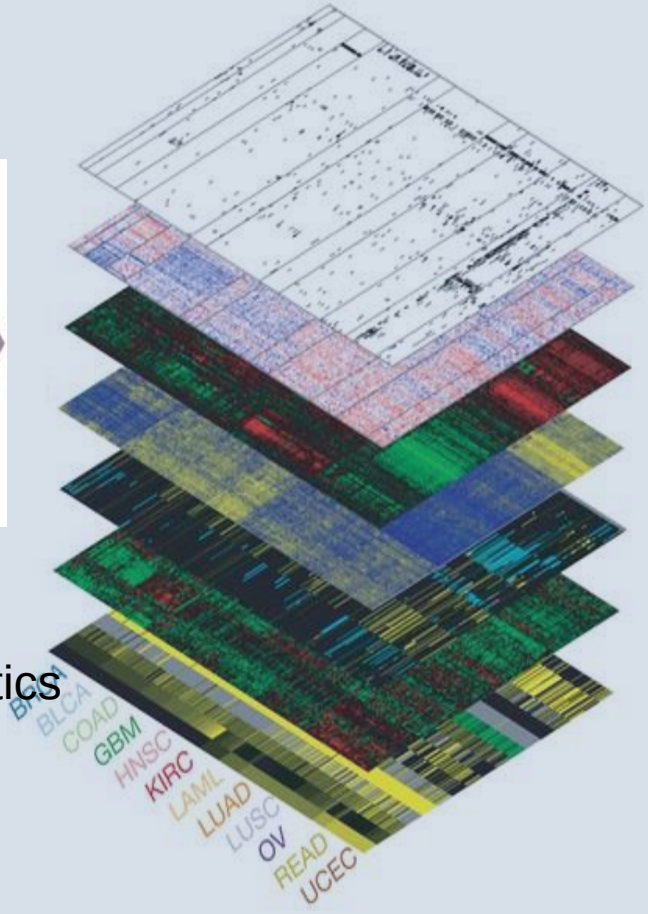
Technologies



Analysis

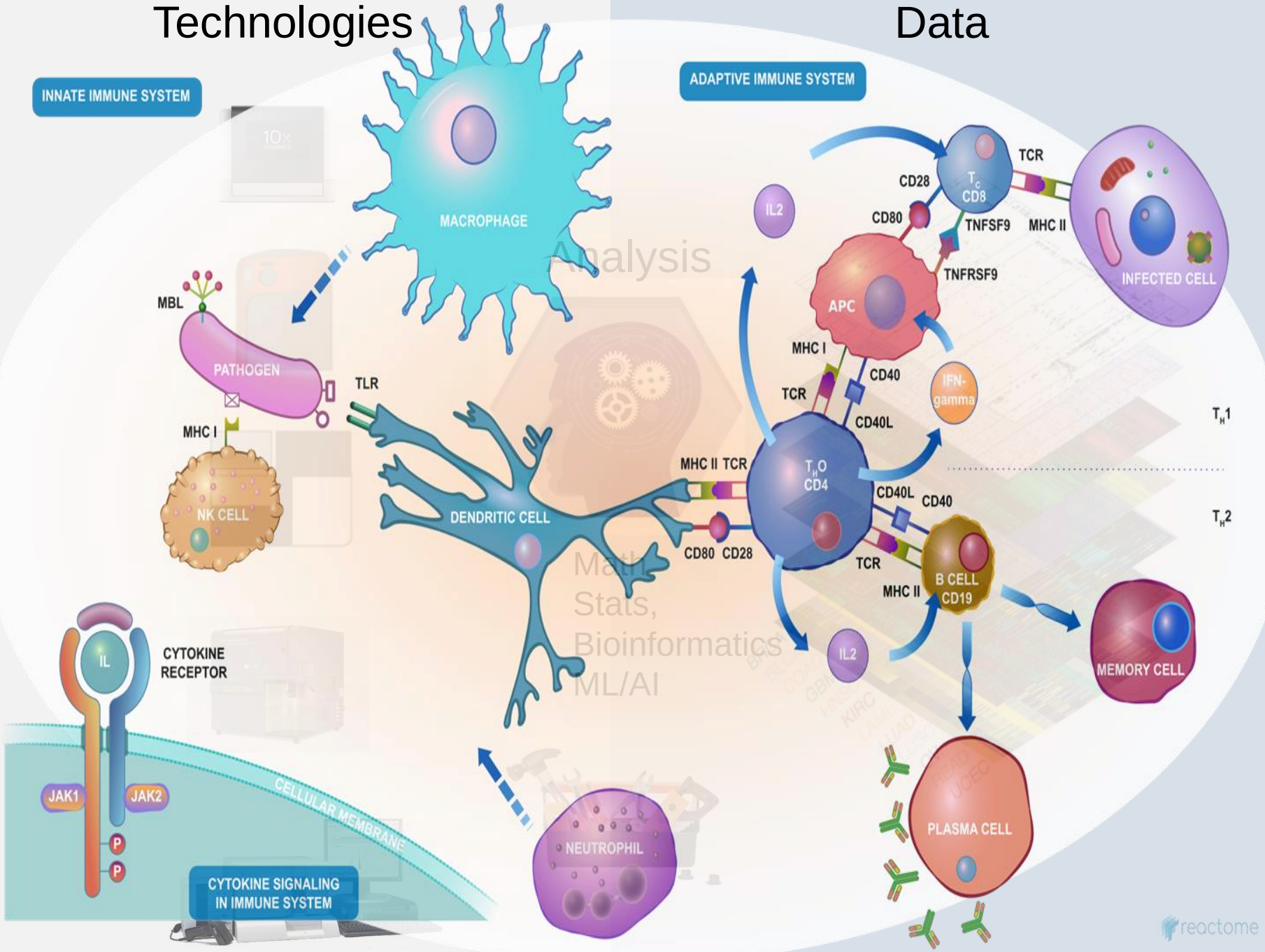
Math
Stats,
Bioinformatics
ML/AI

Data



Technologies

Data



Immune Cell Profiling: Flow Cytometry and Gene Expression

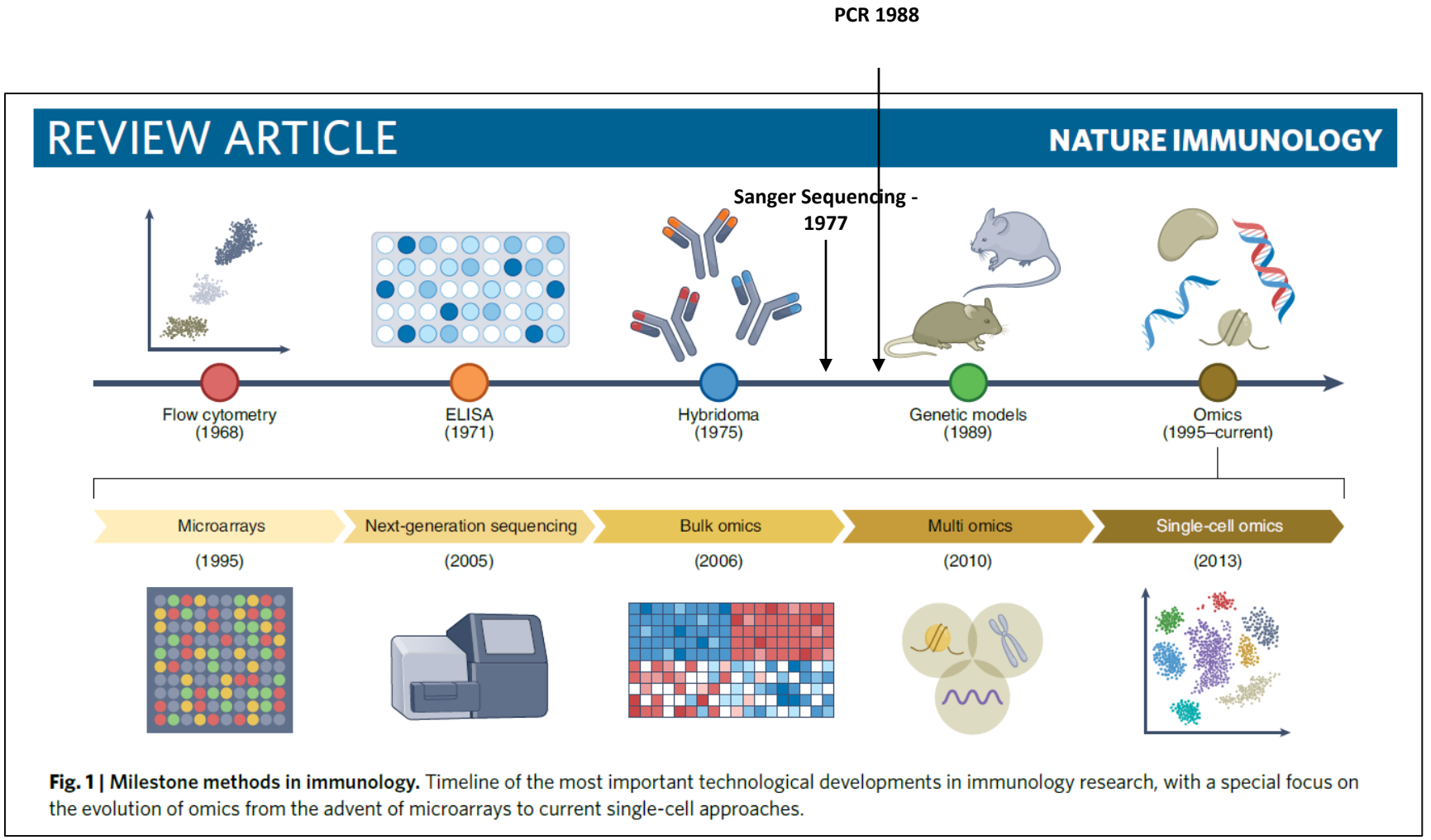
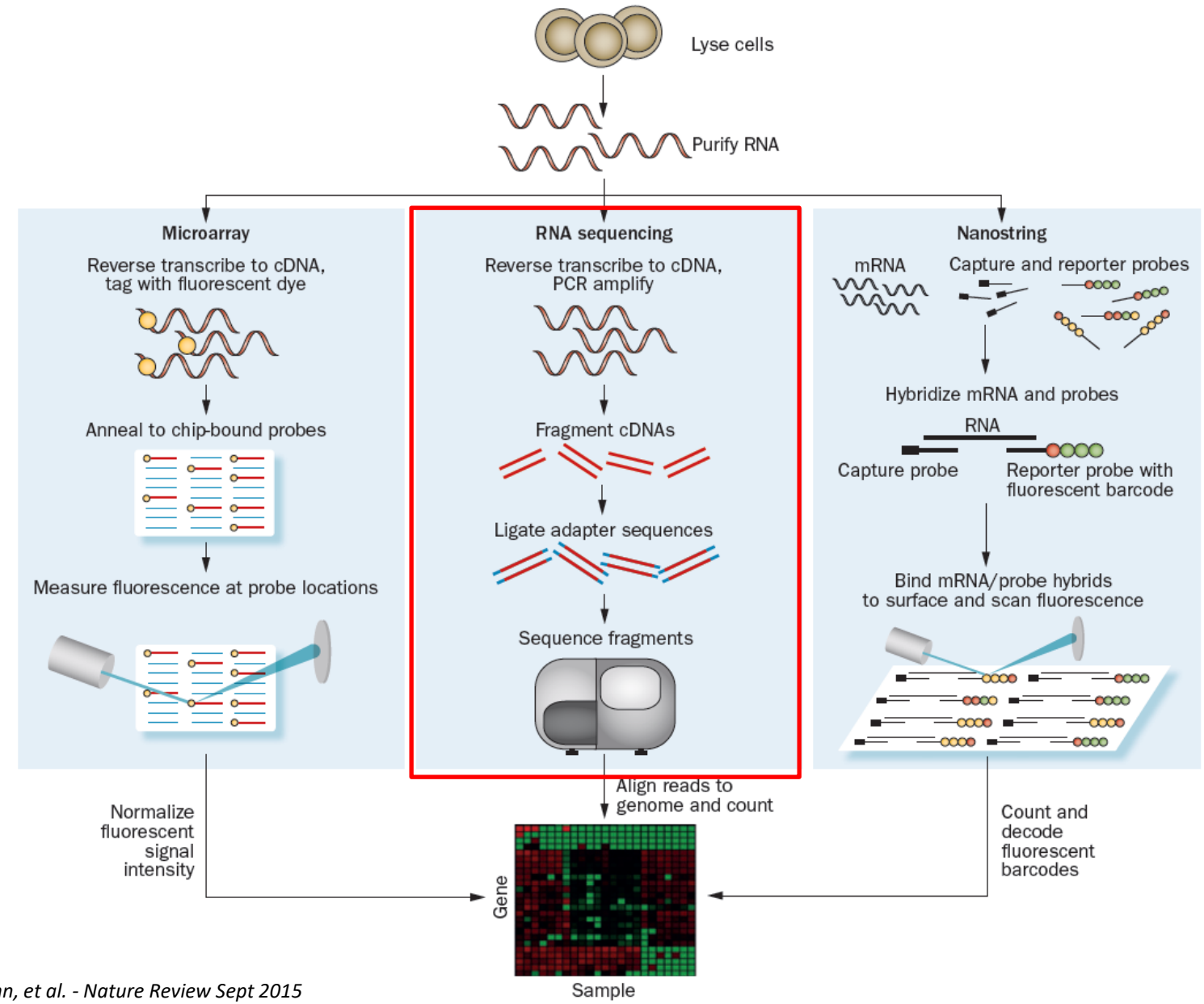


Fig. 1 | Milestone methods in immunology. Timeline of the most important technological developments in immunology research, with a special focus on the evolution of omics from the advent of microarrays to current single-cell approaches.

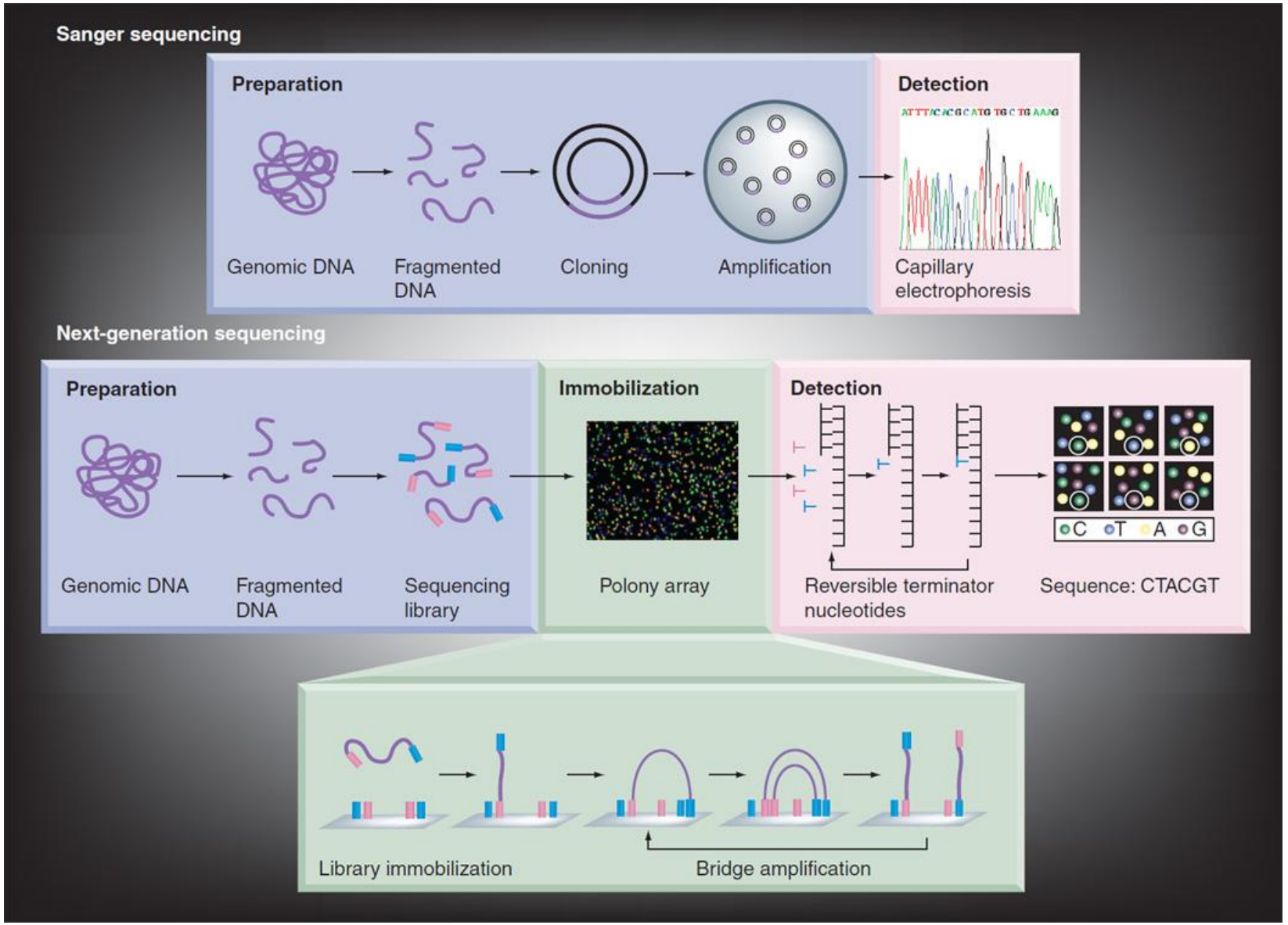
Nature Immunology volume 23, pages1412–1423 (2022)

Gene Expression in “Bulk” Samples



Joerg Ermann, et al. - Nature Review Sept 2015

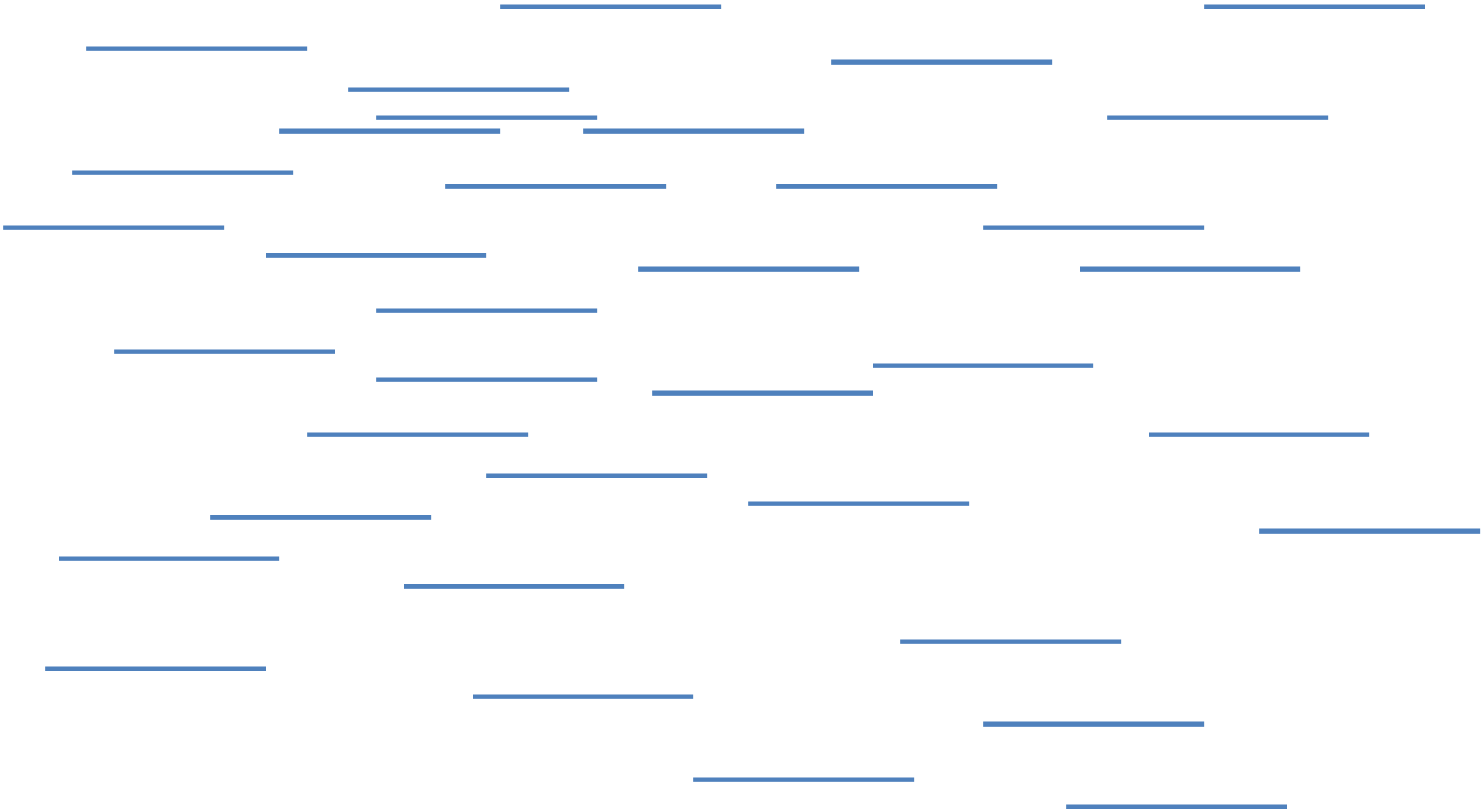
Next Generation Sequencing – The Illumina Platform



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

Lots of sequenced reads…

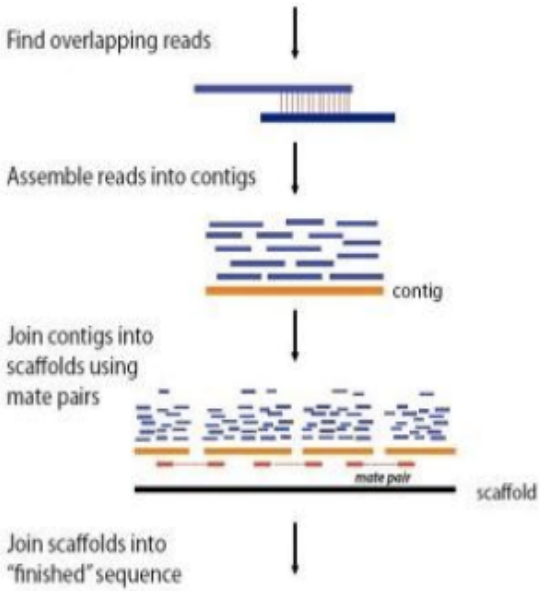
Illumina NovaSeq 6000: up to 20 billion reads, 3.000Gb data, less than 2 days



What do we do with them ?

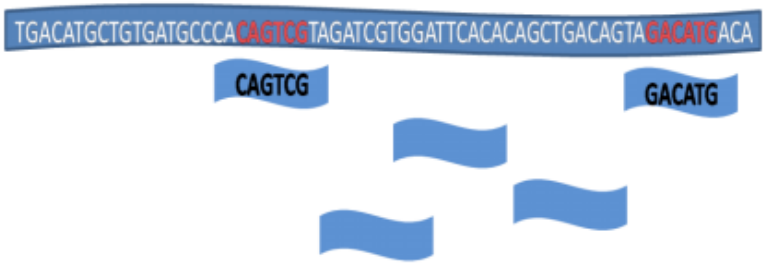
Reads

denovo Assembly



Reference based analysis

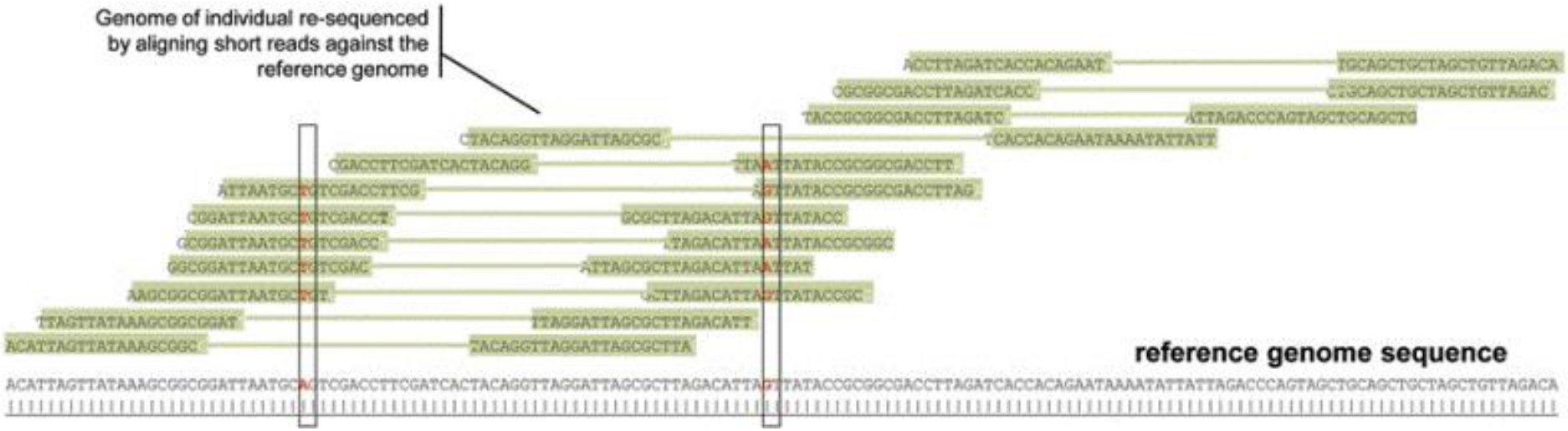
Analysis based on a known reference genome



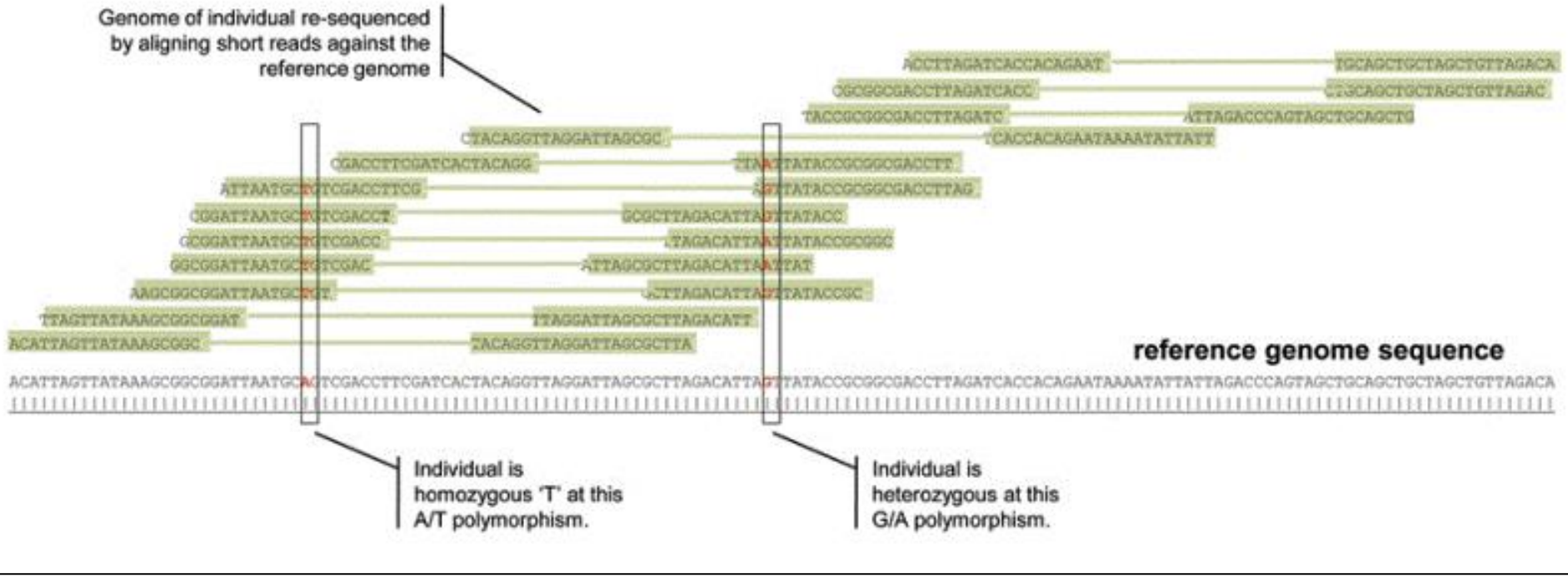
Sequencing of a new organism
Meta-Genomics
Reconstructing cancer genomes

Organism specific experiments
Annotating functional elements

Reads Alignment

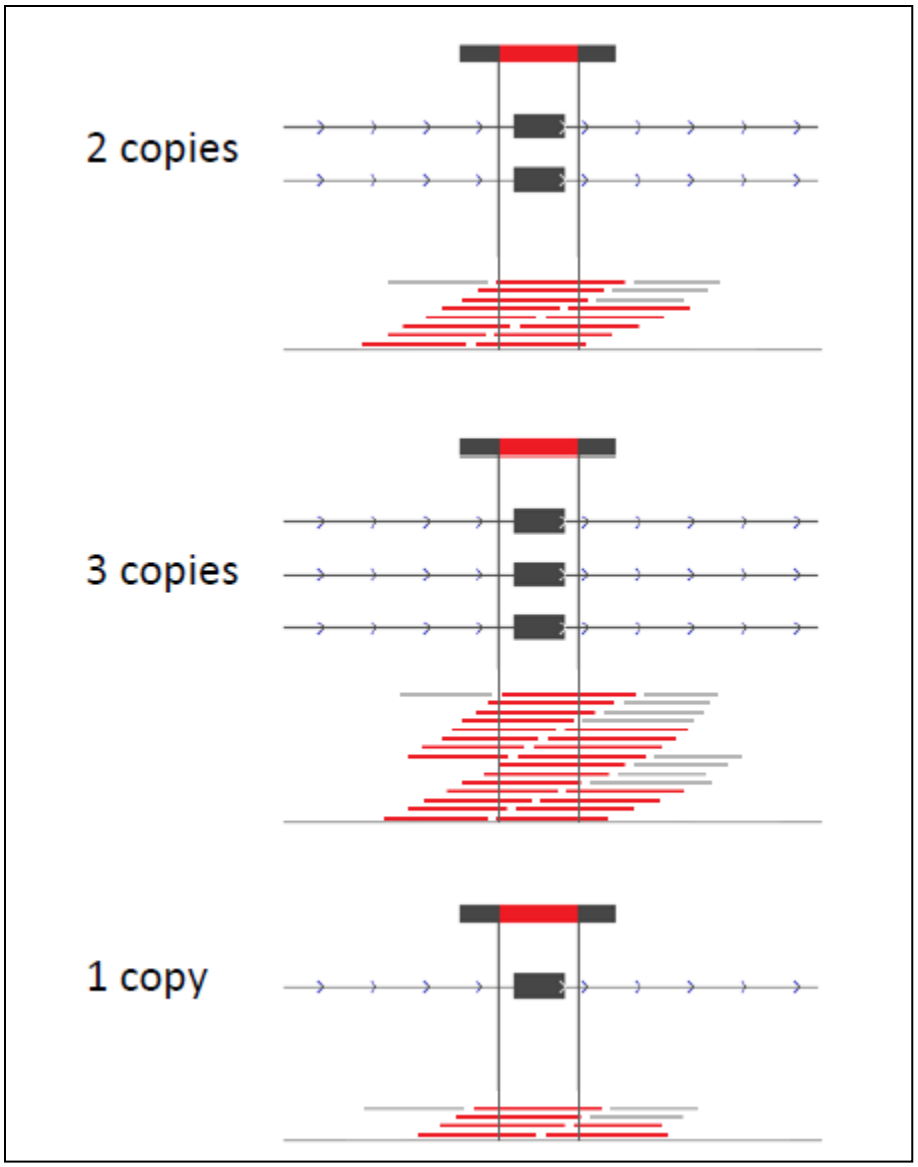


Genome Sequencing: Variant Calling



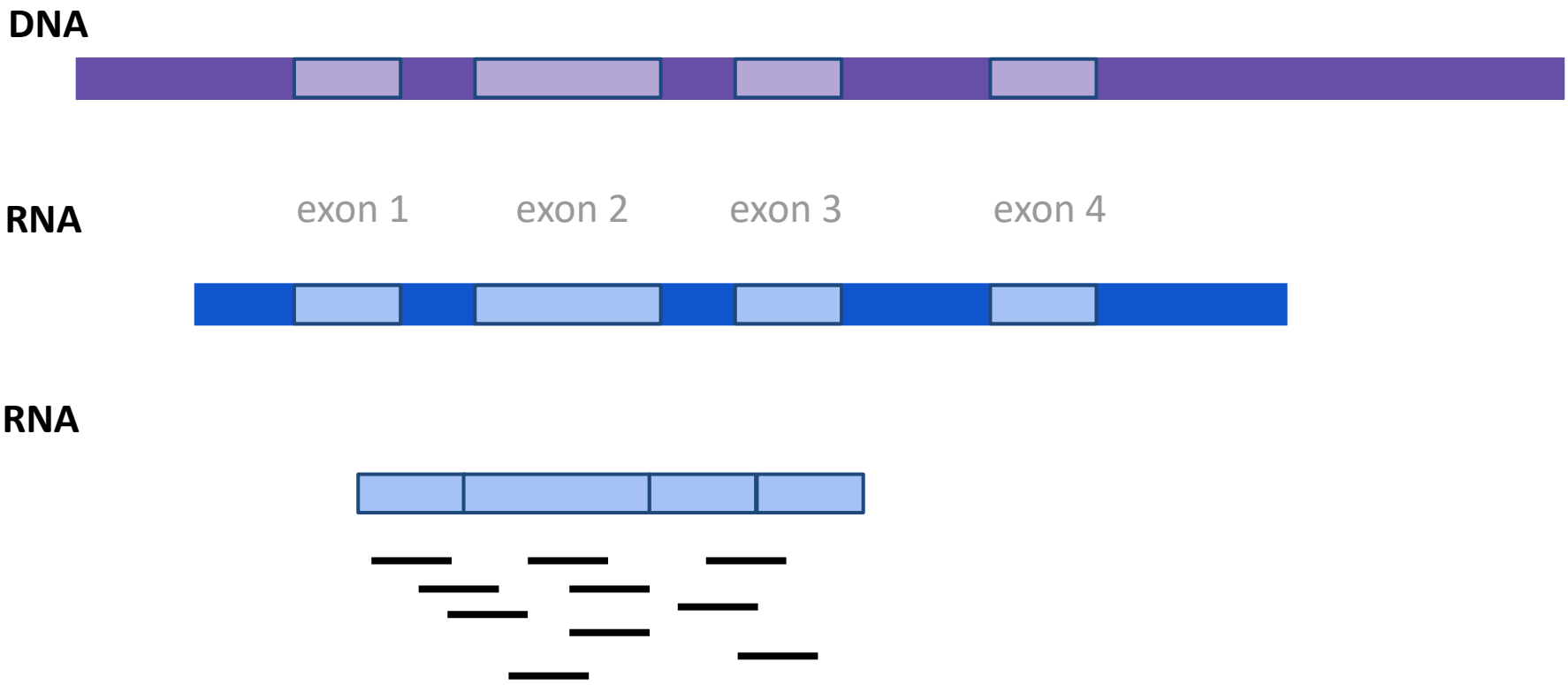
Differences between aligned reads and reference genome can be identified ➔ variants

Genome Sequencing: Copy Numbers



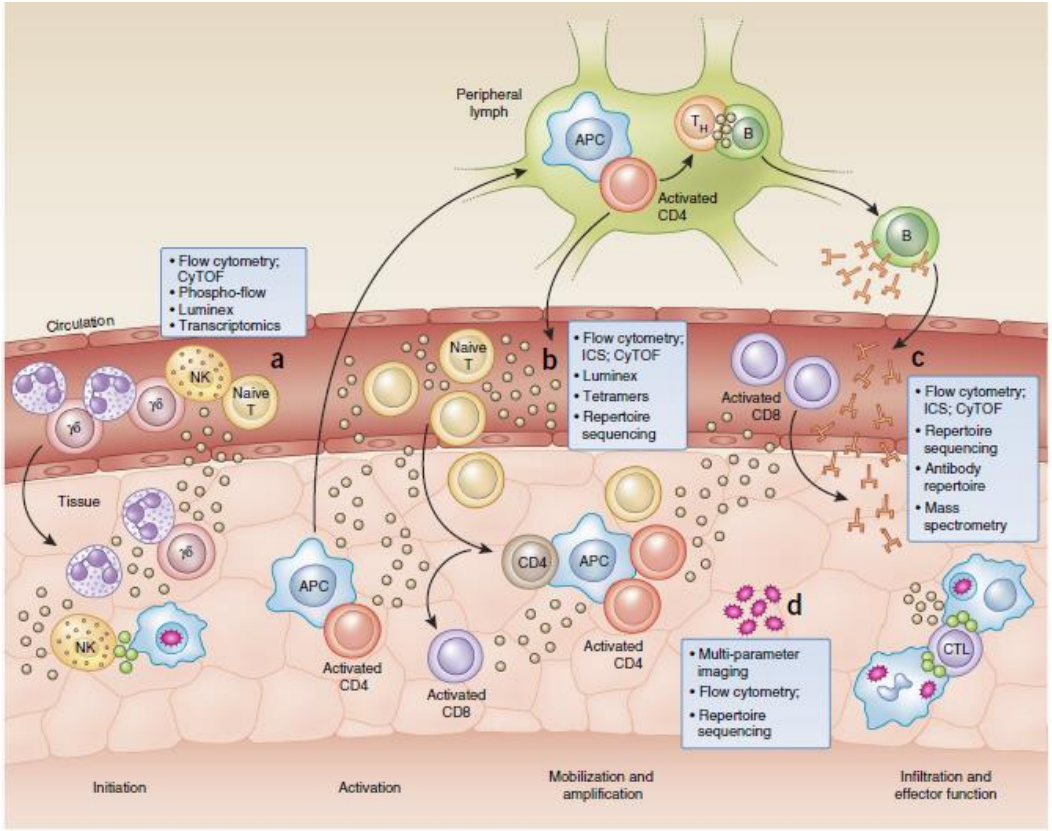
- The number of aligned reads on a given regions is proportional to the number of starting DNA copies of that region
- This information can be used to infer DNA copy number variations (CNVs)

RNA Sequencing

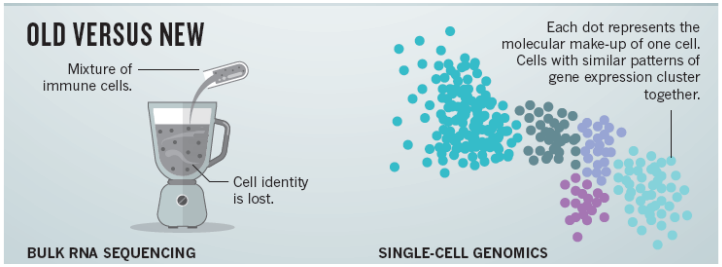


- The number of aligned reads on a given RNA is proportional to the number of its starting molecules
- This information can be used to infer RNA abundances

Immune Systems Heterogeneity



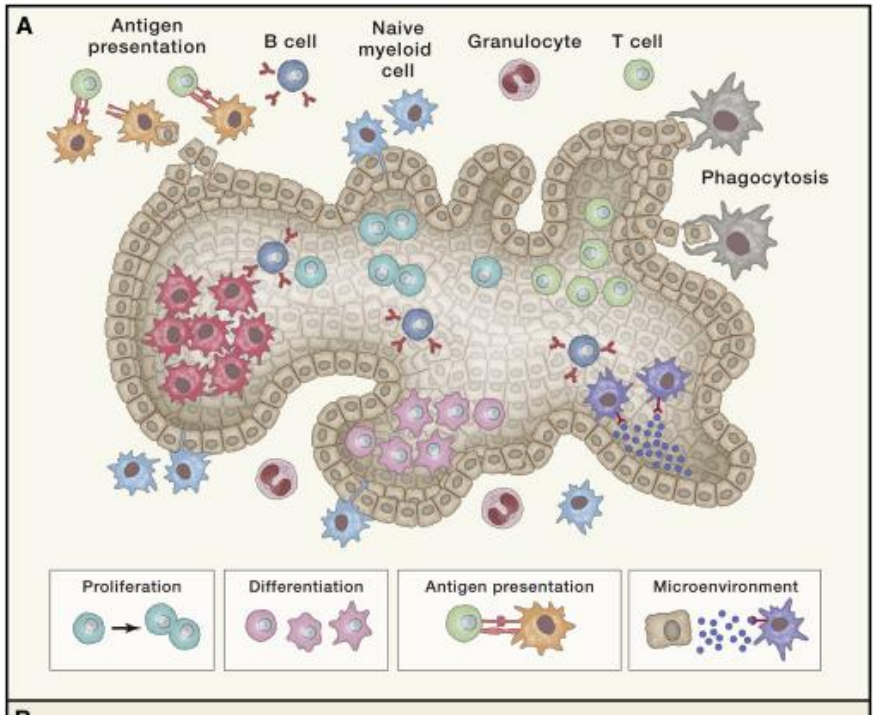
Davis et al. - Nature Immunology **18**, 725–732 (2017)

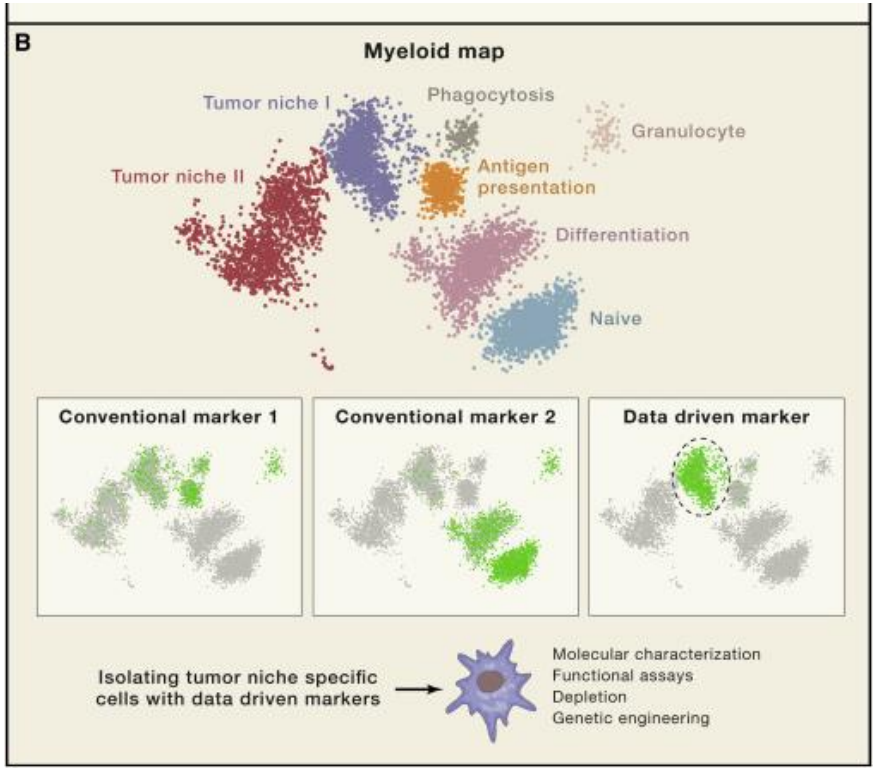


6 JULY 2017 | VOL 547 | NATURE | 27

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

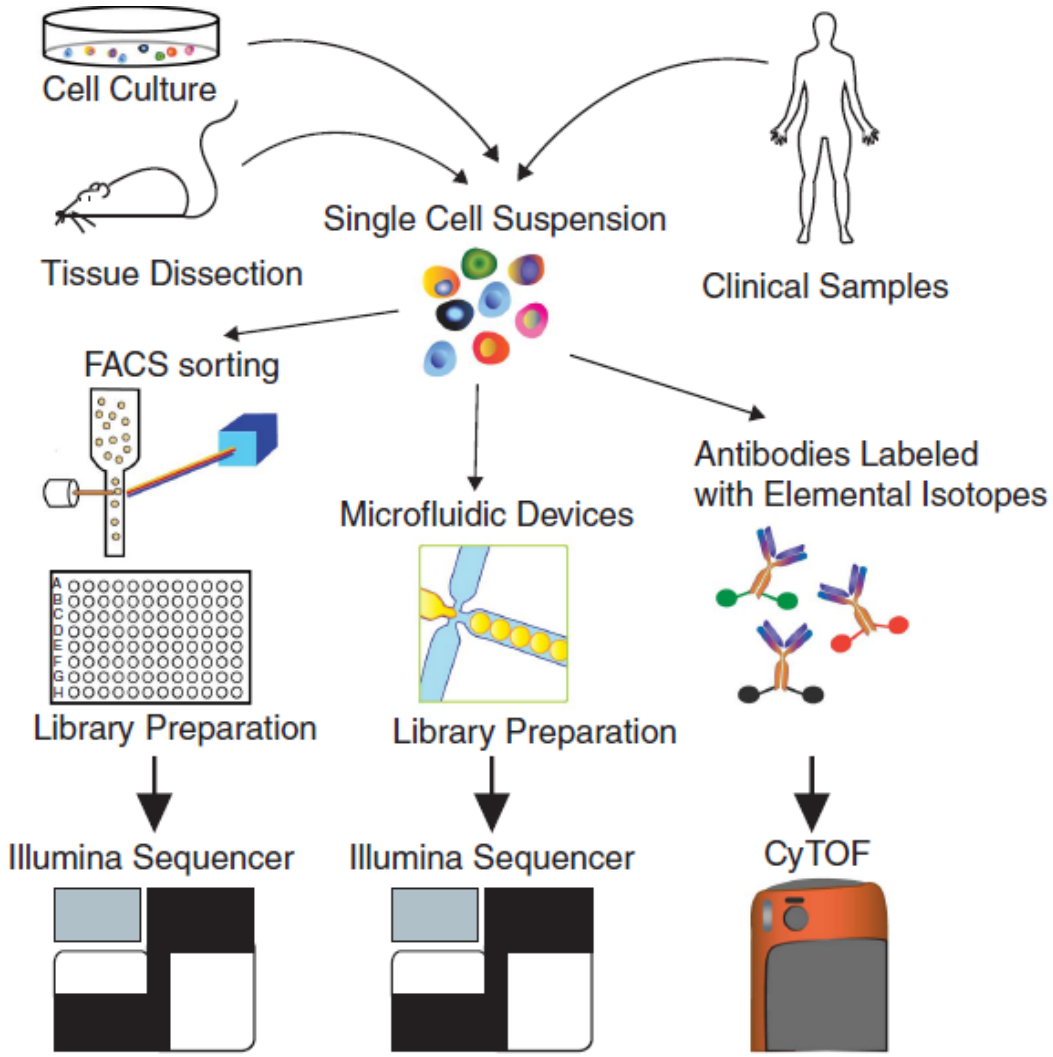
Dissecting tissue heterogeneity at single-cell level





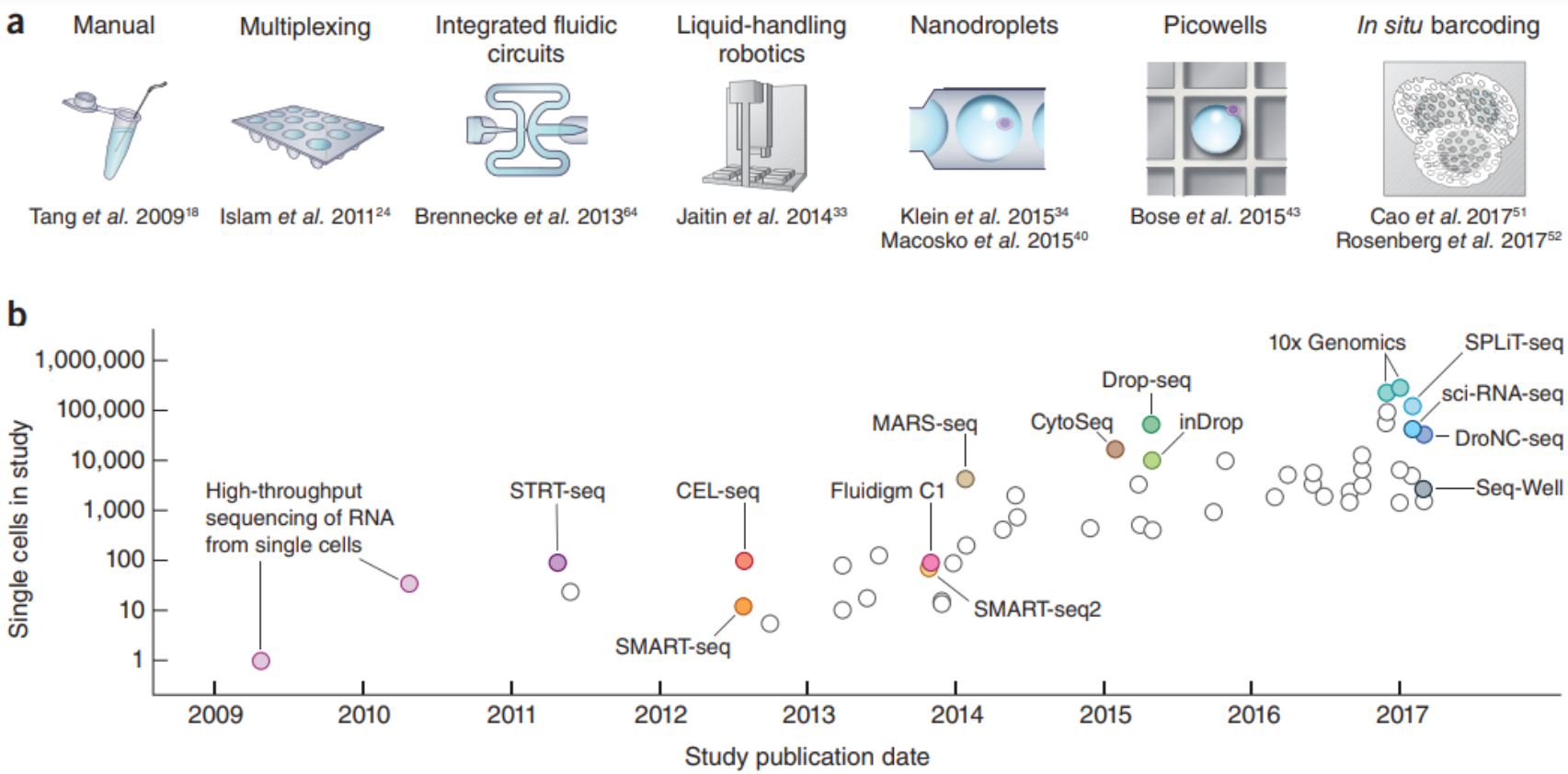
Giladi A, Amit I. Cell. 2018 Jan

Single-Cell Analysis



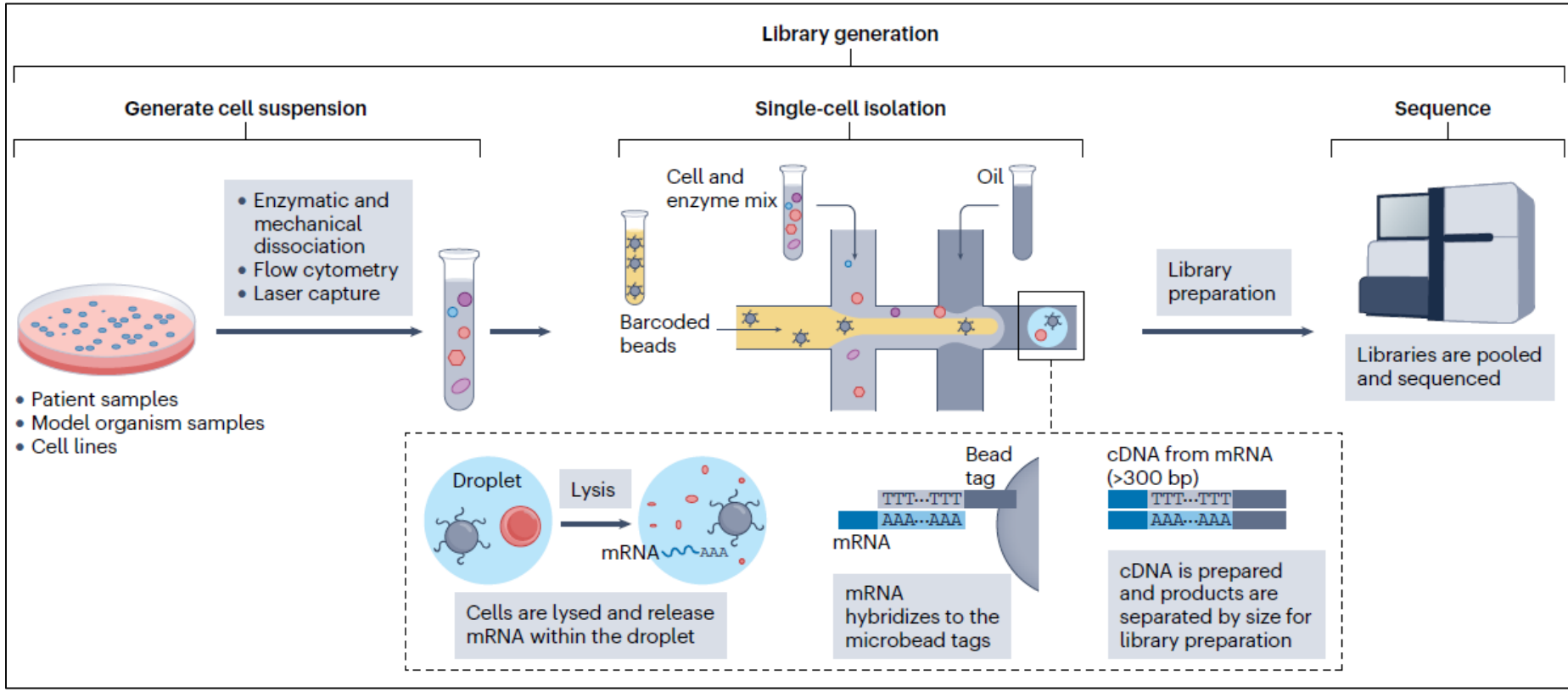
Imm. and Cell Biol. (2016) 94, 225–229;

Development of Single-Cell Technologies



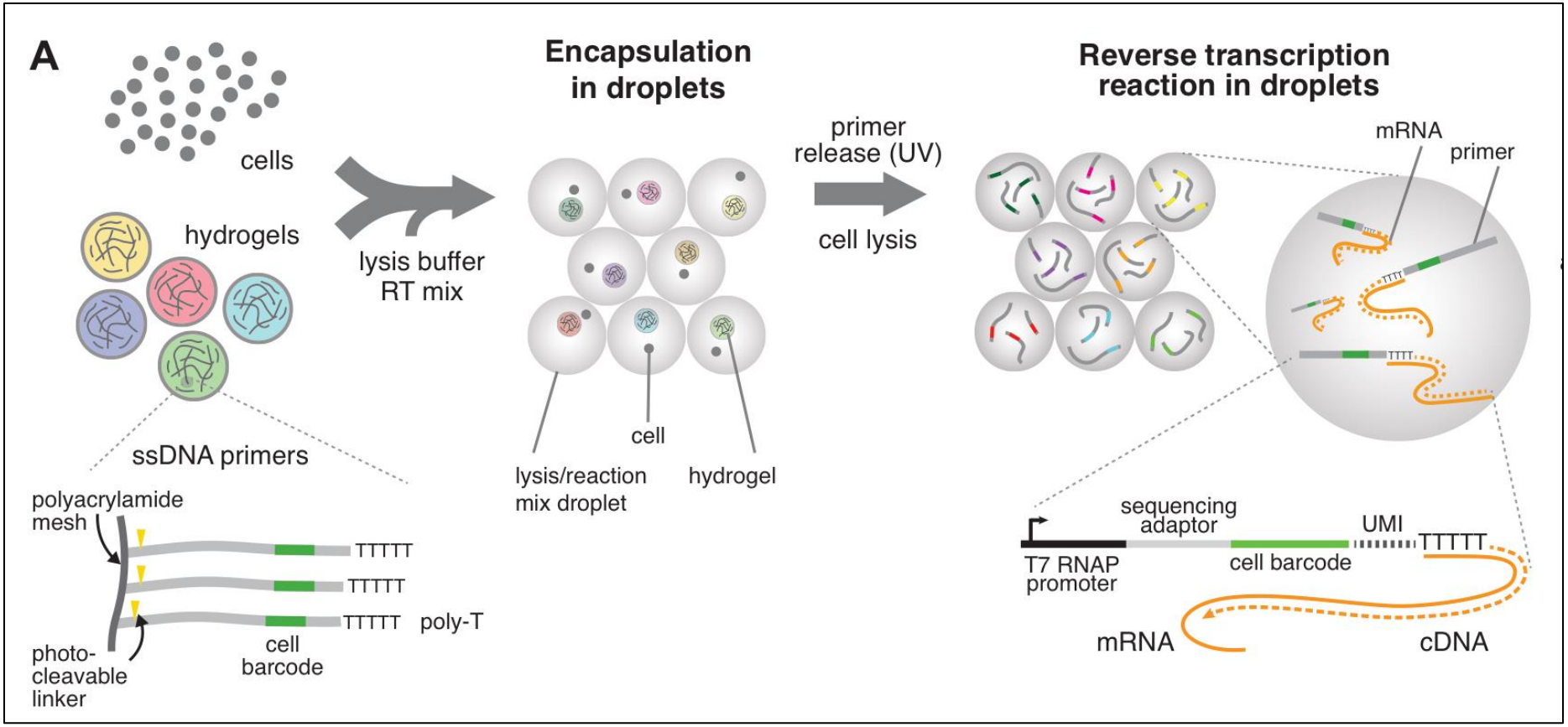
Svensson V, Vento-Tormo R, Teichmann SA.Nat Protoc. 2018

Basic workflow in 10x Chromium



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

InDrop: Barcoding process



Zilionis et al., Nature Protocols 2017

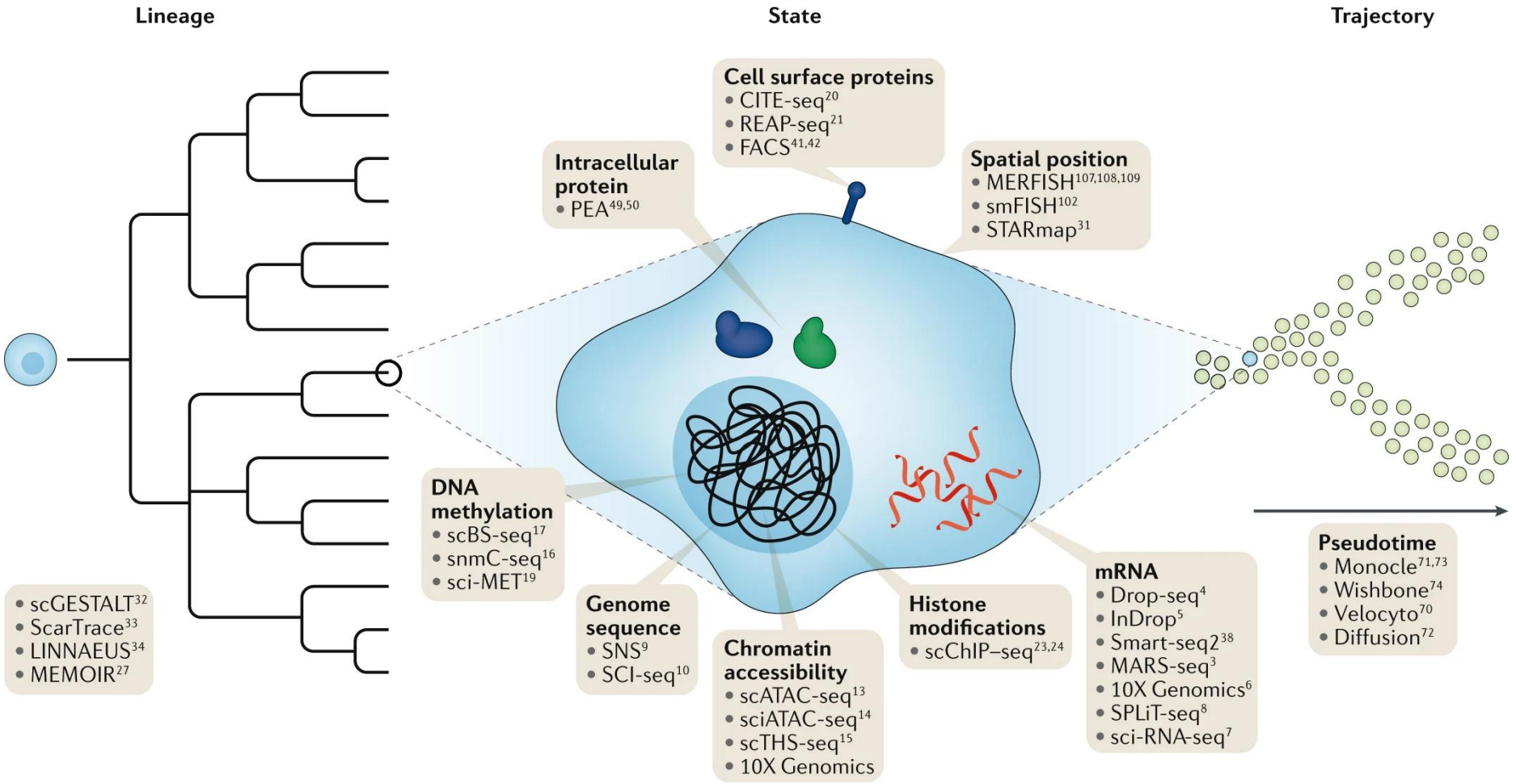
Single-Cell Sequencing Methods

TABLE 1 | Summary of single-cell RNA sequencing methods.

Method	Fluidigm C1 system (SMART-seq)	Fluidigm C1 system (mRNA Seq HT)	SMART-seq2	10X Genomics Chromium system	MARS-seq
cDNA coverage	Full-length	3′ counting	Full-length	5′/3′ counting	3′ counting
UMI	No	No	No	Yes	Yes
Amplification technology	Template switching-based PCR	Template switching-based PCR	Template switching-based PCR	Template switching-based PCR	*in vitro* transcription
Multiplexing of samples	No	Yes	No	Yes	Yes
Single cell isolation	Fluidigm C1 machine	Fluidigm C1 machine	FACS	10X Genomics Chromium single cell controller	FACS
Cell size limitations	Homogenous size of 5–10, 10–17, or 17–25 μM	Homogenous size of 5–10, 10–17, or 17–25 μM	Independent of cell size	Independent of cell size	Independent of cell size
Required cell numbers per run	≥10,000	≥10,000	No limitation	≥20,000	No limitation
Visual quality control check	Microscope examination	Microscope examination	No	No	No
Long term storage	No, must process immediately	No, must process immediately	Yes	No, must process immediately	Yes
Throughput	Limited by number of machines	Limited by number of machines	Limited by operator efficiency	Up to 8 samples per chip	Process is automated
Cost	+ + + + +	+ + +	+ + + +	+	+ +
Sample Preparation Scenario 1 (~5000 single cell)	Targeted cell No: 4992 cells	Targeted cell No: 4800 cells	Targeted cell No: 4992 cells	Targeted cell No: 5000 cells	Targeted cell No: 4992 cells
	26 rounds of 2 runs (2 C1 machines; concurrent)	3 rounds of 2 runs (2 C1 machines; concurrent)	26 rounds of 2 96-well plates	1 run	13 runs of 1 384-well plate
	~26 weeks	~3 weeks	~26 weeks	~2–3 days	~7 weeks
Sample Preparation Scenario 2 (~96 single cell)	Targeted cell No: 96 cells	Targeted cell No: Minimum 800 cell	Targeted cell No: 96 cells	Targeted cell No: Minimum 500 cells	Targeted cell No: 96 cells
	1 run (1 C1 machine)	1 run (1 C1 machine)	1 run of 96-well plates	1 run	1 run of 384-well plate
	~1 week	~1 week	~1 week	~2–3 days	~2–3 days

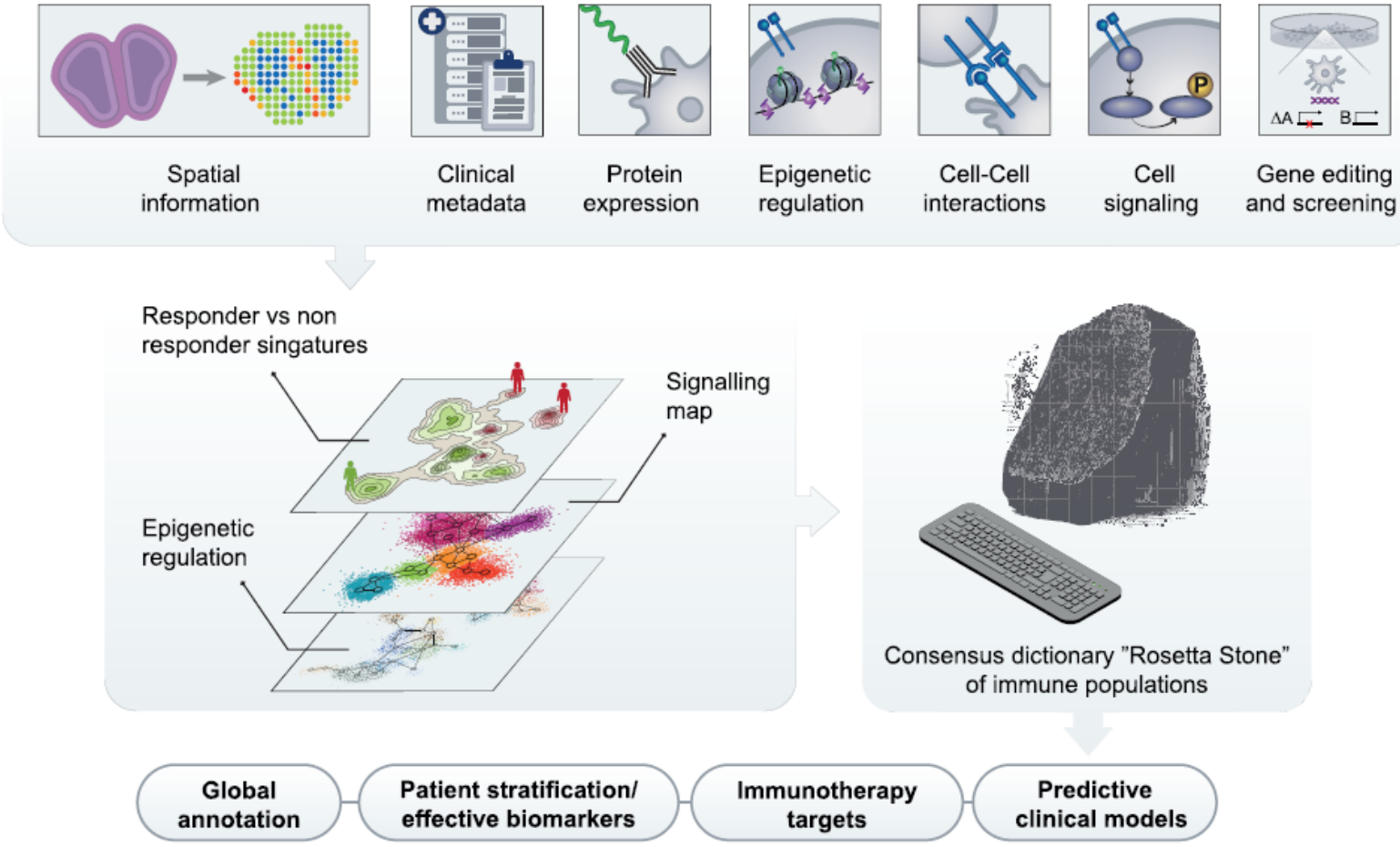
See P, Lum J, Chen J, Ginhoux F.Front Immunol. 2018 Oct 23;9:2425

Single-cell multi-omics



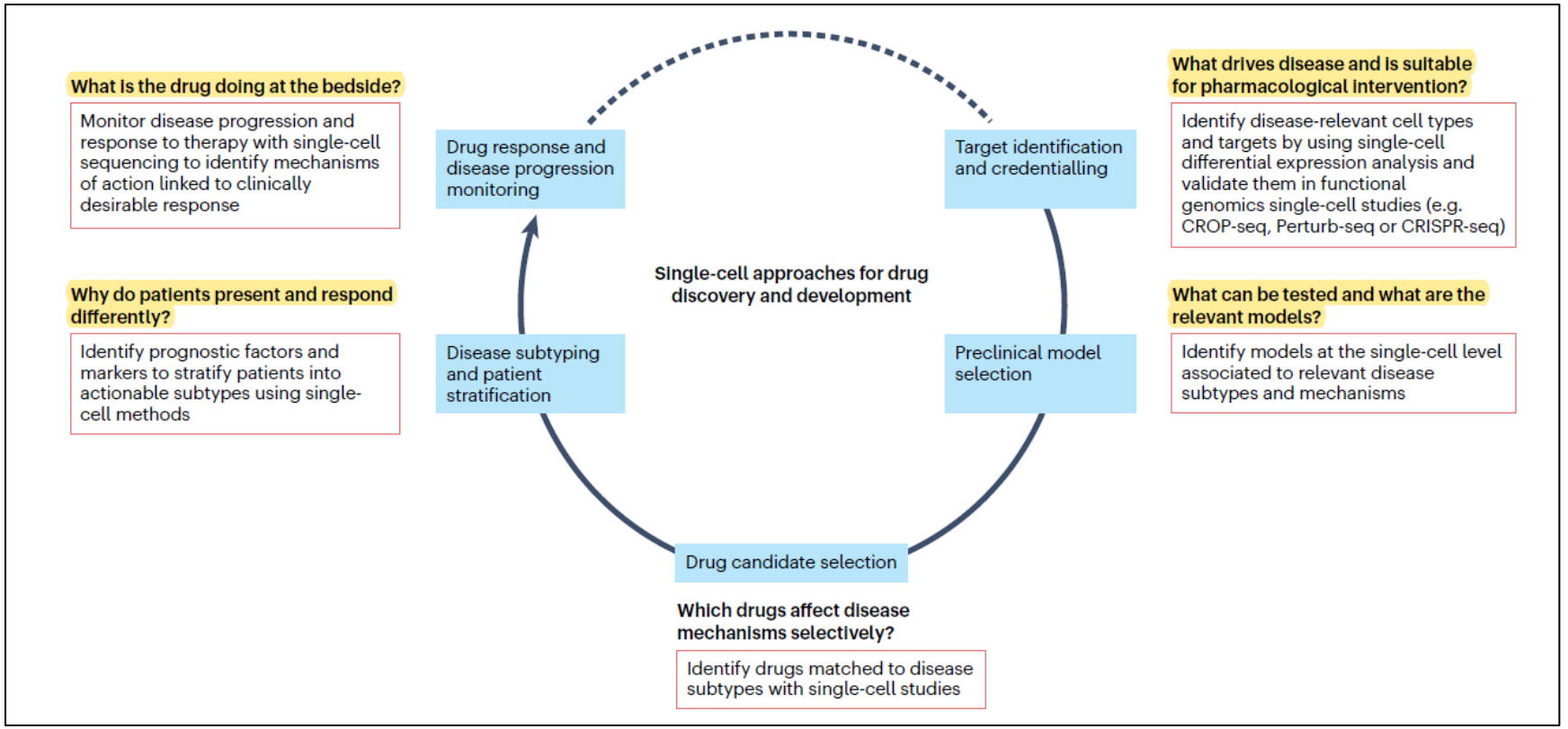
Stuart T, Satija R.Nat Rev Genet. 2019

Systems Biology Through Single-Cell Multi-omics



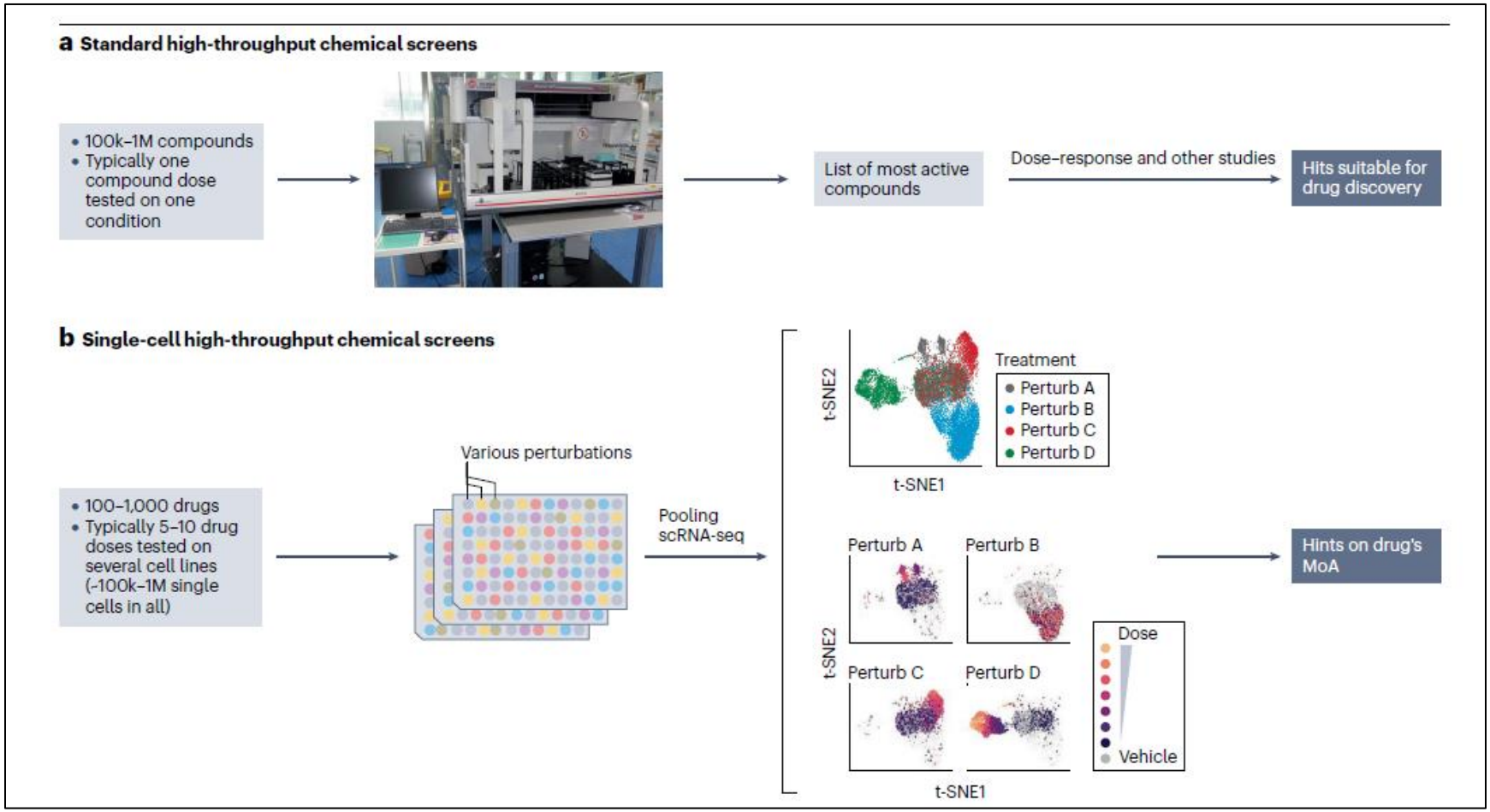
Ginhoux F, Yalin A, Dutertre CA, Amit I. Immunity. 2022

Applications T Drug Discovery



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

Applications To Drug Discovery



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

Single-Cell for Biomarker Discovery and Patient Stratification

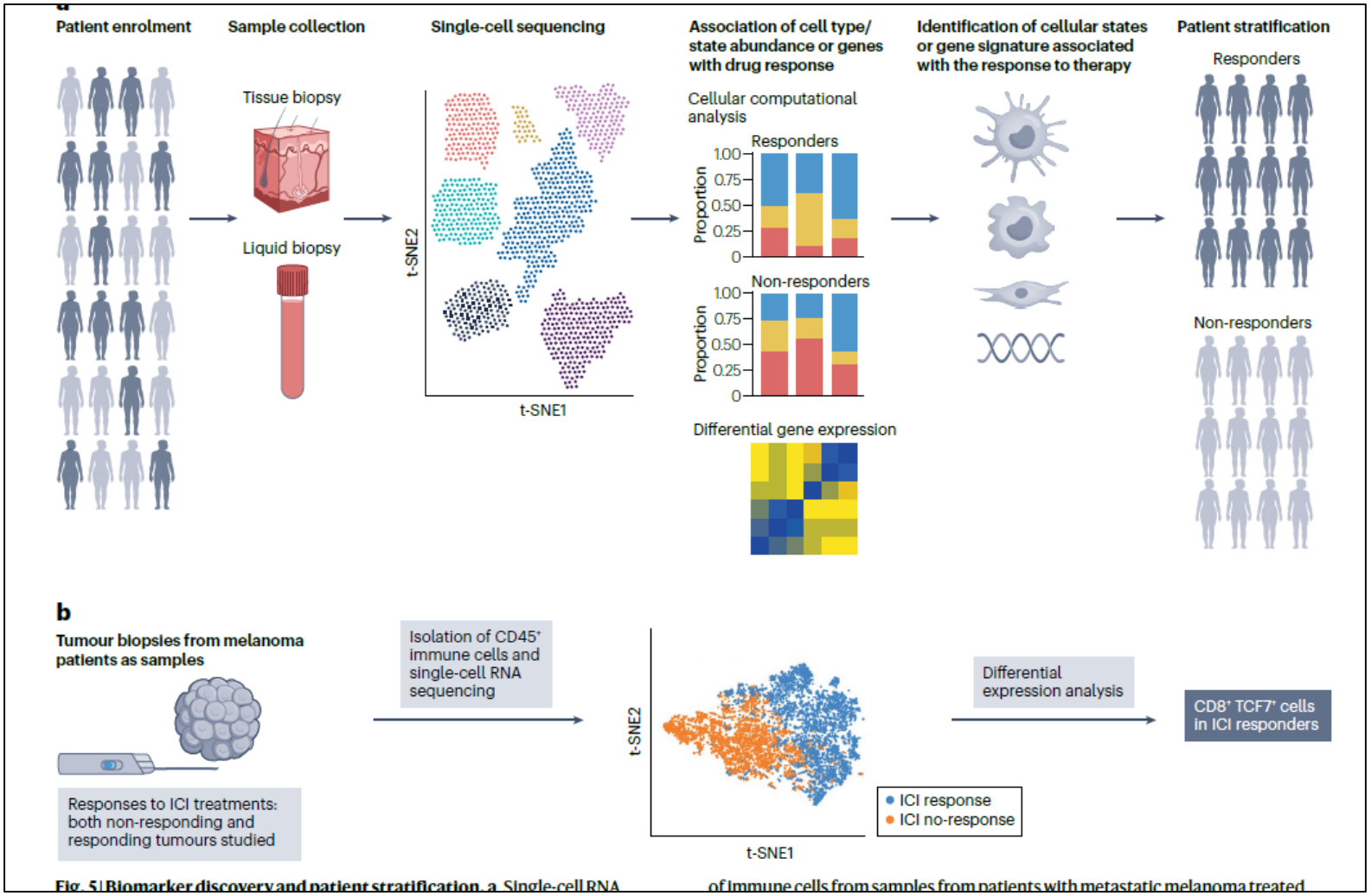
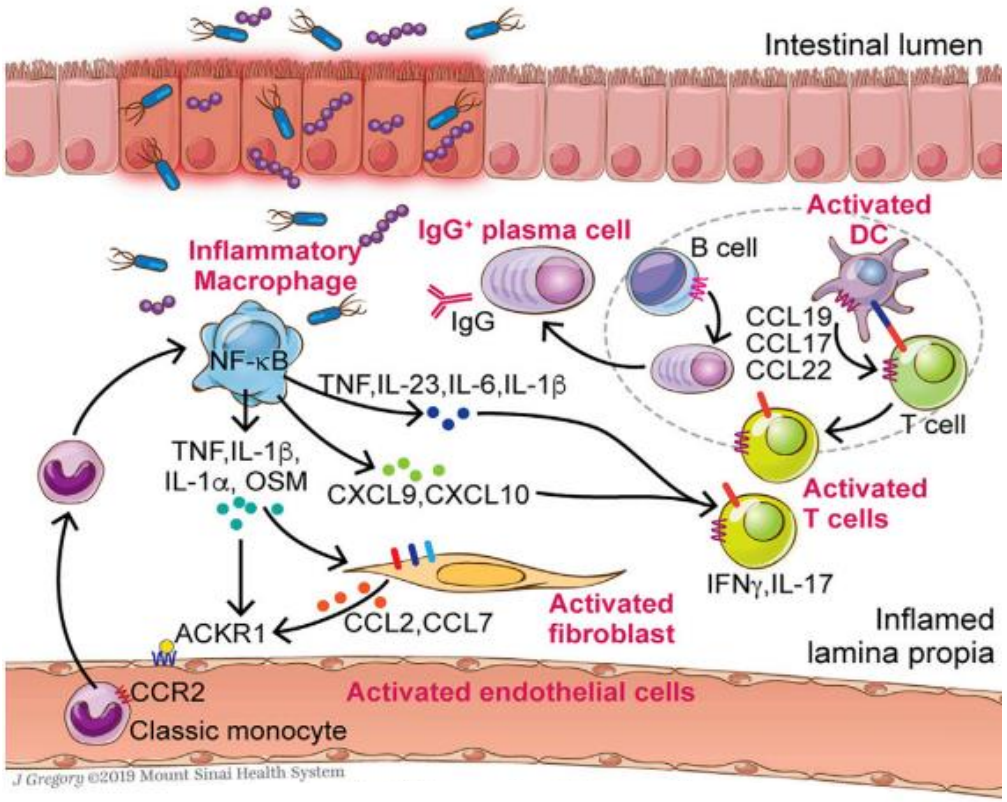


Fig. 5 | Biomarker discovery and patient stratification. a Single-cell RNA ... of immune cells from samples from patients with metastatic melanoma treated

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

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

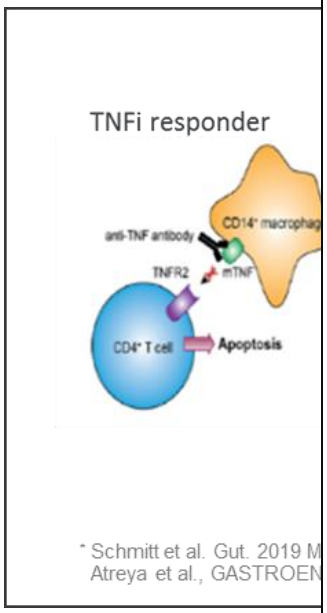
GIMATS^{high} Module in CD inflamed ileums associates with resistance to anti-TNF responder CD patients



- 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

Working Hypot
responders

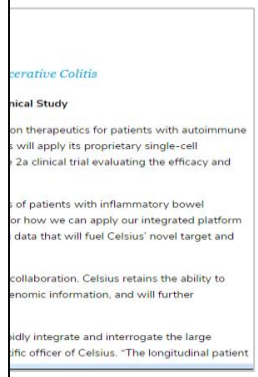
Results of Novel Clinical Study Show Adults with Moderately to Severely Active Ulcerative Colitis Achieved Higher Rates of Clinical Response, Clinical Remission, and Endoscopic Improvement at 12 Weeks with Guselkumab and Golimumab Combination Therapy Versus Either Monotherapy Alone

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

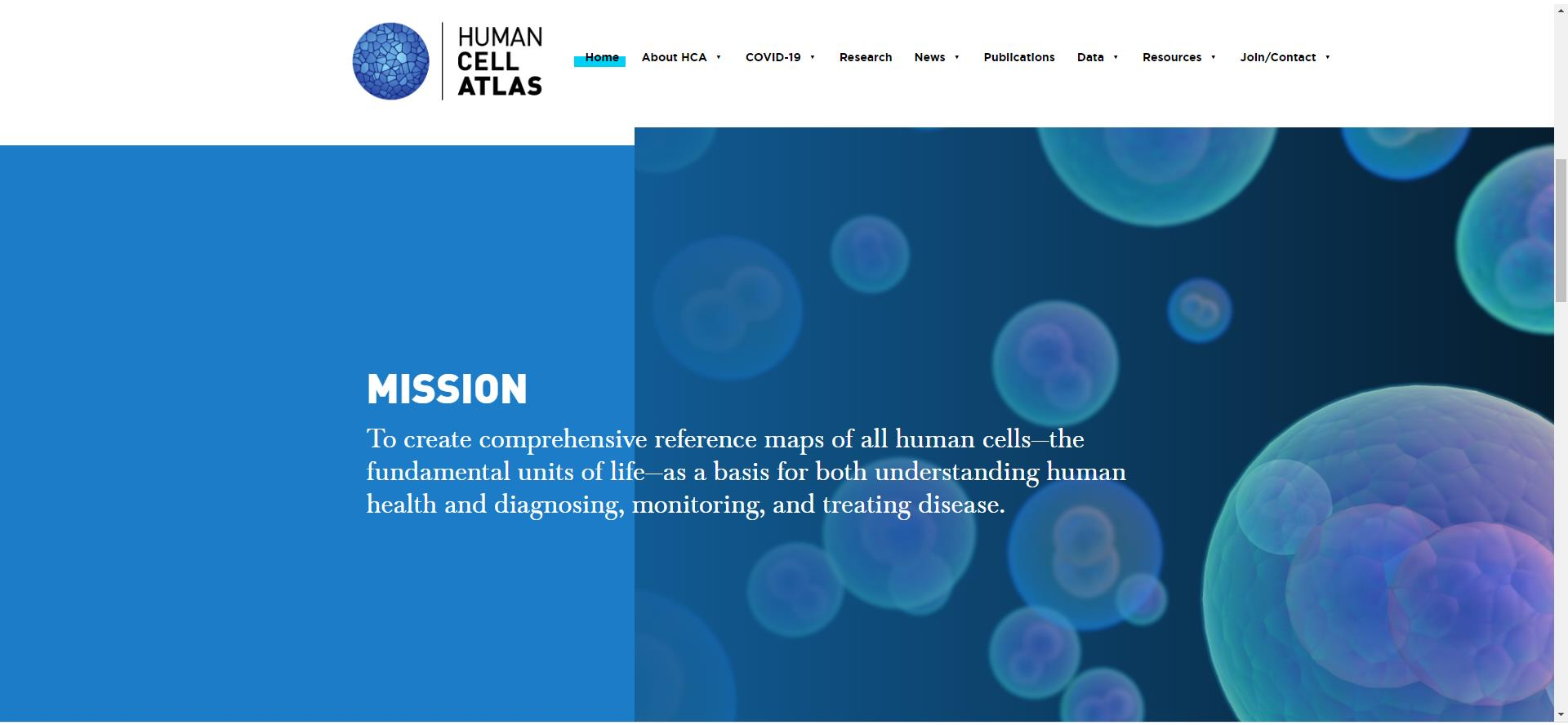
Janssen
PHARMACEUTICAL COMPANIES OF
Johnson & Johnson

f single-cell
in Phase 2a
apy


erative Colitis
nical Study
on therapeutics for patients with autoimmune
will apply its proprietary single-cell
2a clinical trial evaluating the efficacy and
of patients with inflammatory bowel
or how we can apply our integrated platform
data that will fuel Celsius' novel target and
collaboration. Celsius retains the ability to
enomic information, and will further
idly integrate and interrogate the large
fic officer of Celsius. "The longitudinal patient

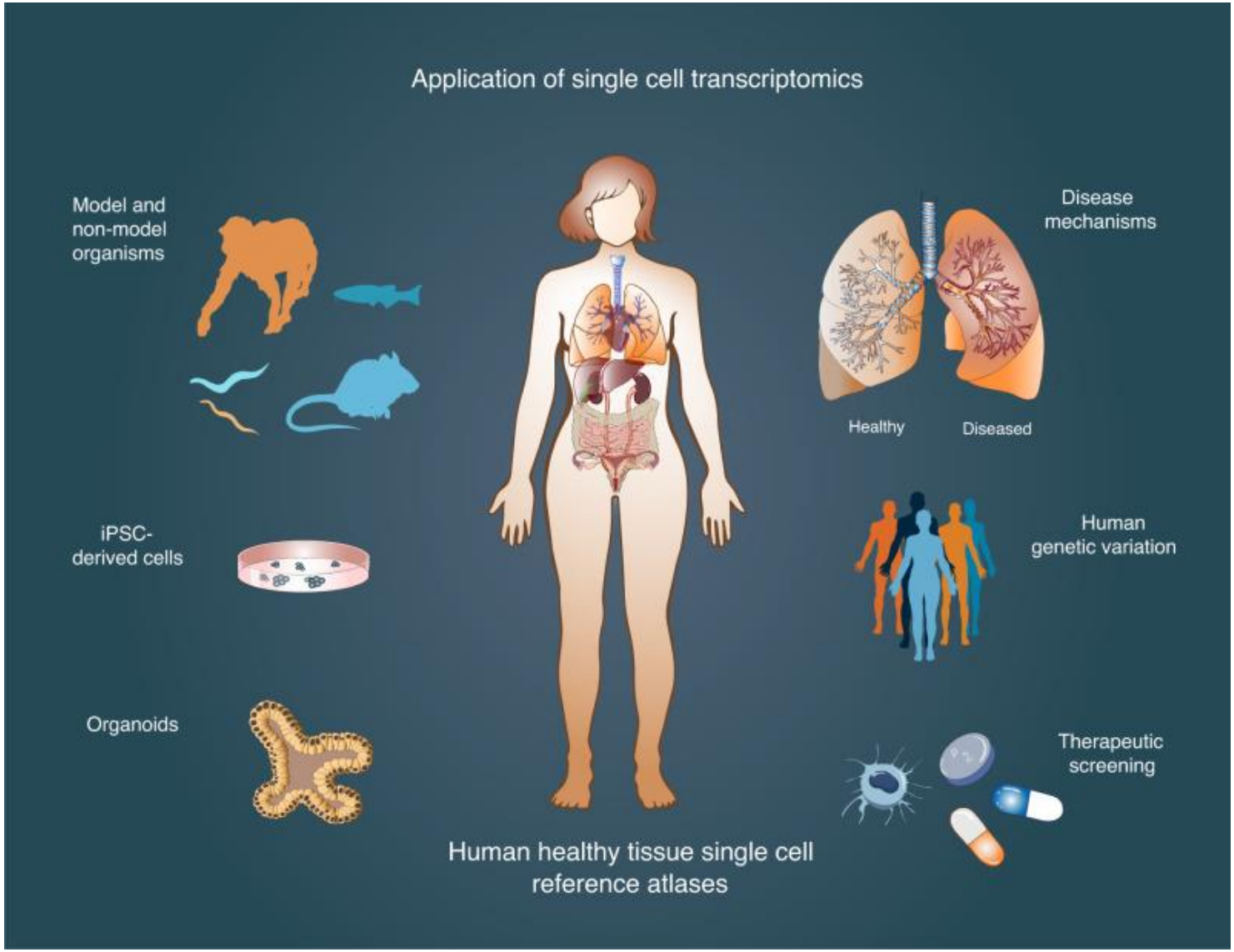
non-

Human Cell Atlas



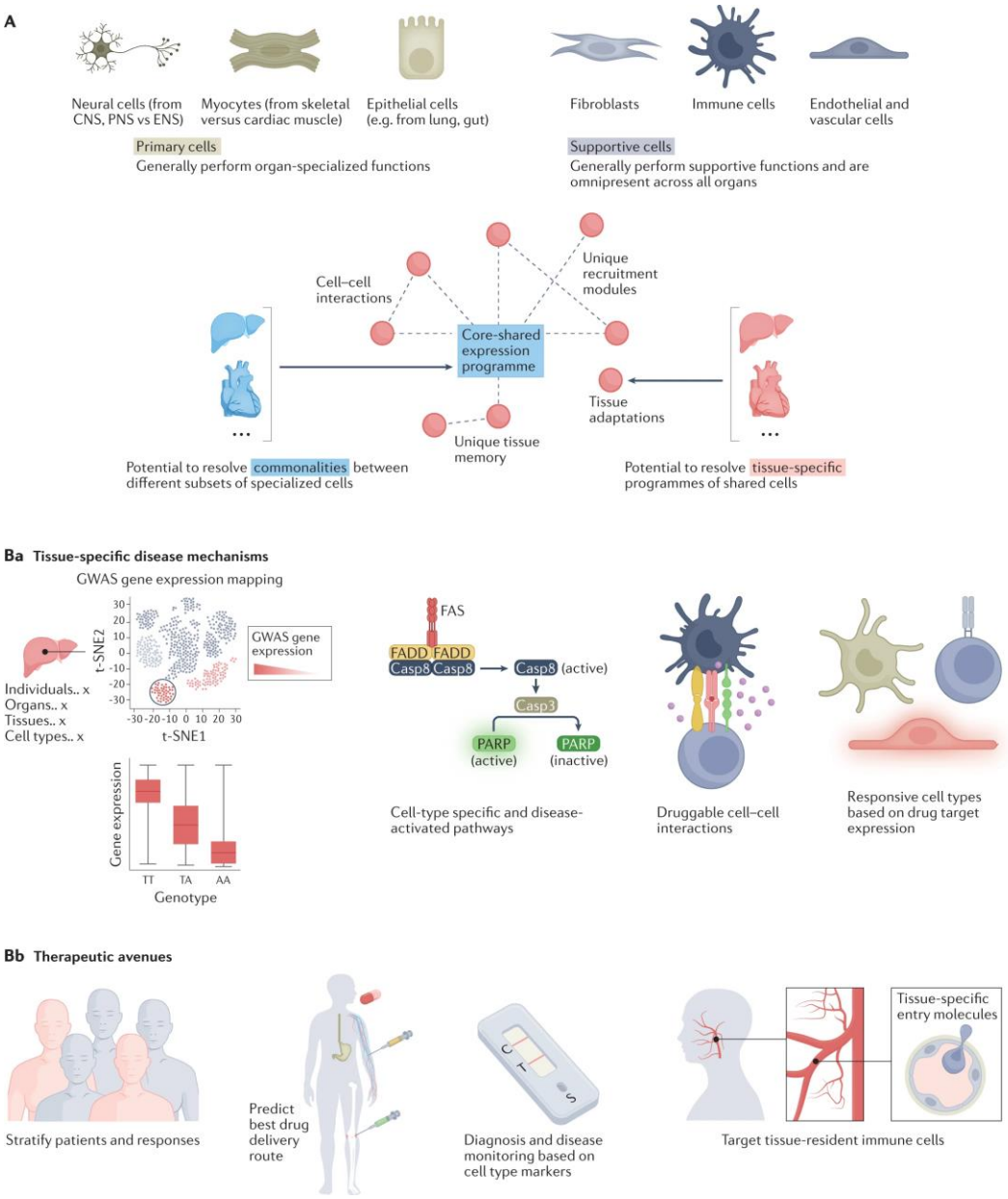
https://www.humancellatlas.org/

Single-Cell Atlas



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

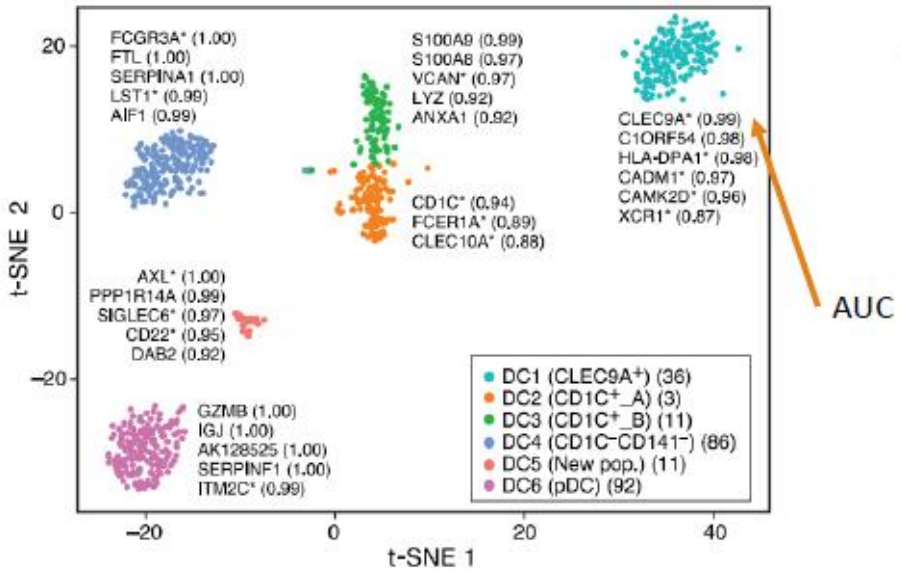
Single-Cell Cross-Tissue Comparisons



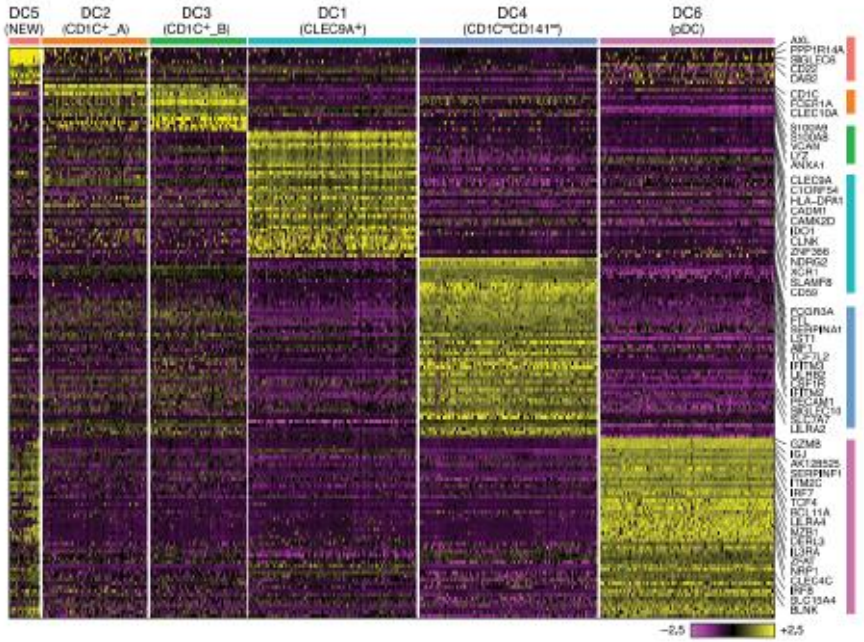
Elmentaite R, Domínguez Conde C, Yang L, Teichmann SA.Nat Rev Genet. 2022 Feb 25.

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

Enrich and sort cells of interest → Generate counts and QC the data → Identify clusters of cells that are similar to each other → Find discriminative markers per cluster → Isolate cells using newly identified markers → Validate the identify of the cells using scRNA-seq → Confirm the identity of these cells in independent individuals → Perform functional analysis of selected types



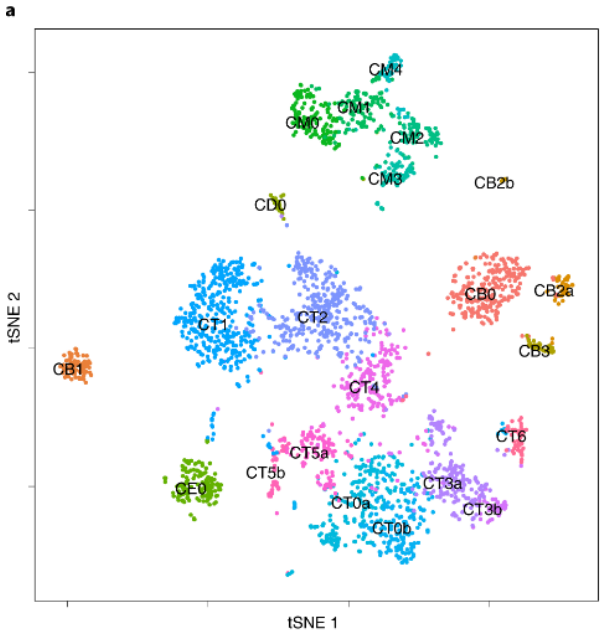
AUC



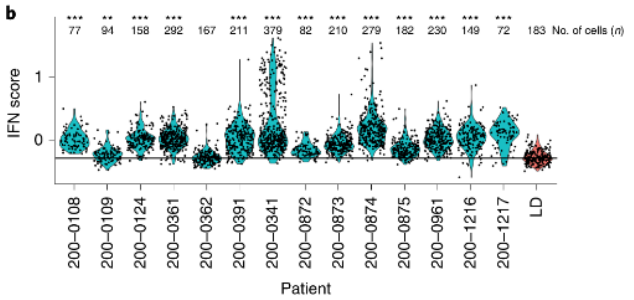
2.400 HLA-DR^{+}lineage^{-} cells

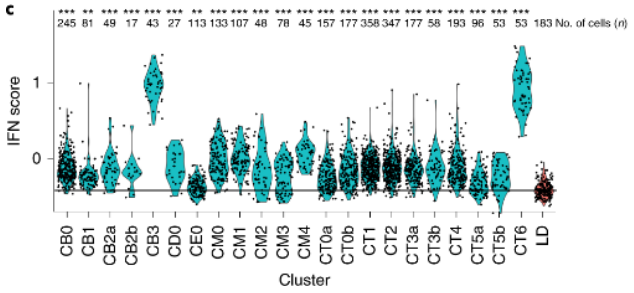
Villani et al. Science 21 Apr 2017

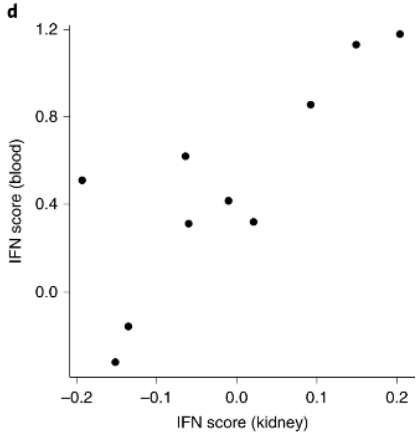
Immune cell landscape in kidneys of patients with lupus nephritis



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





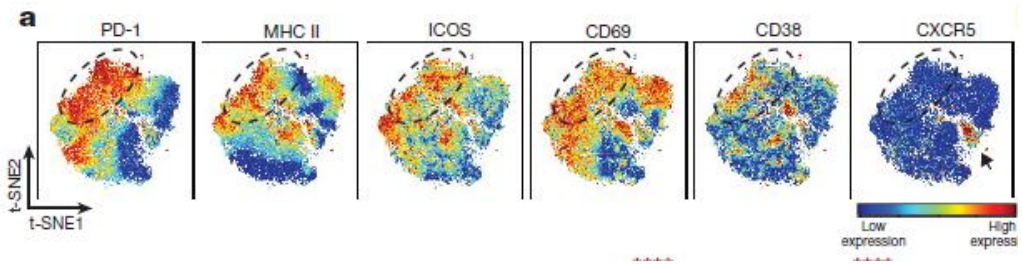


- First single-cell dissection of LN
- 24 patients, 10 ctrls
- 21 subsets of leukocytes, including clusters of myeloid cells, T-cells, NK, B-cells
- CXCR4, CX3CR1 broadly expressed
- Use of urine liquid biopsies and kidney samples

Arazi et al. Nat Imm 2019

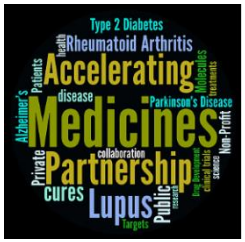
Novel pathological cell types: expanded peripheral Th subsets in RA

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

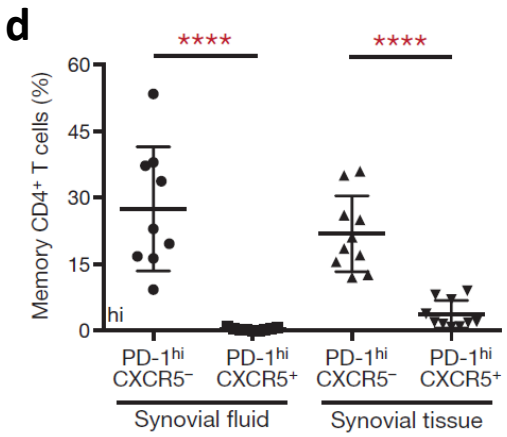


Pathologically expanded peripheral T helper cell subset drives B cells in RA

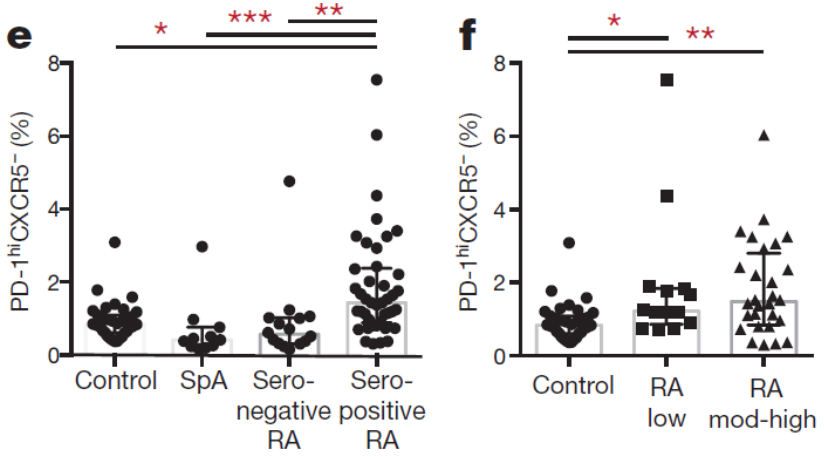
Synovial PD-1hi CXCR5−CD4+ T cells express factors associated with B-cell help (ICOS, IL21, MAF).



synovial fluid



blood

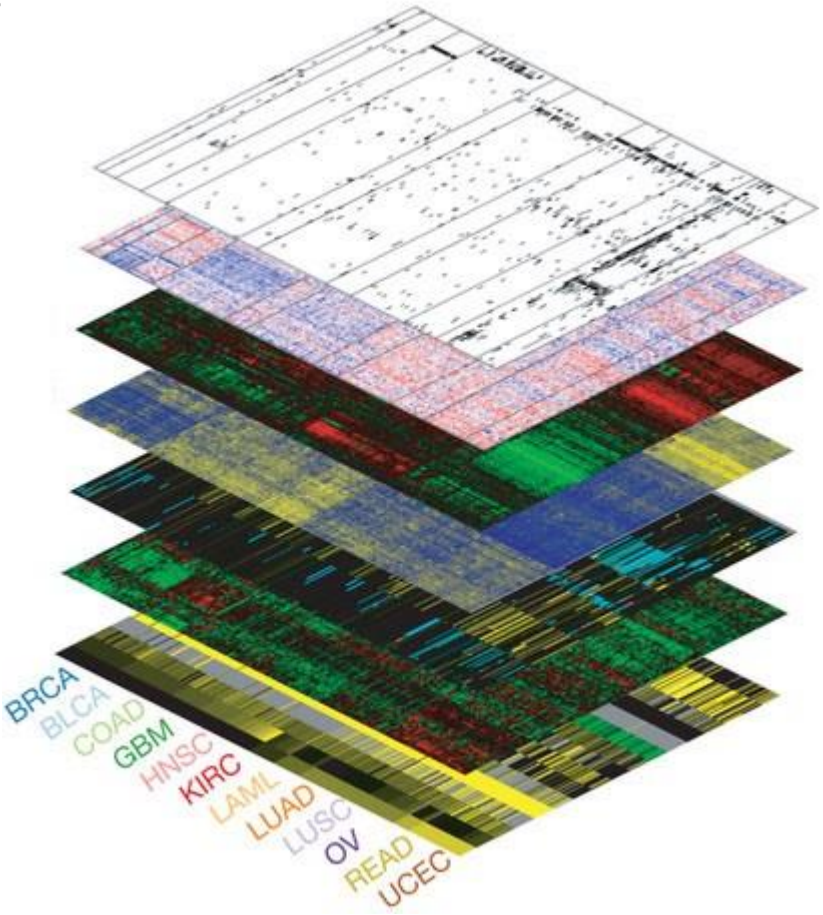


Rao et al. - Nature. 2017 Feb 1;542(7639)

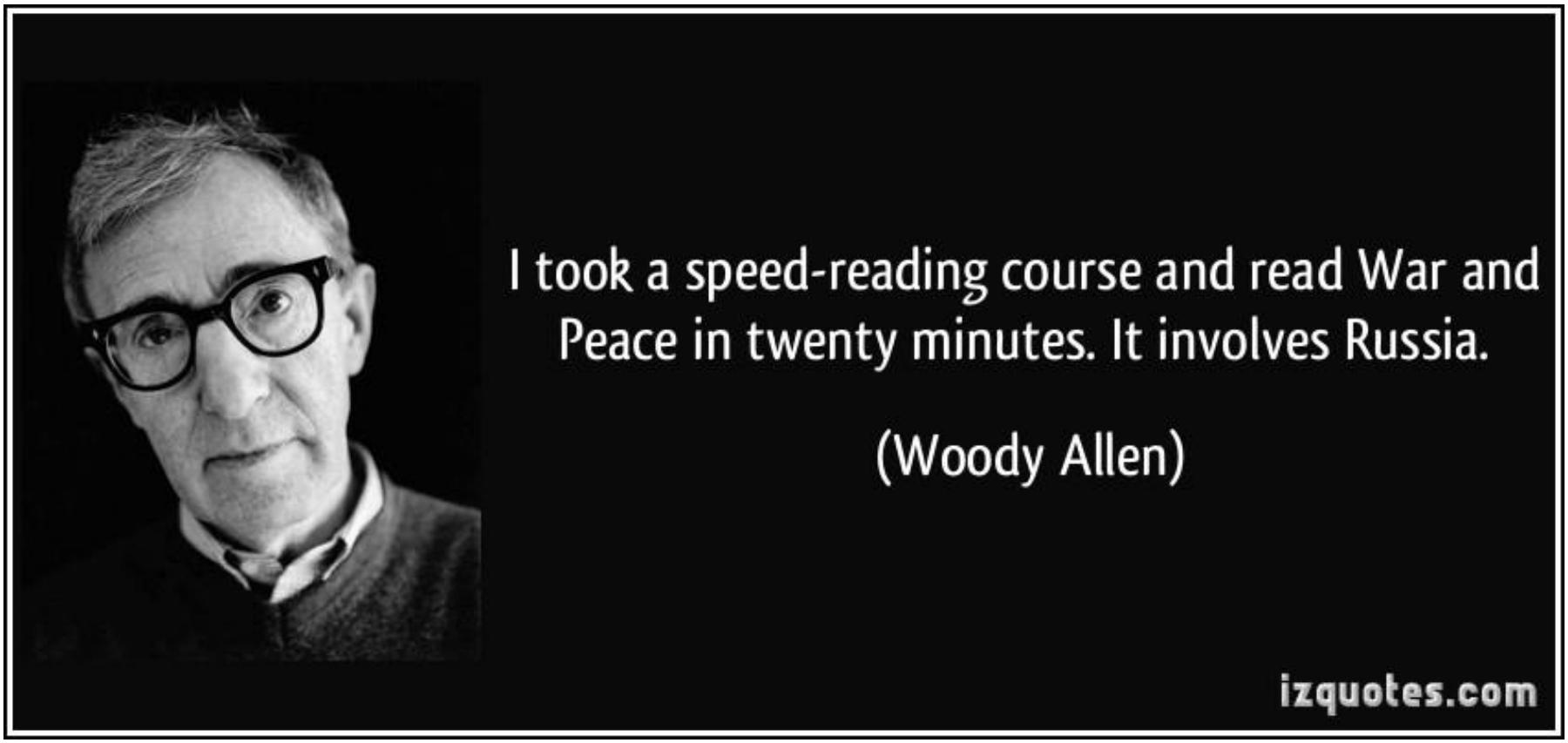
DATA AGGREGATION AND VISUALIZATION

Data Dimensionality

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



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



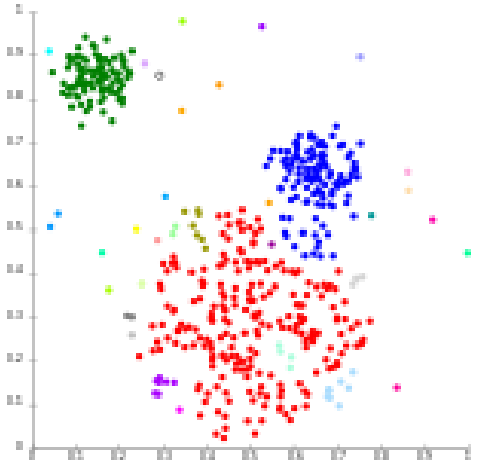
Data Aggregation

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

Clustering

Finding a partition such that:

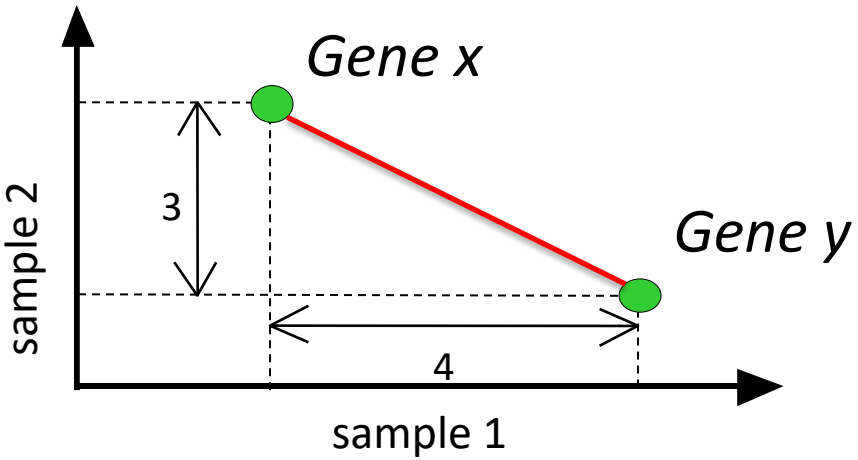
- Distance between objects within the same cluster is **minimised**
- Distance between objects from different clusters is **maximised**



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



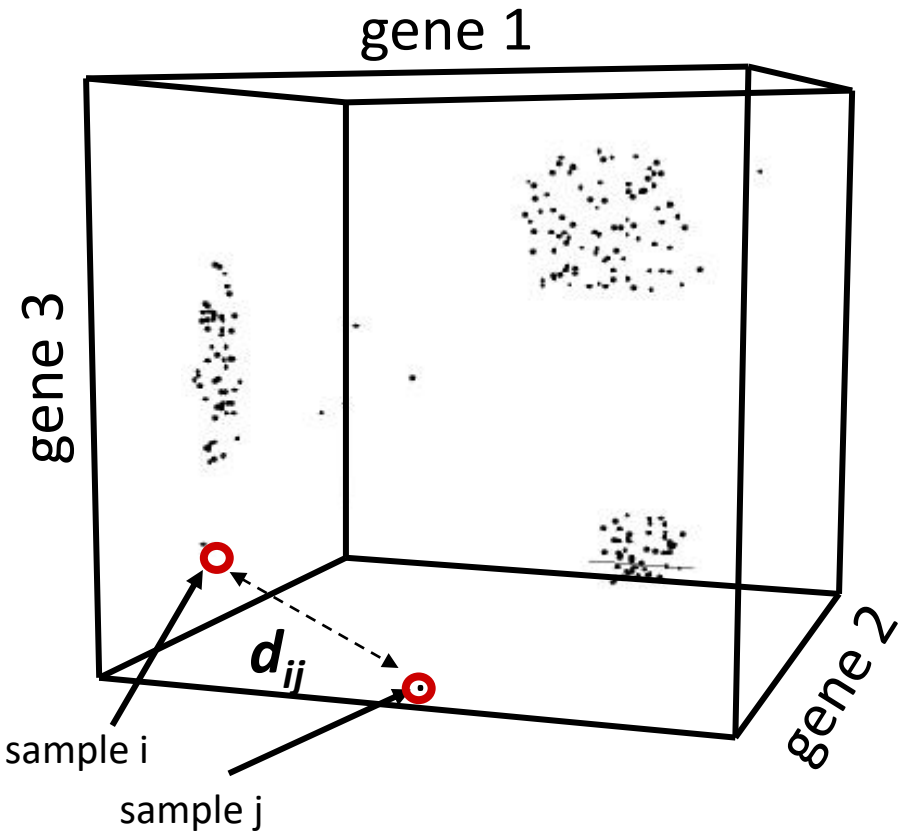
- Euclidean distance : $\sqrt[2]{4^2 + 3^2} = 5$
- Manhattan distance : $4 + 3 = 7$
- "sup" distance : $\max\{4,3\} = 4$

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

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

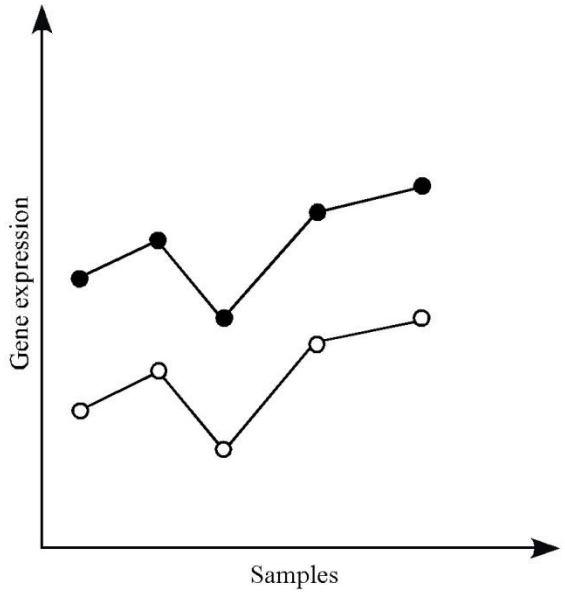
Geometrical Distances as measures of similarity

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

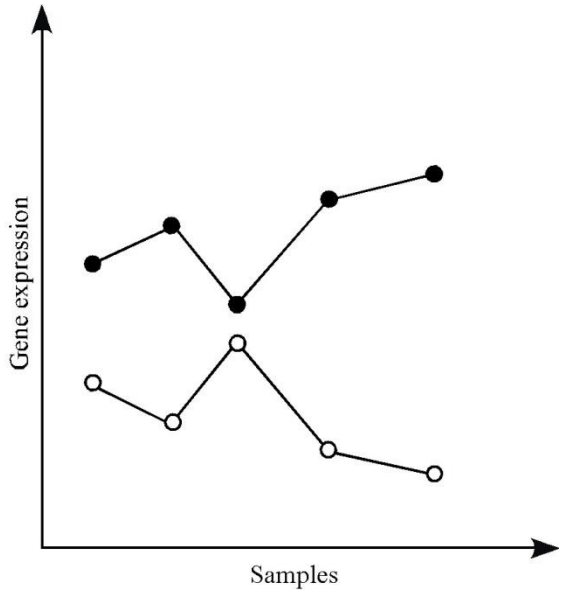


- N genes = N dimensions
- each sample can be represented as a point in the N-dimensional space

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

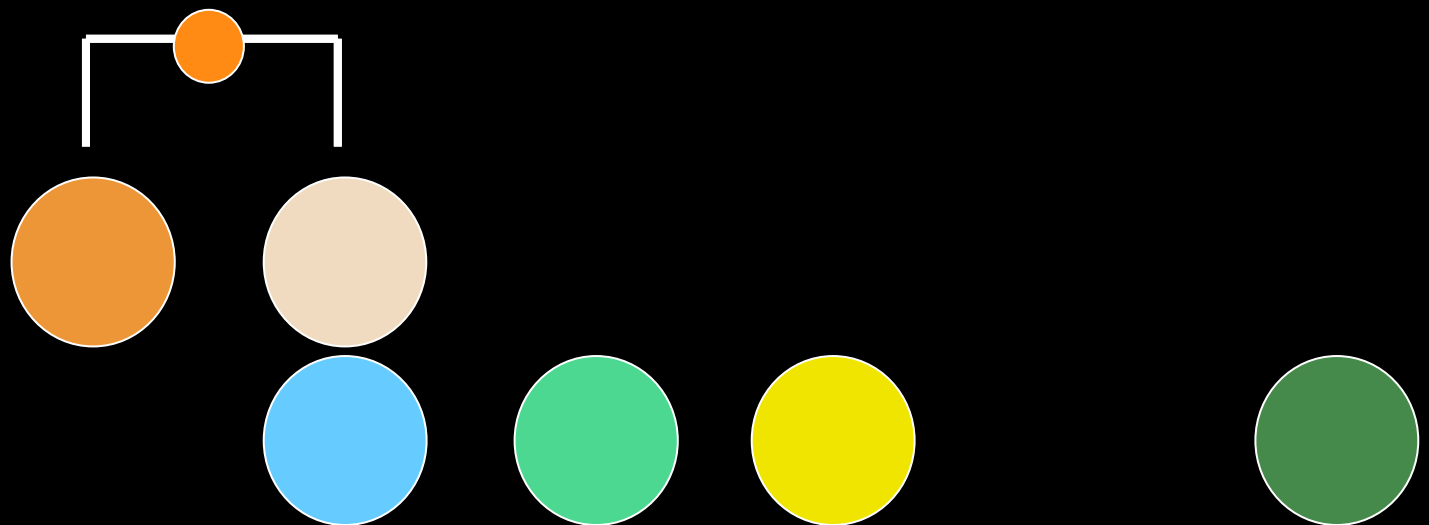
At the beginning, each object (gene) is a cluster. In each of the subsequent steps, the two *closest* clusters are merged into one cluster until there is only one cluster left.

Hierarchical Clustering

Similarity distance: color

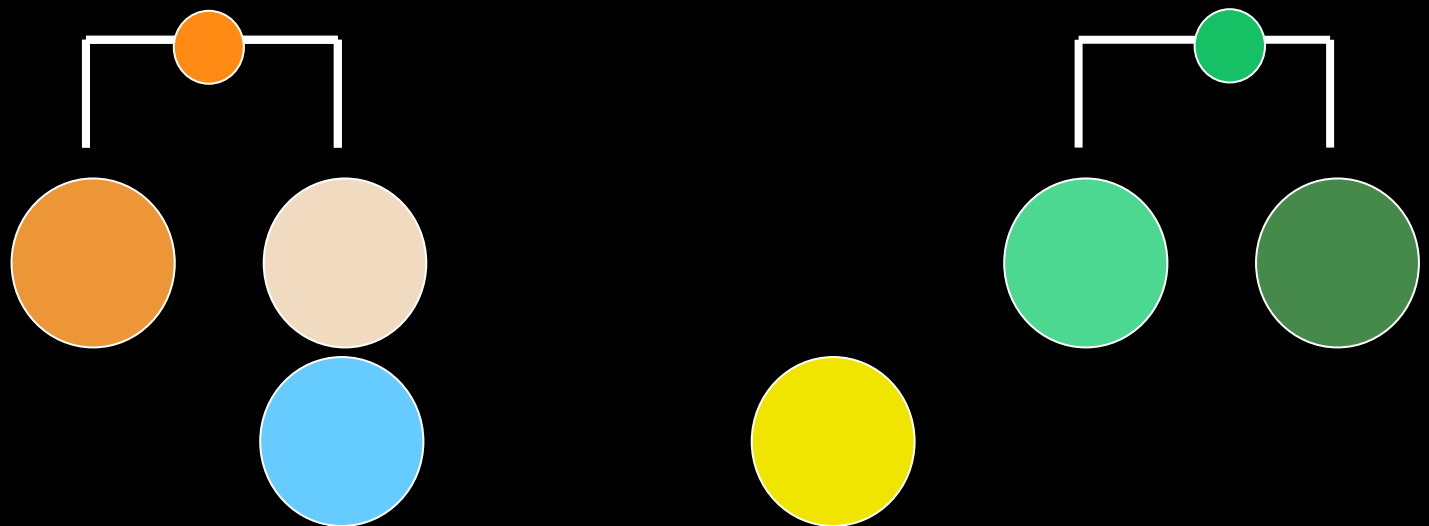
Hierarchical Clustering

Similarity distance: color



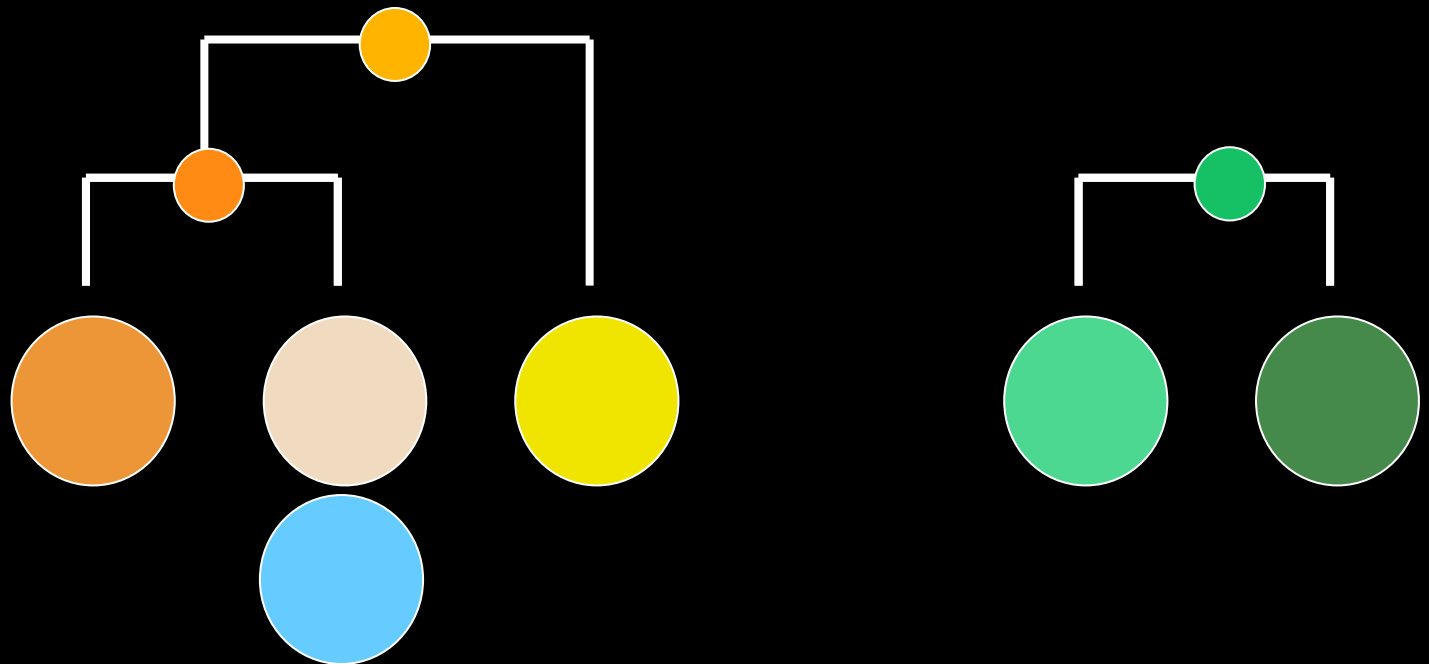
Hierarchical Clustering

Similarity distance: color



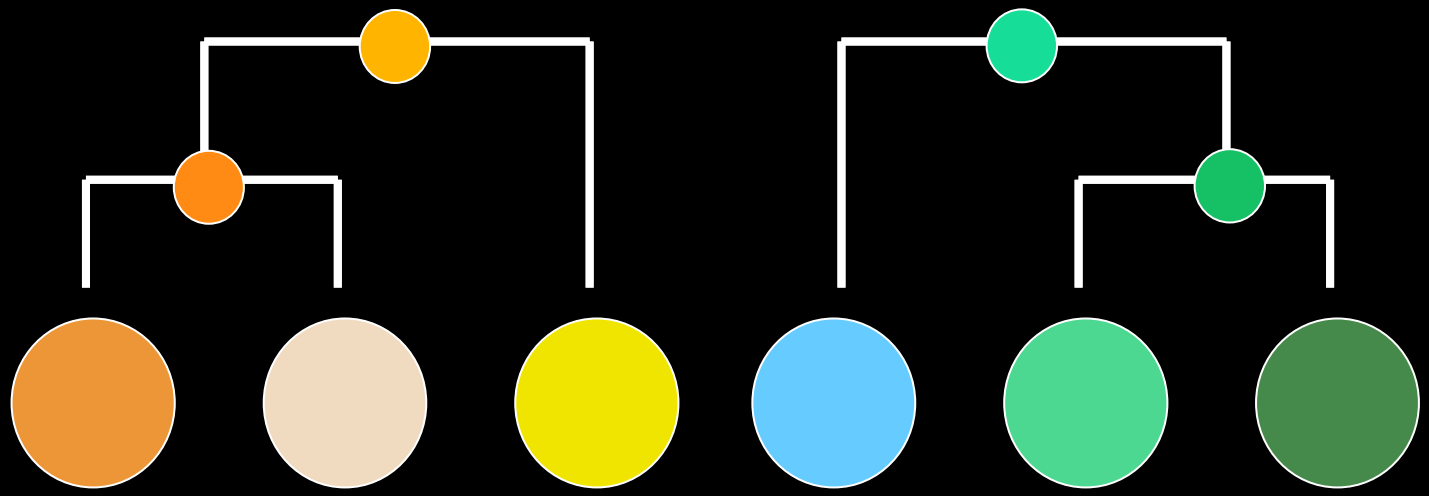
Hierarchical Clustering

Similarity distance: color



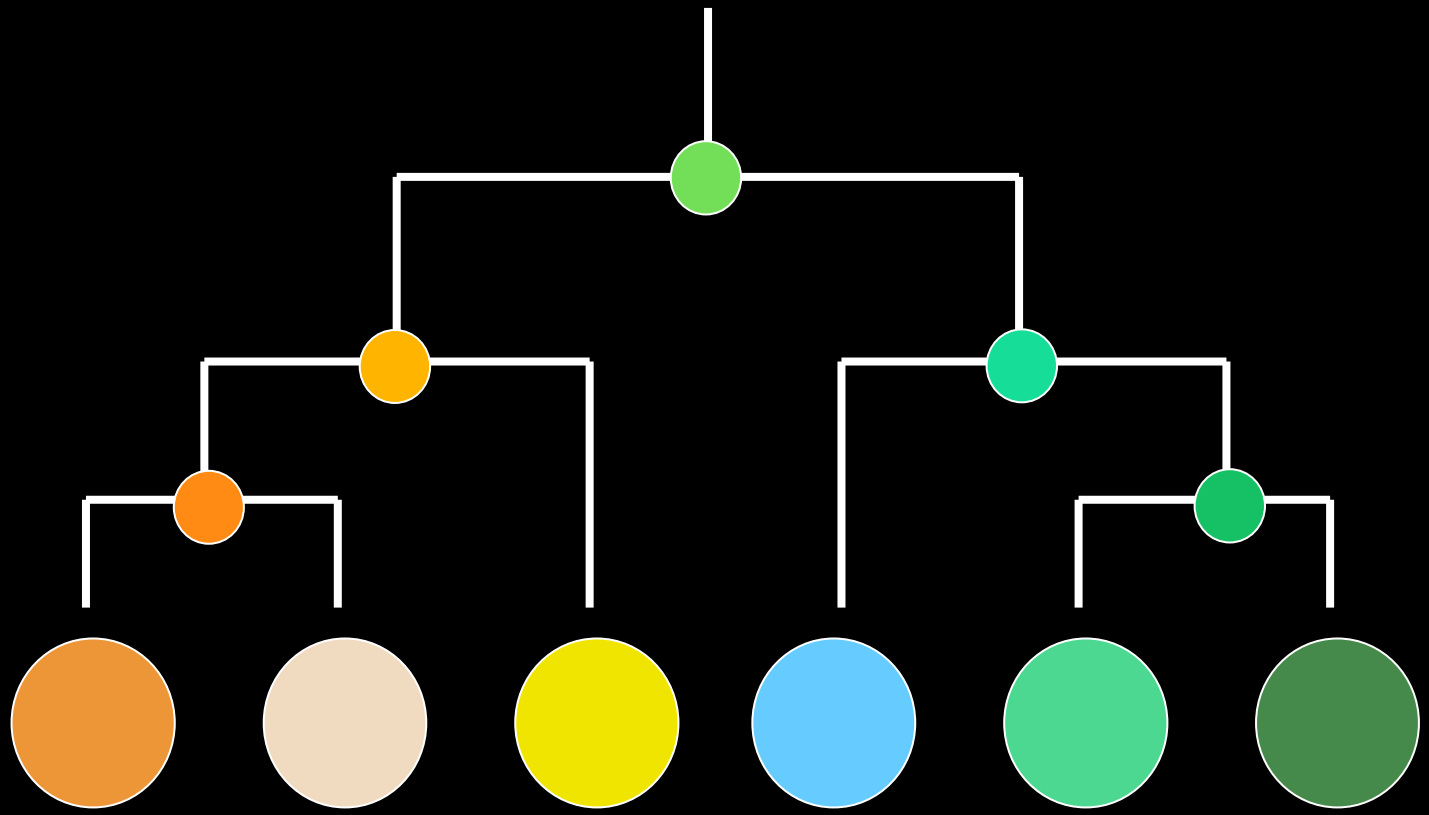
Hierarchical Clustering

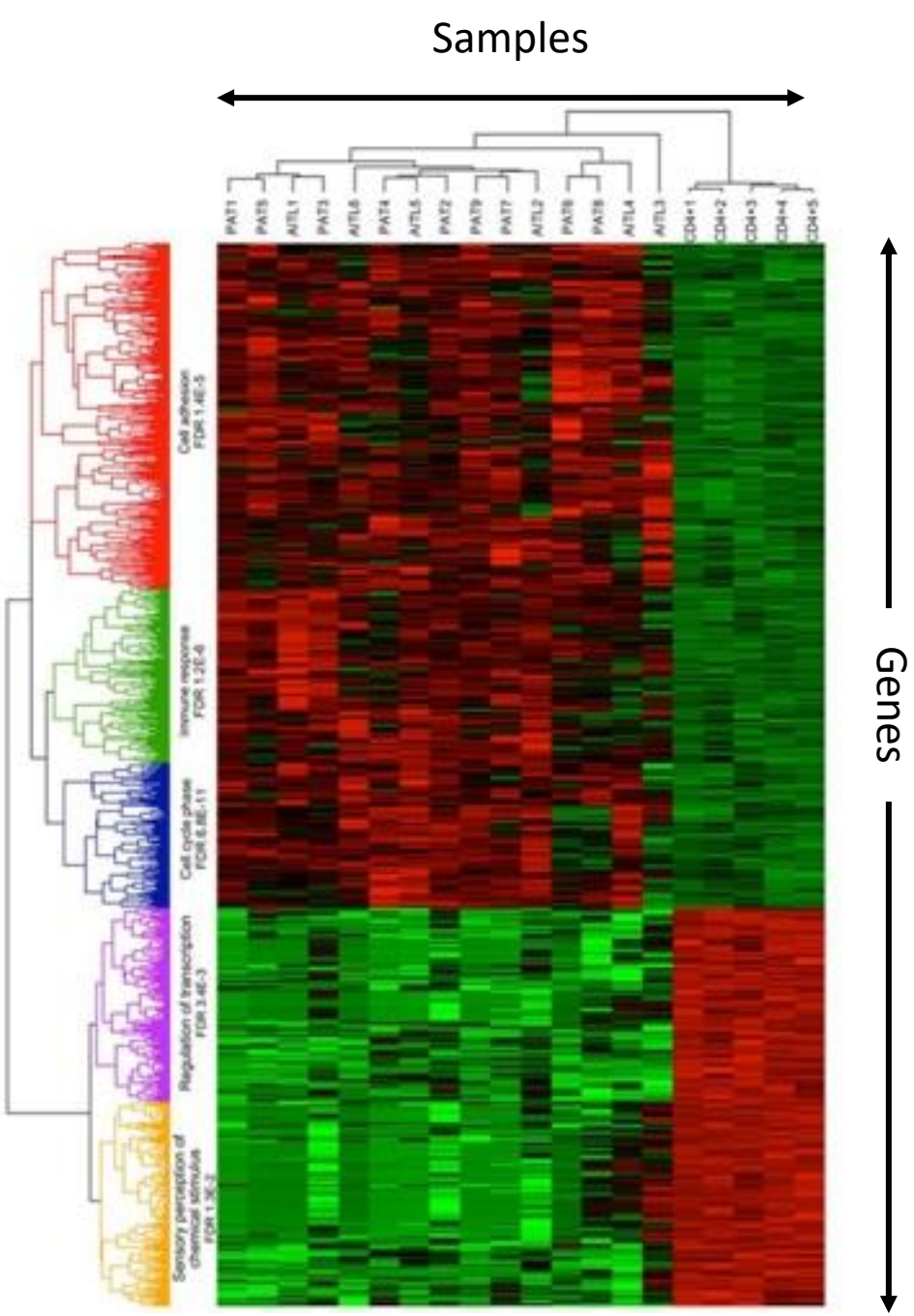
Similarity distance: color



Hierarchical Clustering

Similarity distance: color




Samples
Genes
PAT1
PAT5
AITL1
PAT3
AITL6
PAT4
AITL5
PAT2
PAT9
PAT7
AITL2
PAT8
PAT6
AITL4
AITL3
CD4+1
CD4+2
CD4+3
CD4+4
CD4+5
Cell adhesion
FDR 1.4E-5
Immune response
FDR 1.2E-8
Cell cycle phase
FDR 6.8E-11
Regulation of transcription
FDR 3.4E-3
Sensory perception of
chemical stimulus
FDR 1.3E-2

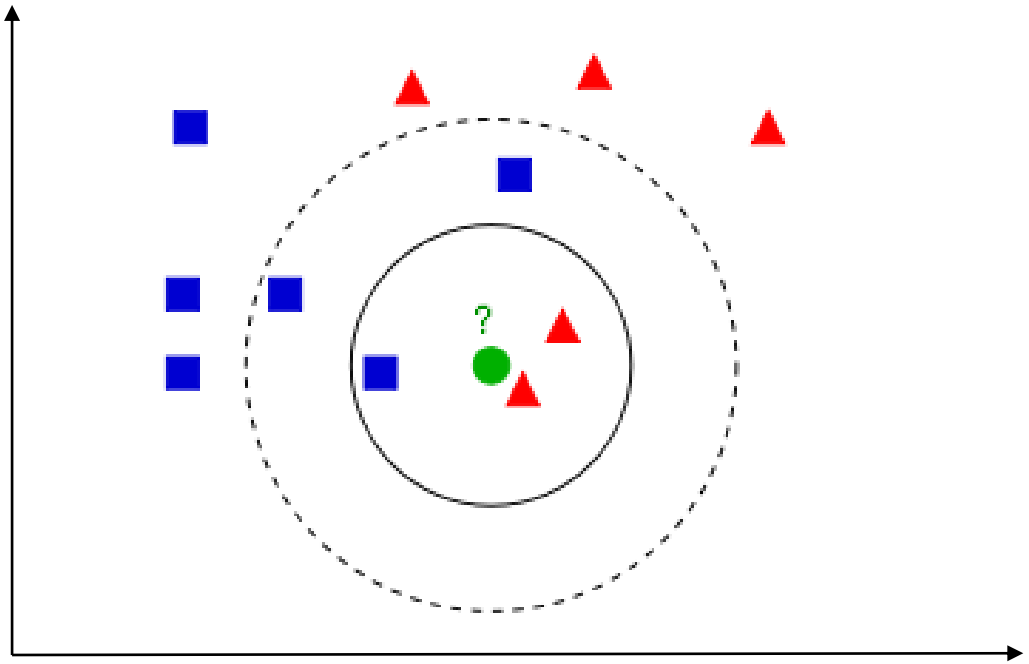
Supervised Clustering (Classification)

- 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

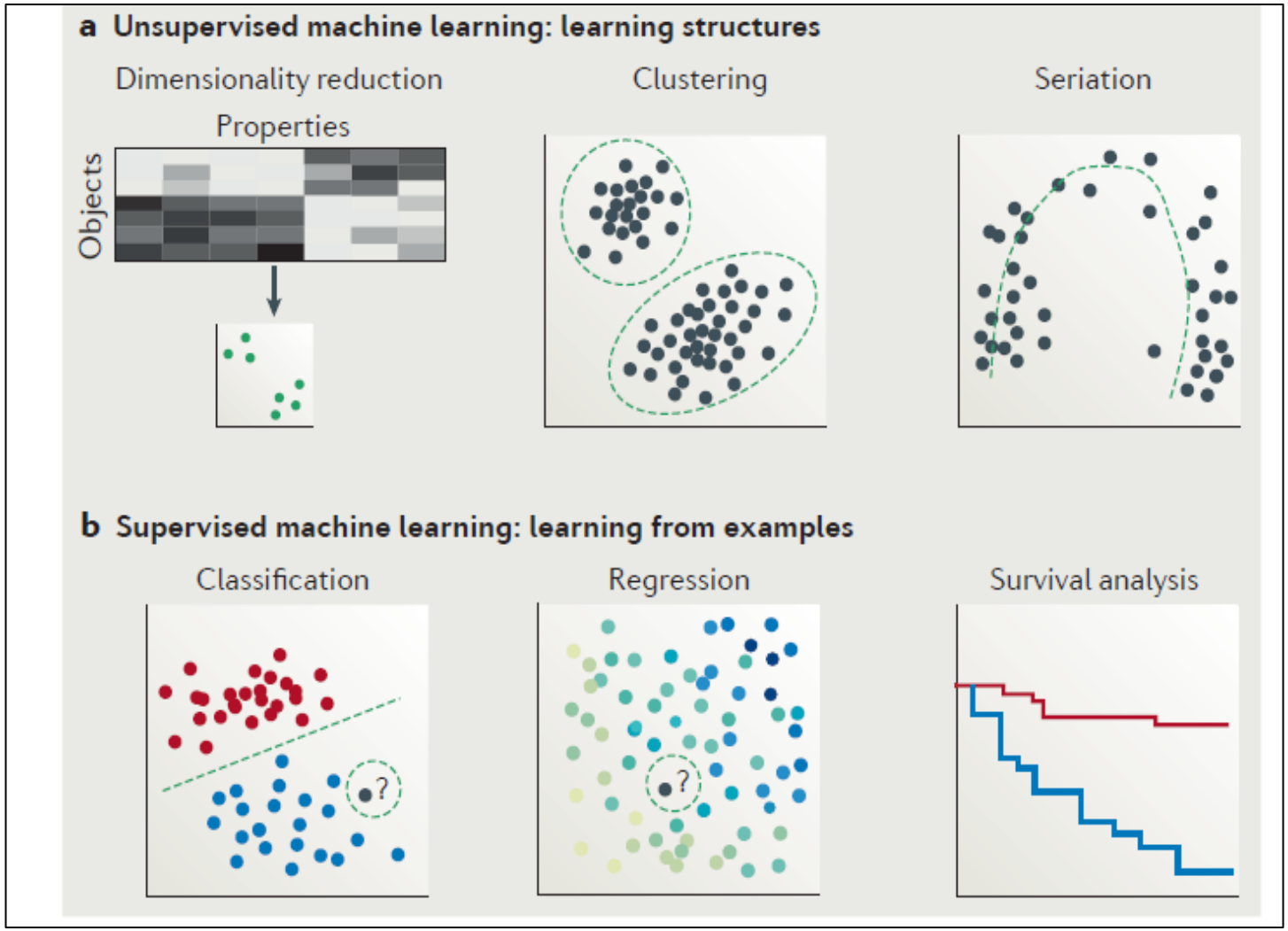
k-nearest neighbors classification (k-NN)

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.

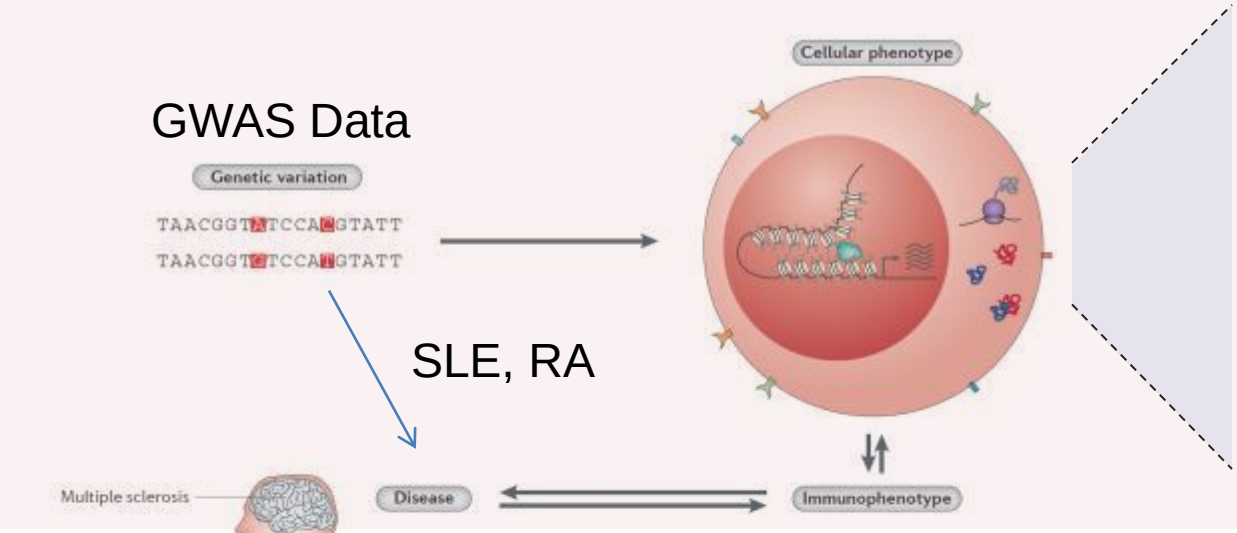


Supervised and Unsupervised Machine Learning

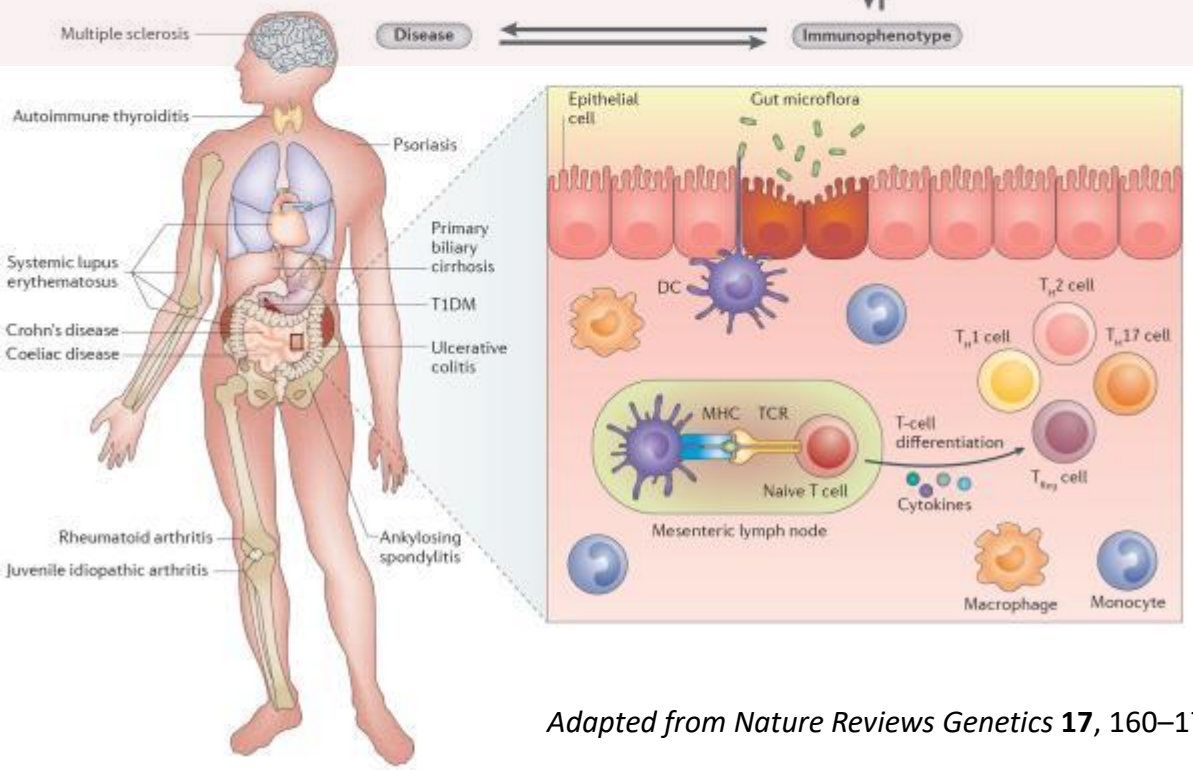


Nature Reviews Immunology 16, 449–462 (2016)

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

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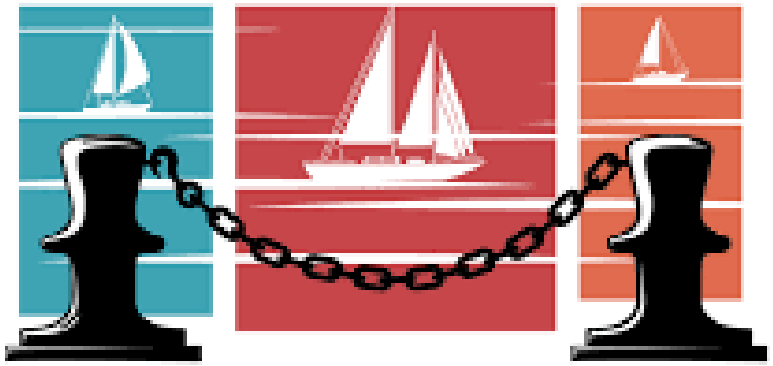
JUNE 20-23



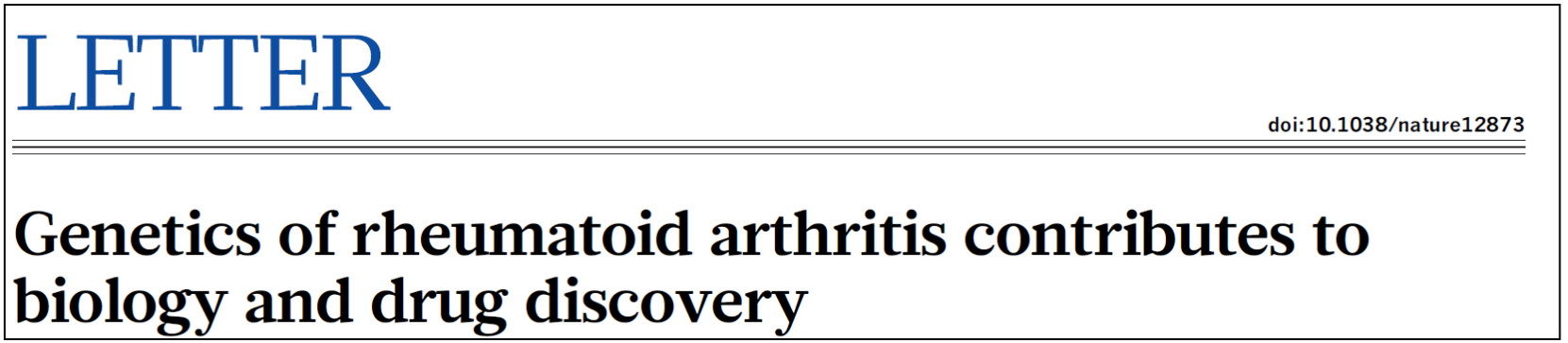
Deep Dive into Case Studies:

From Systems Immunology to Novel Therapeutic Insights

Emanuele de Rinaldis

Workshop in Systems Immunology
June 19thth, 2023

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



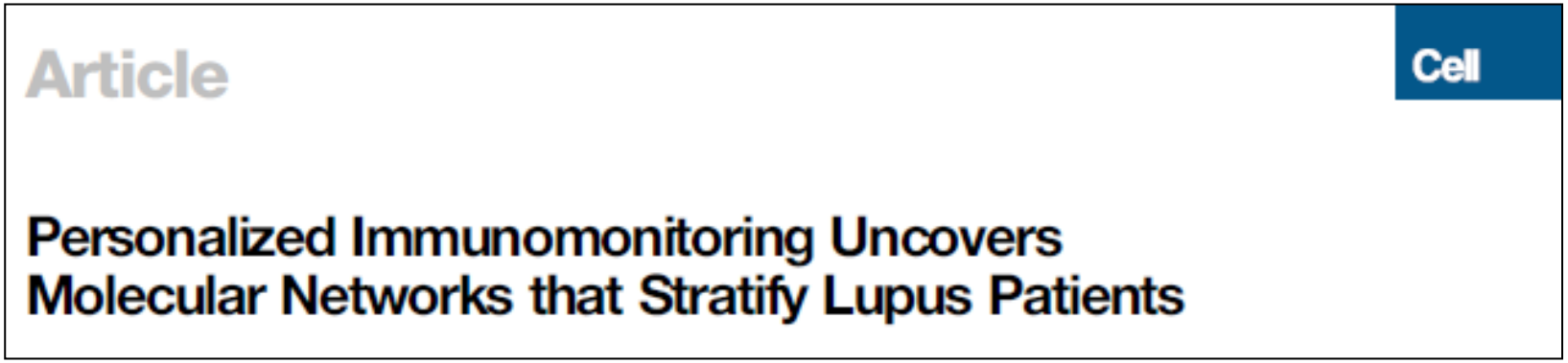
LETTER

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 fine-mapping

Characterization of results

Data integration and genes prioritization

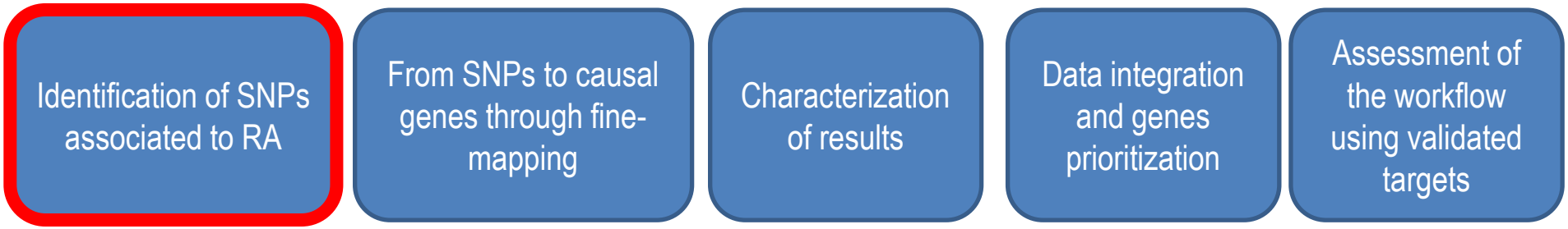
Assessment of the workflow using validated targets

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

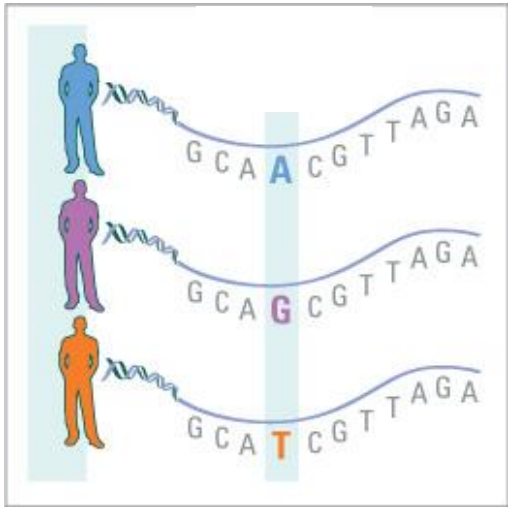
Toolbox

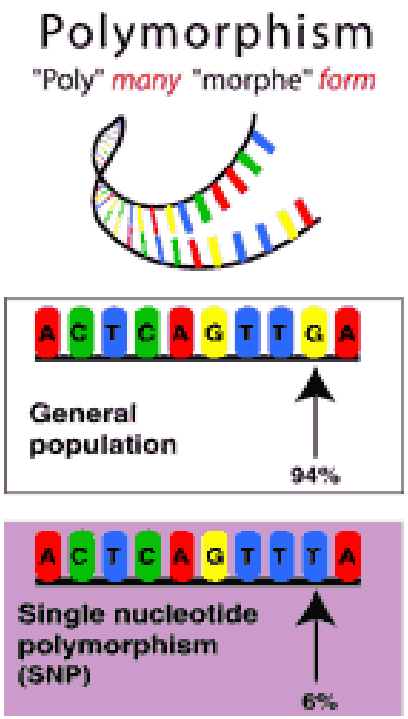
- **Genome Wide Association Studies (GWAS) and Meta-Analysis**
  - Genome variability and SNPs
  - Logistic Regression
  - Linkage Disequilibrium
  - Imputation
  - Manhattan Plots
- **Multiple Testing**
- **Network Analysis**
- **Fine-mapping and data integration**
  - Epigenetics data
  - Transcriptional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

- **Statistical enrichment**

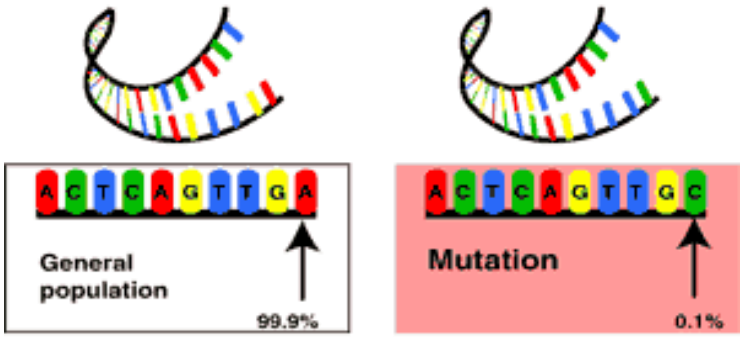

Identification of SNPs associated to RA
From SNPs to causal genes through fine-mapping
Characterization of results
Data integration and genes prioritization
Assessment of the workflow using validated targets

Genetic Variability



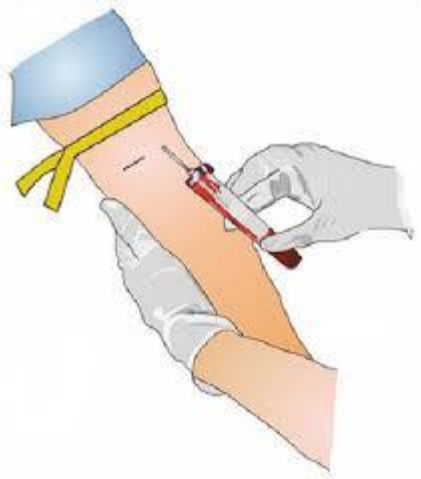


SNP: common (>1%) variant of one **S**ingle **N**ucleotide

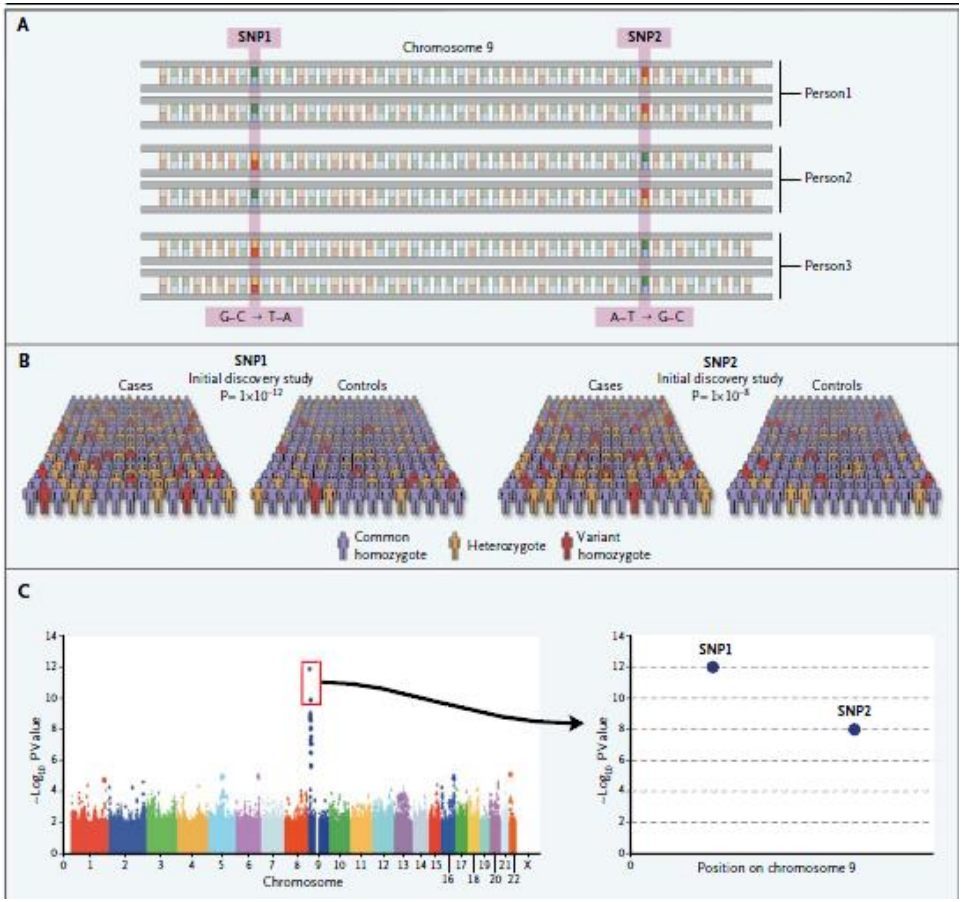


Mutation: typically a rare variant, associated with a disease

Genome-Wide Association Studies (GWAS)



Extraction of germline DNA (e.g. blood)



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

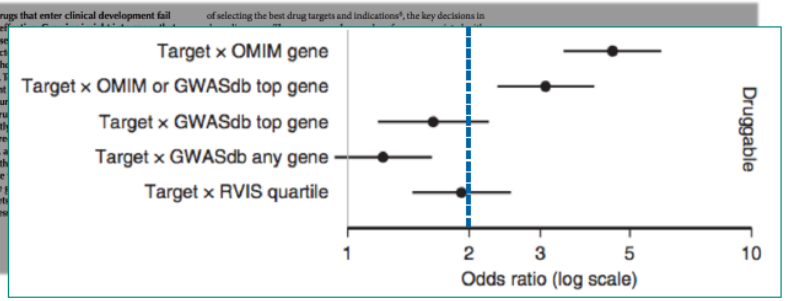
Genome-wide association study: An approach used in genetics research to look for associations between many - typically hundreds of thousands - specific genetic variations - most commonly single-nucleotide polymorphisms - and particular diseases

Why Pharma Is Investing in Genetics

The support of human genetic evidence for approved drug indications

~2-fold increase in success for genetic targets

Matthew R Nelson[1], Hannah Tipney[2], Jeffery L Painter[1], Judong Shen[1], Paola Nicoletti[3], Yufeng Shen[3,4], Aris Floratos[3,4], Pak Chung Sham[5,6], Mulin Jun Li[6,7], Junwen Wang[6,7], Lon R Cardon[8], John C Whittaker[2] & Philippe Sanseau[2]



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

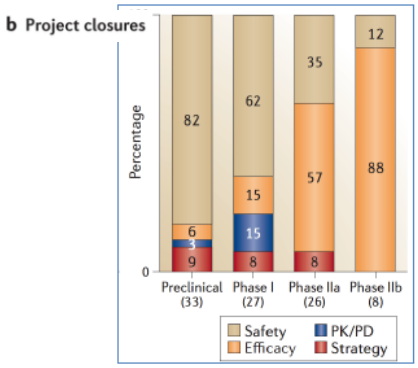
Emily A. King*, J. Wade Davis, Jacob F. Degner

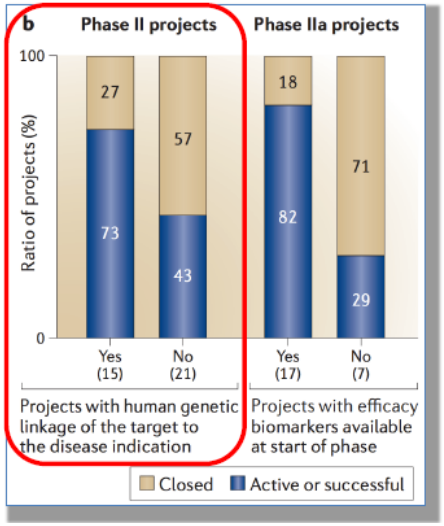
Department of Computational Genomics, AbbVie, North Chicago, Illinois, United States of America

* 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)

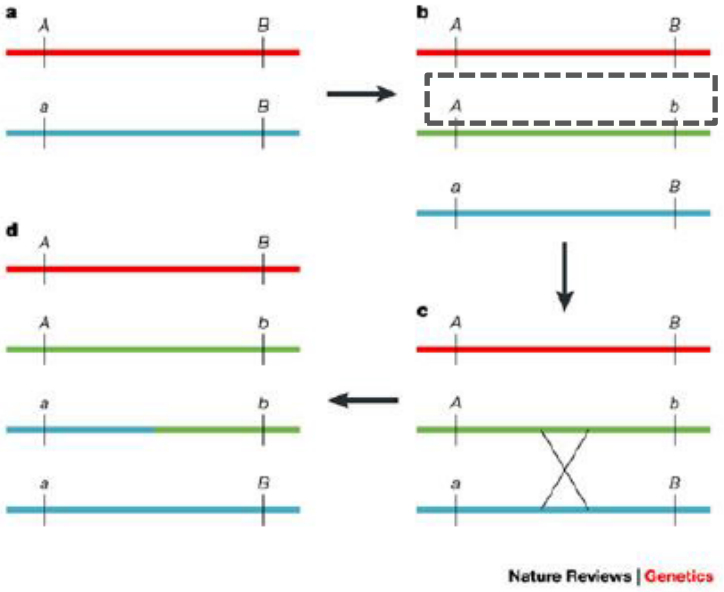
Linkage Disequilibrium

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

Genotypes are not independent but correlated

Set of alleles inherited together from a single parent

haplotypes



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

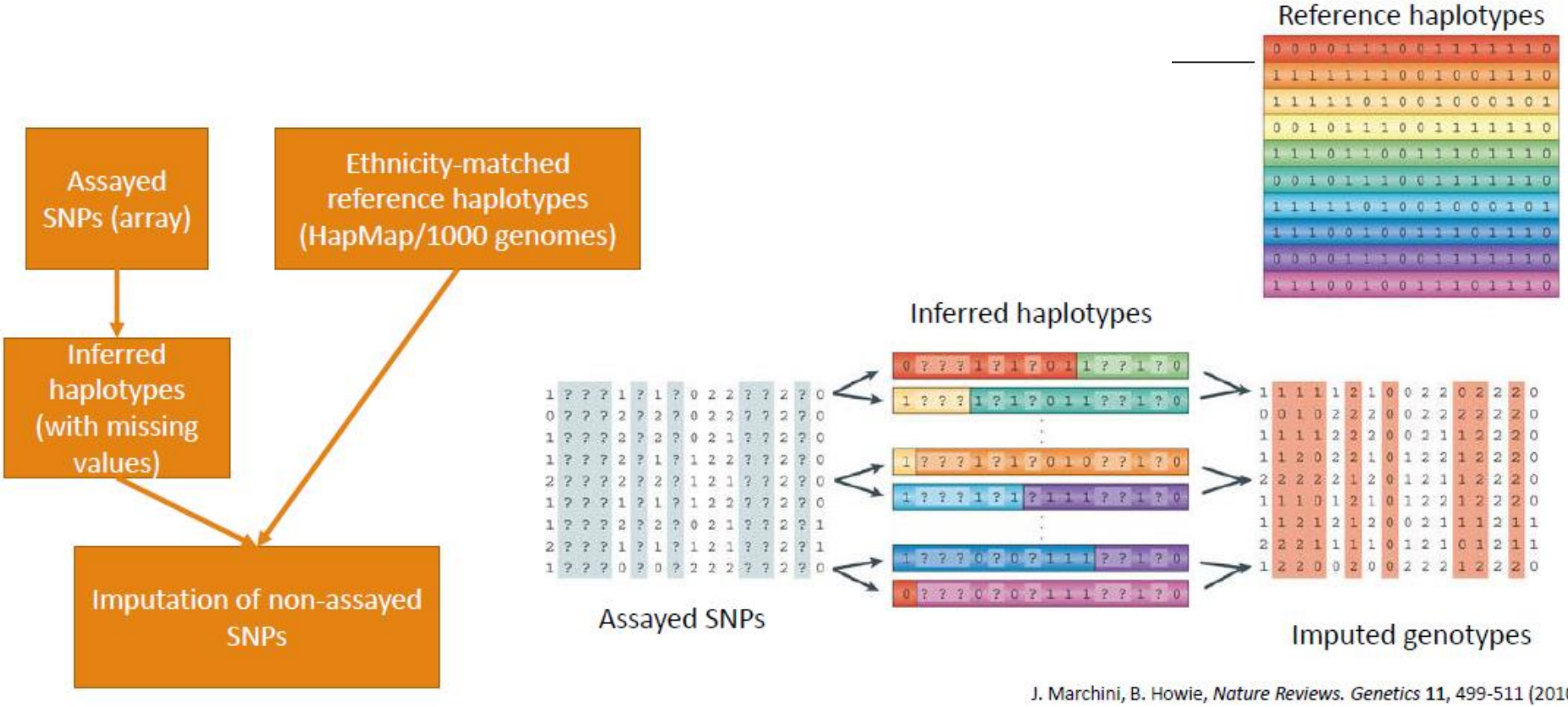
Correct for multiple testing

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

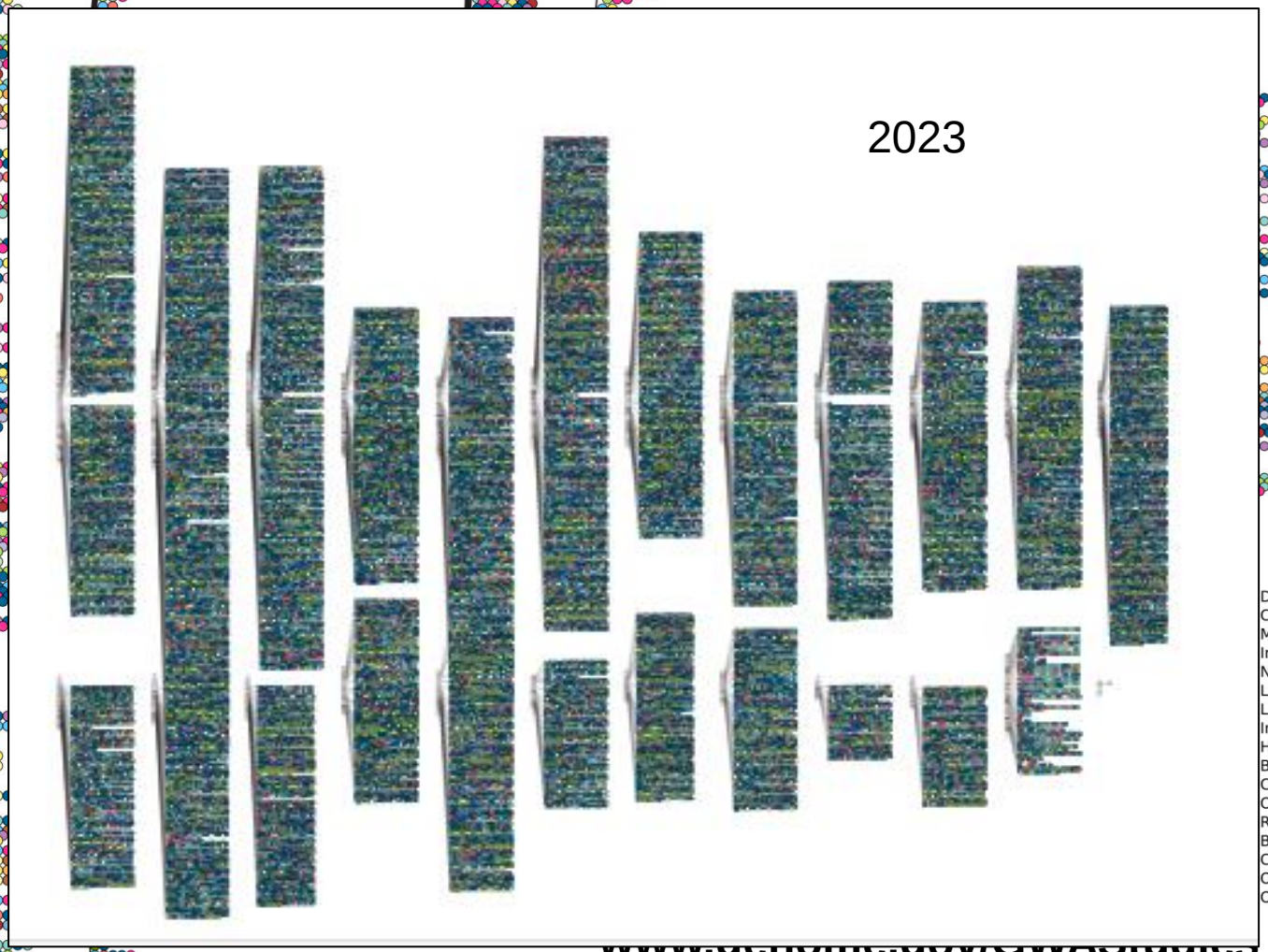
Genome-wide threshold = $5x10^{-8}$

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



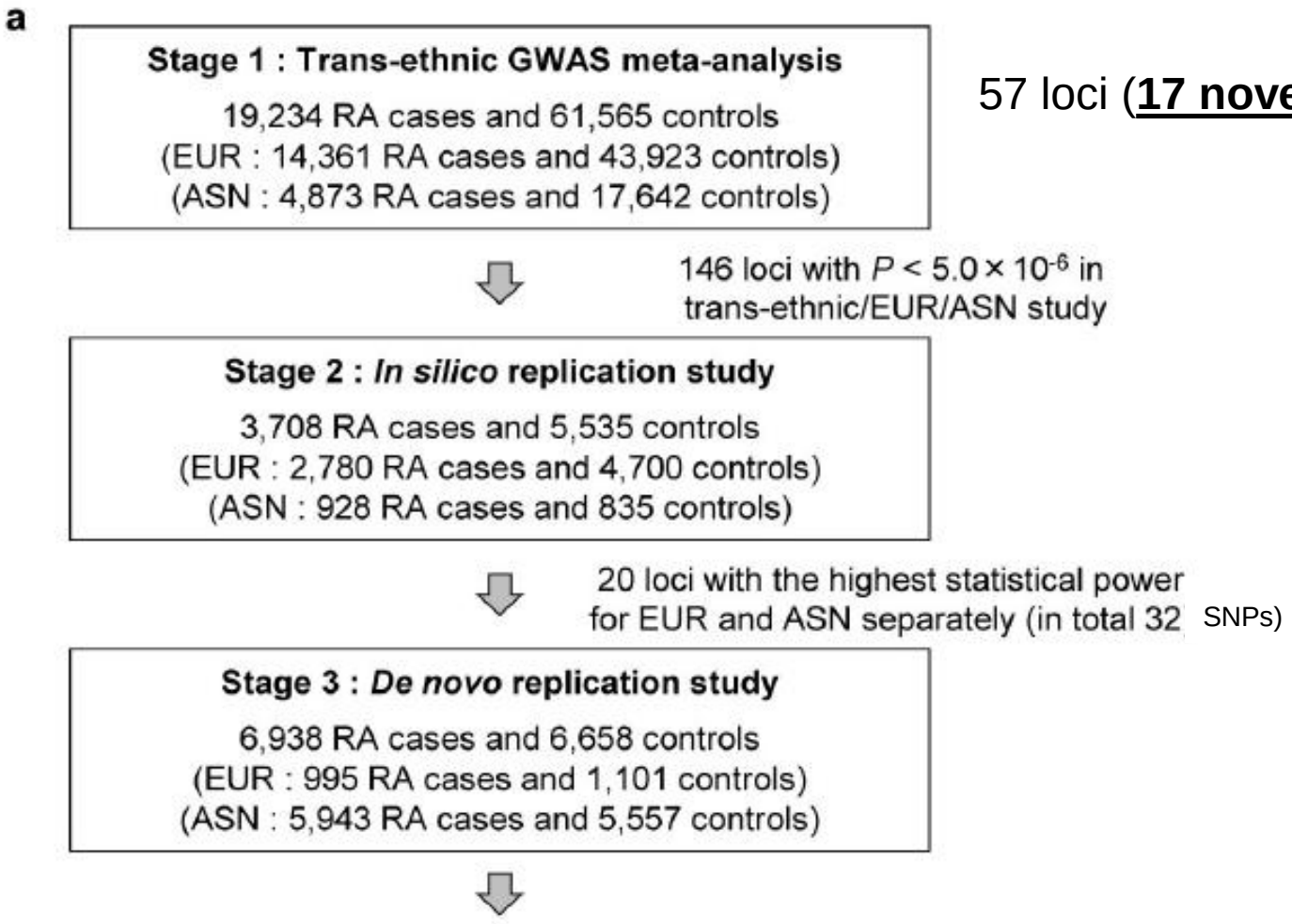
Digestive system disease
Cardiovascular disease
Metabolic disease
Immune system disease
Nervous system disease
Liver enzyme measurement
Lipid or lipoprotein measurement
Inflammatory marker measurement
Hematological measurement
Body measurement
Cardiovascular measurment
Other measurement
Response to drug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

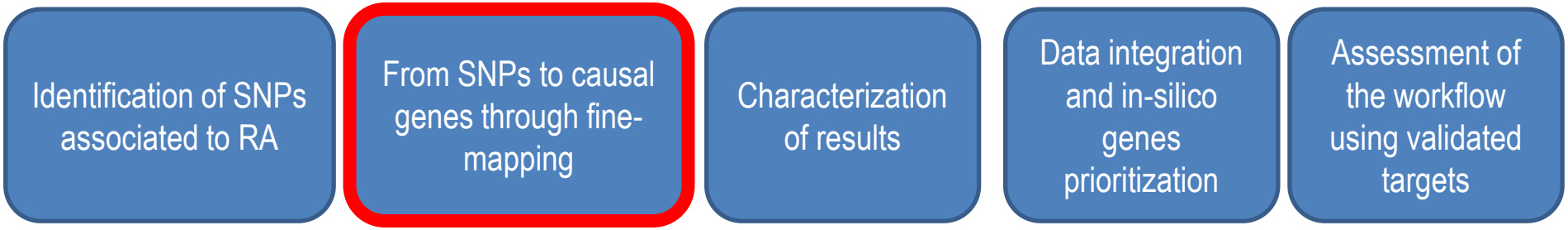


57 loci (**<u>17 novel</u>**) pval<10-8

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
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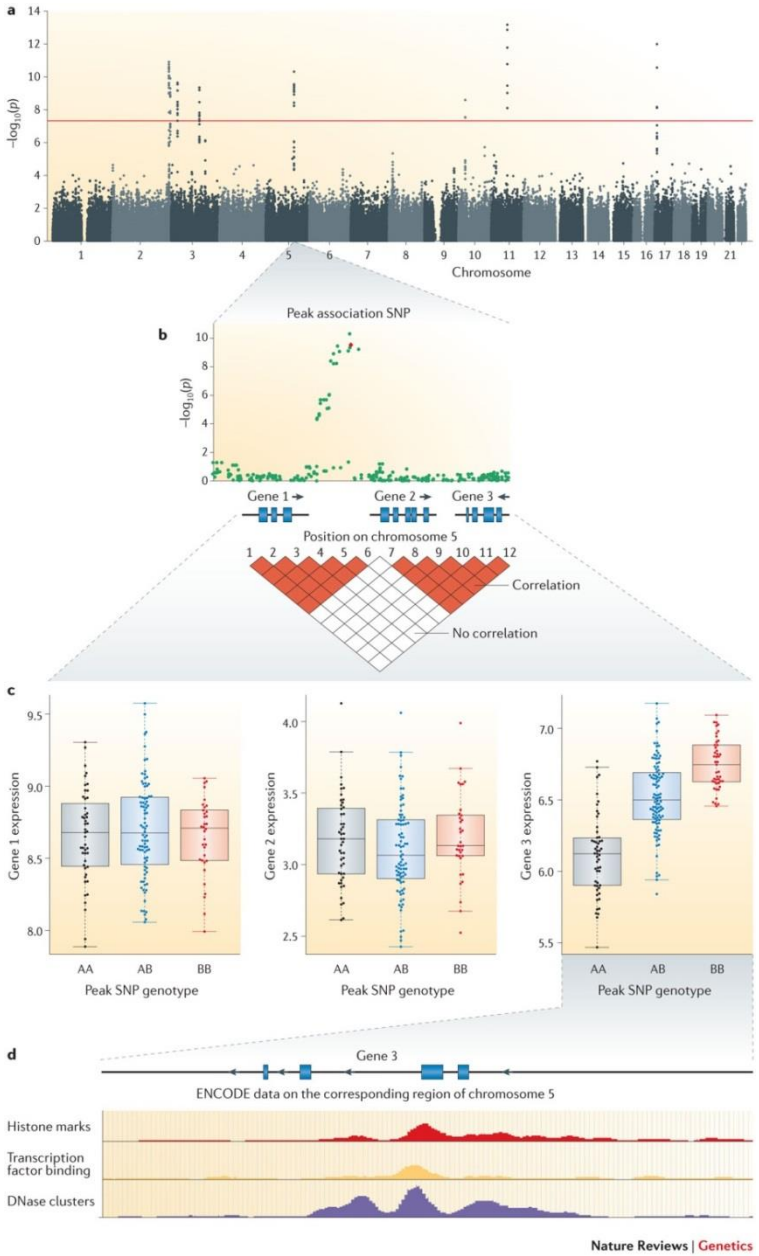
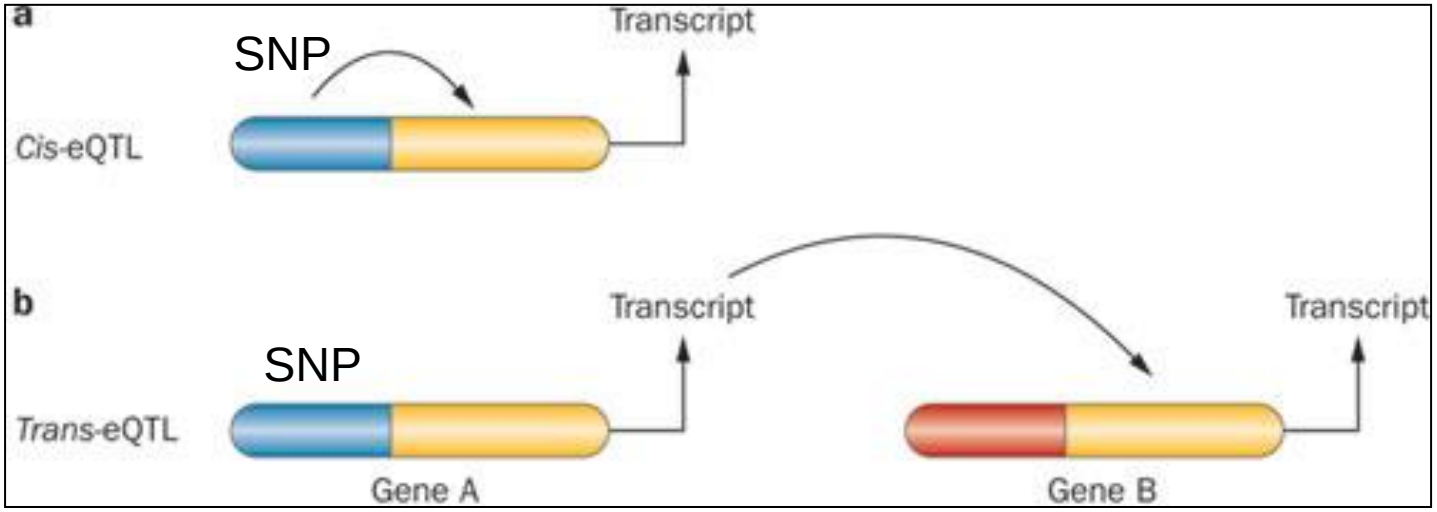
Assessment of the workflow using validated targets

Fine-Mapping: overlaying different information to identify causal genes

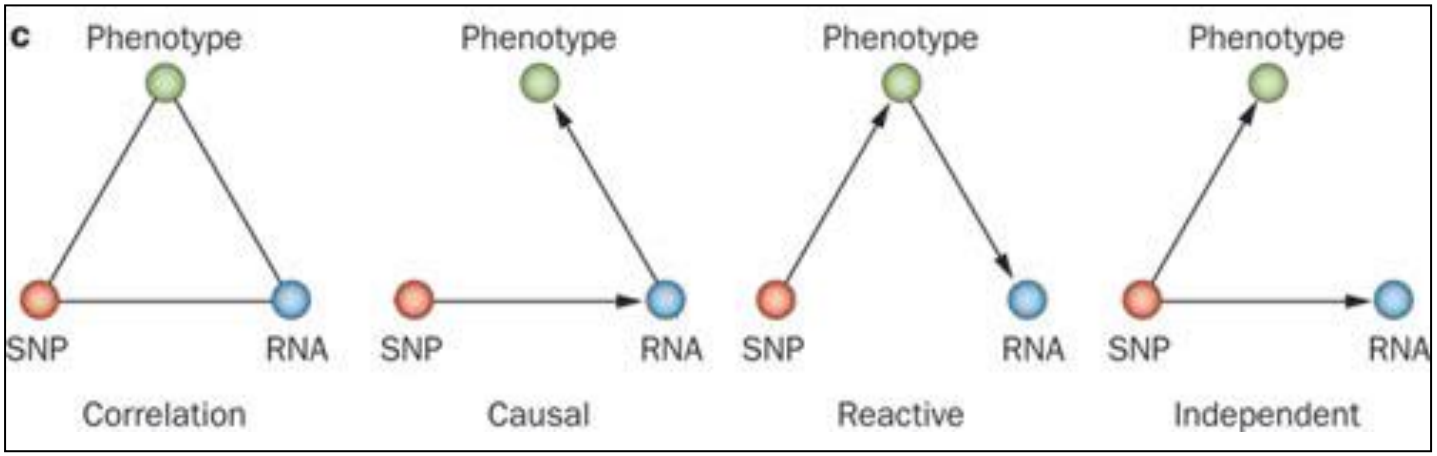
M. Civelek & A. J. Lusis – Nat. Rev. Genetics (2013)



Combining DNA and RNA information – eQTLs and Causal Networks



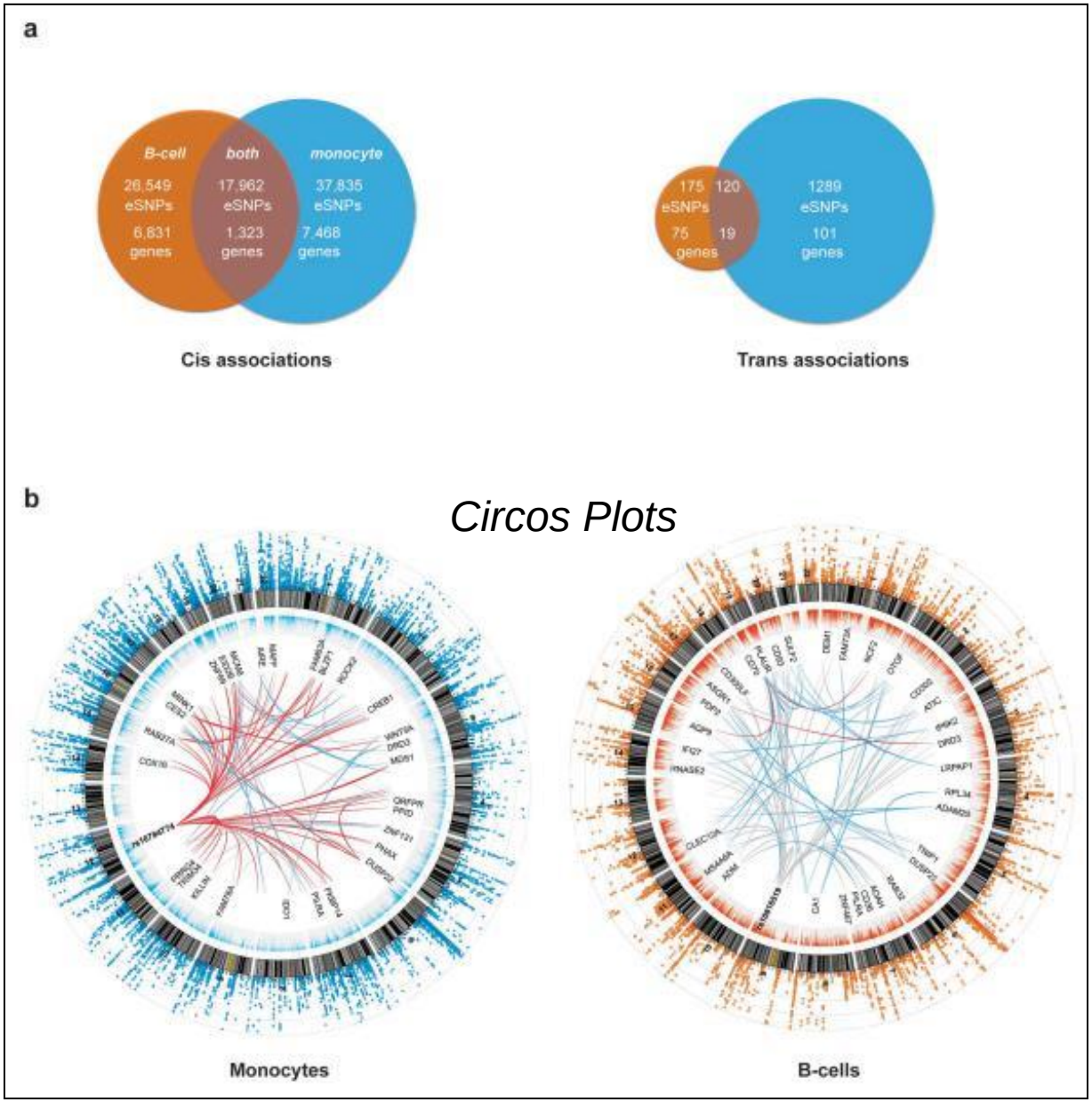
- DNA/RNA combined analysis can highlight eSNPs
- eSNPs exert an effect on genes' transcription



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

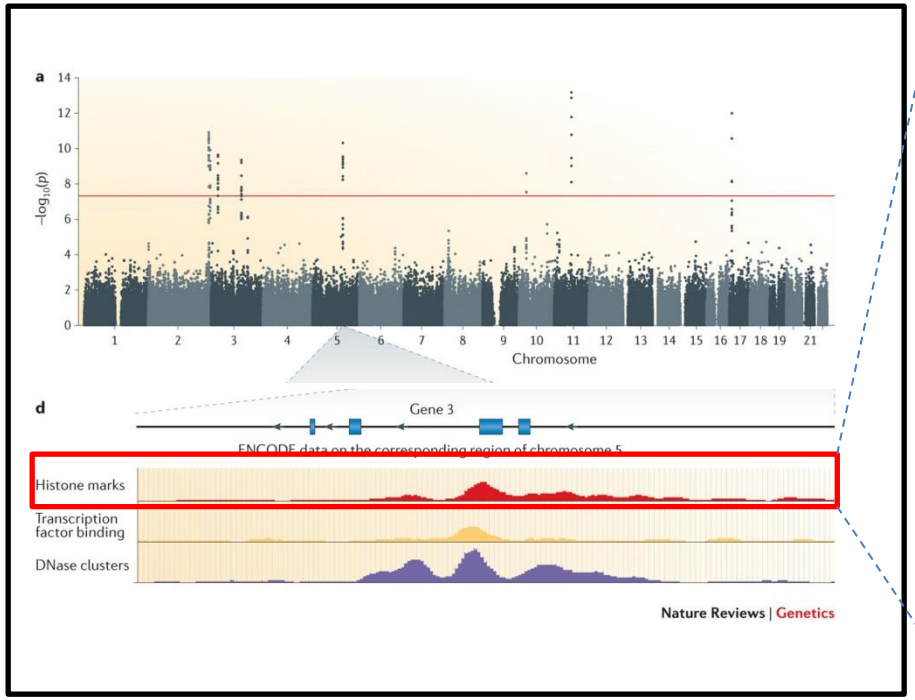
Cell type-specific eQTLs in B-cell and monocytes



[Nat Genet.](#) *2012 Mar 25;44(5):502-10. doi: 10.1038/ng.2205.*

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

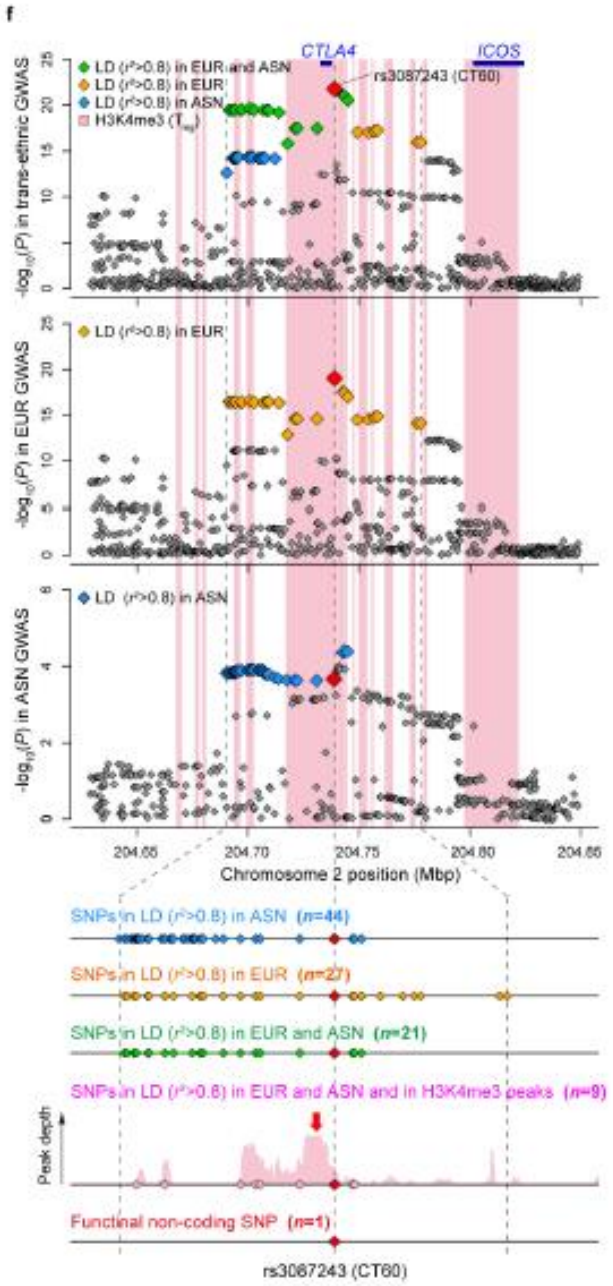
100 RA risk loci



d

Cell types	*P* for H3K4me3 enrichment
T_{reg} primary cells	$\leq 1.0\times10^{-5}$
$CD4^+$ memory primary cells	3.0×10^{-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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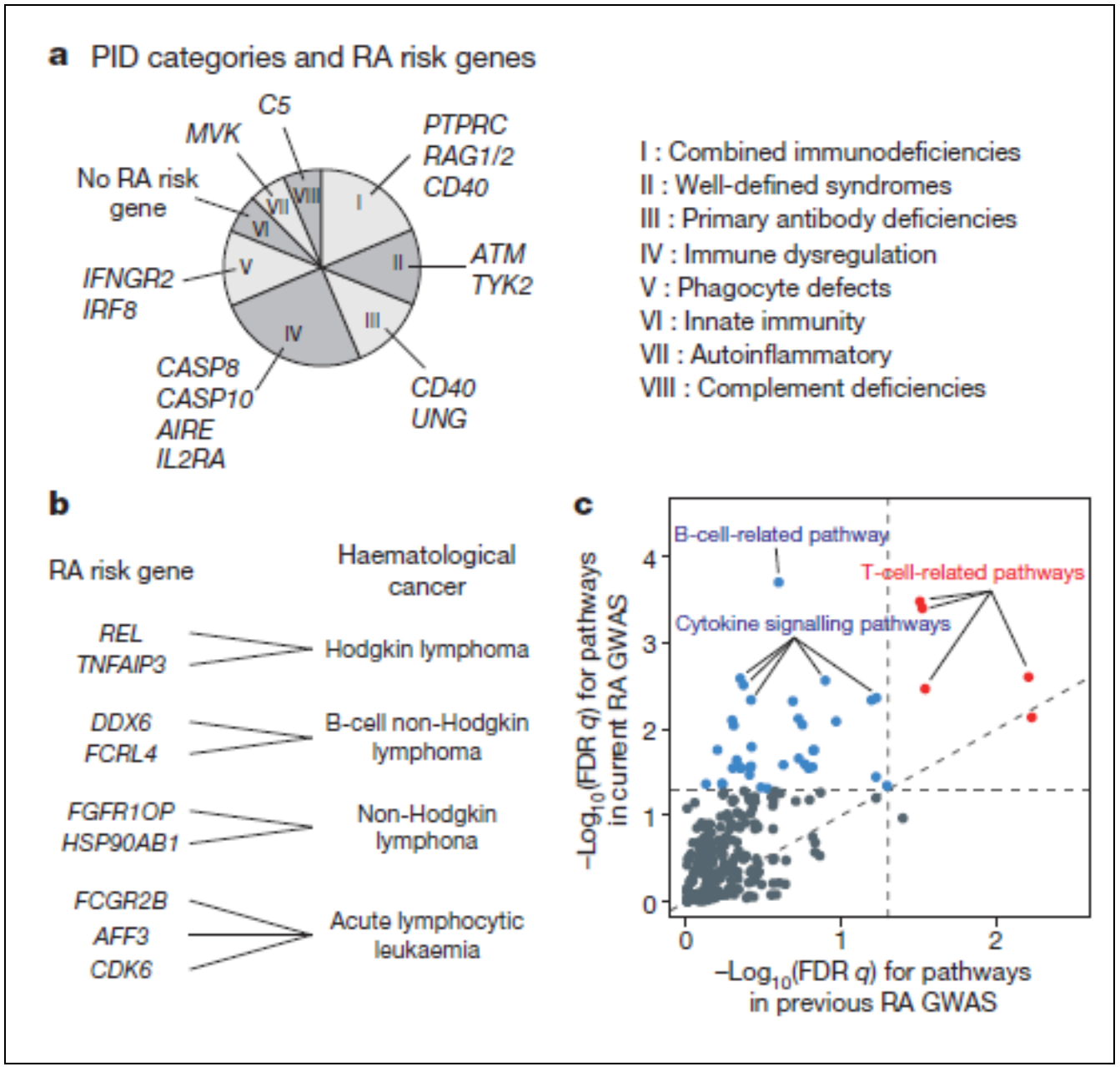
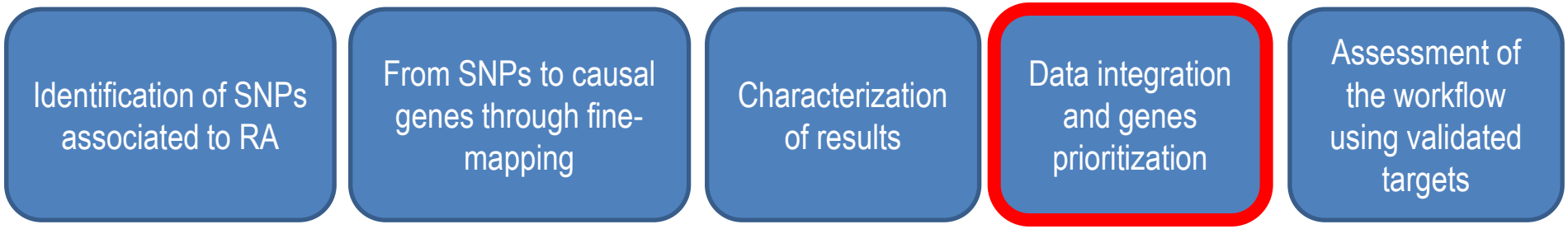
Assessment of the workflow using validated targets

Characterization of Results (100 loci, 377 genes)

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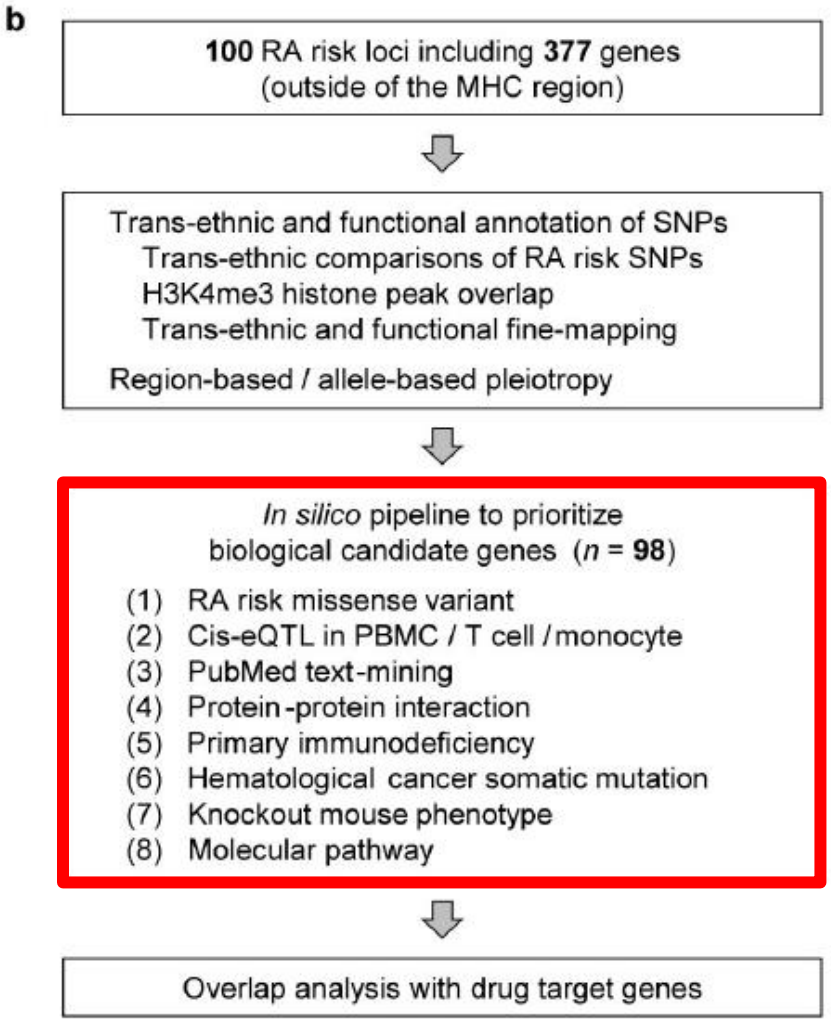
In-silico genes prioritization



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

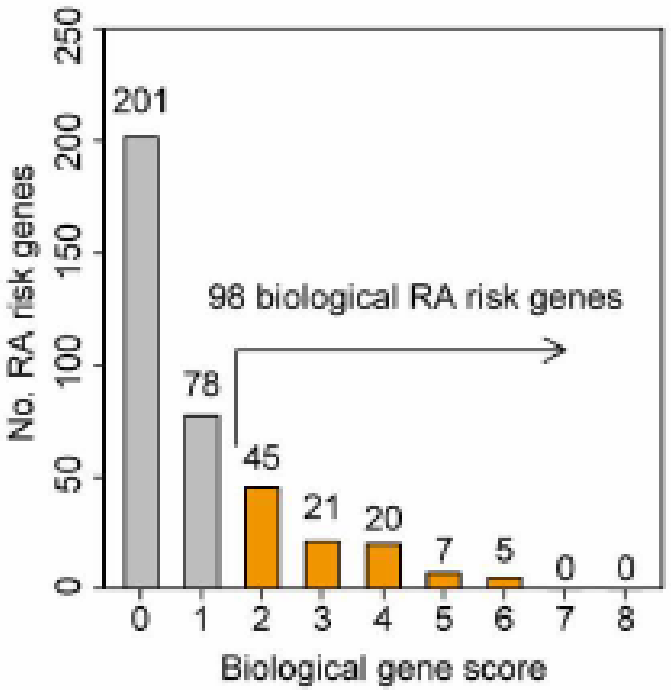
Prioritization of biological candidate genes from RA risk loci

a

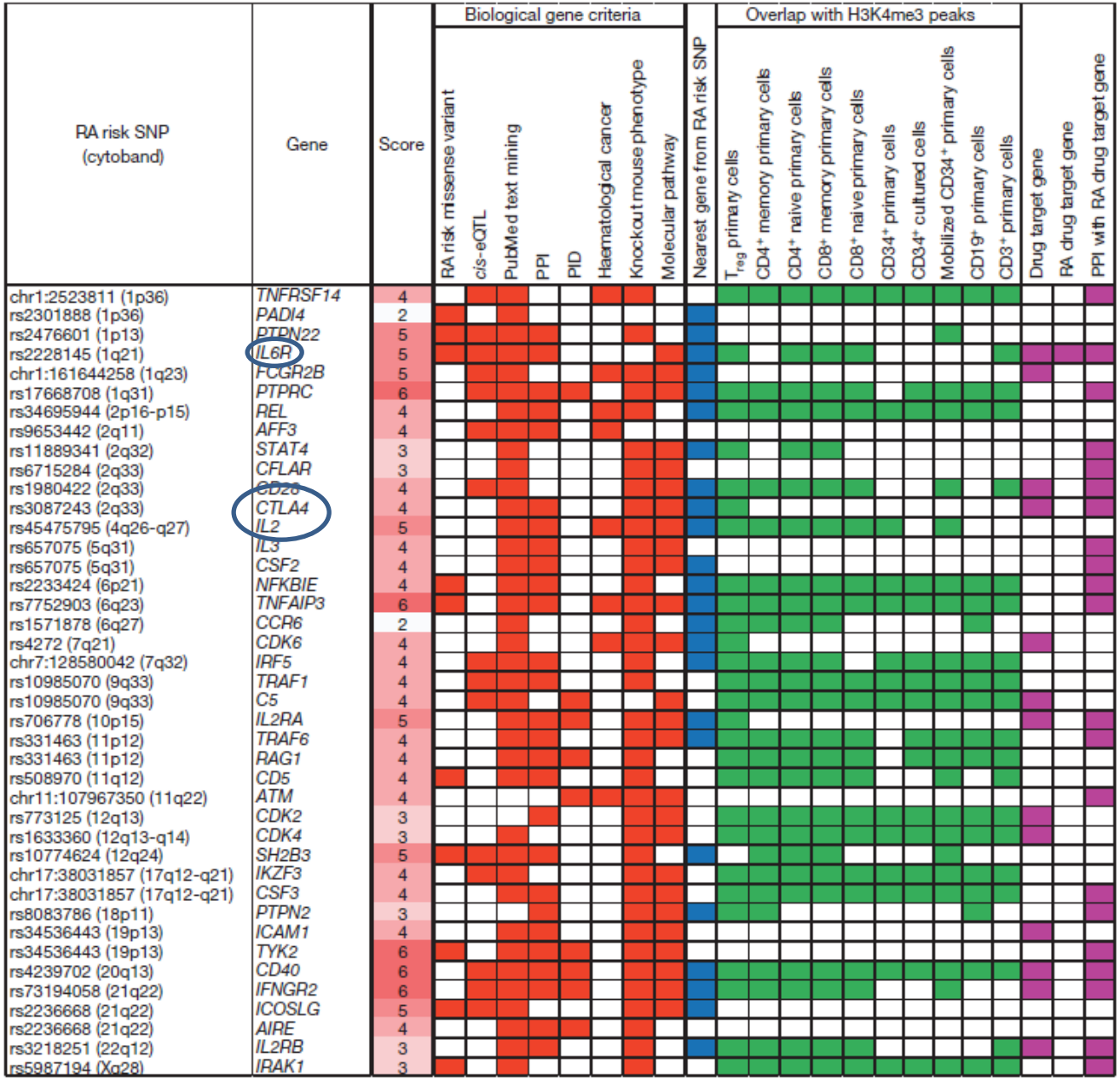
Biological RA risk gene prioritization criteria

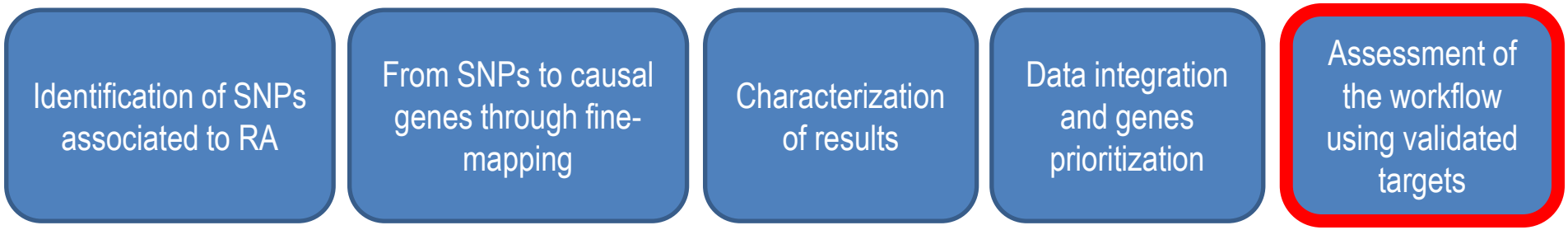
(1) RA risk missense variant (n = 19)
(2) Cis-eQTL (n = 51)
(3) PubMed text-mining (n = 90)
(4) Protein-protein interaction (n = 63)
(5) Primary immunodeficiency (n = 15)
(6) Hematological cancer (n = 17)
(7) Knockout mouse phenotype (n = 86)
(8) Molecular pathway (n = 35)

b



Data Integration and Gene Prioritization




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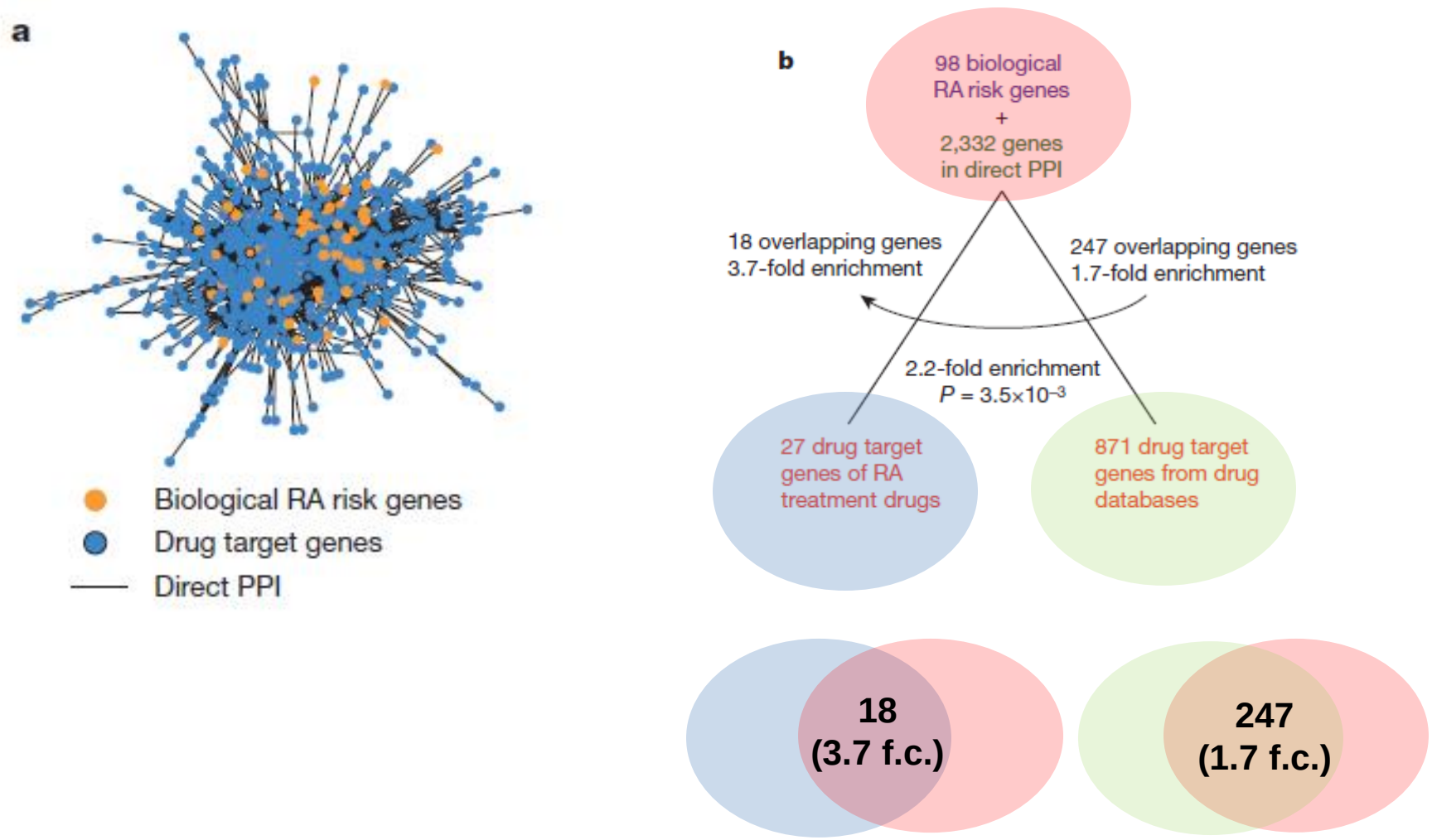
Assessment of the workflow using validated targets

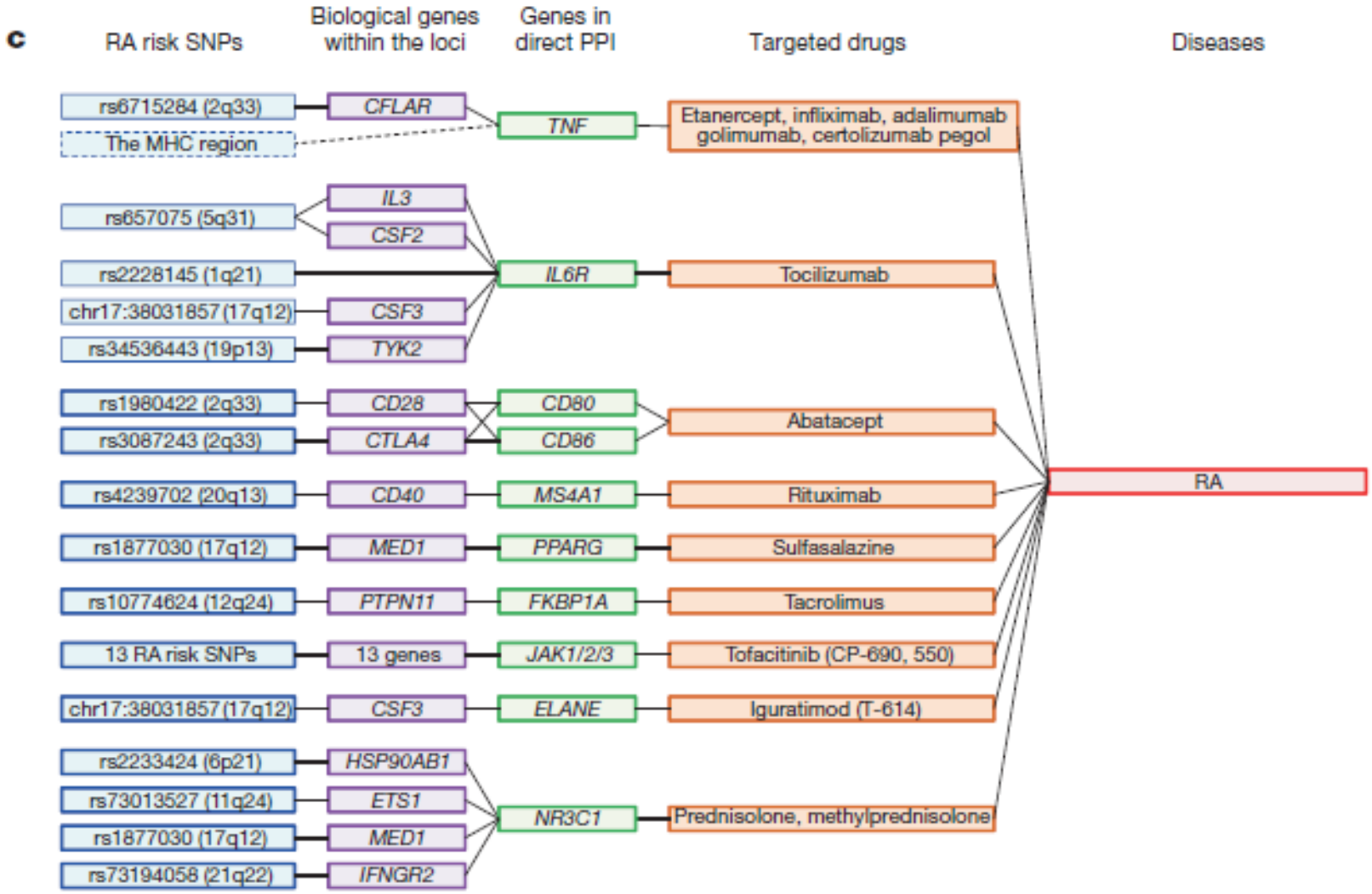
- 98 biological RA risk genes (score >=2)
- +2.322 genes in PPI



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

Mapping RA risk SNPs to drug targets

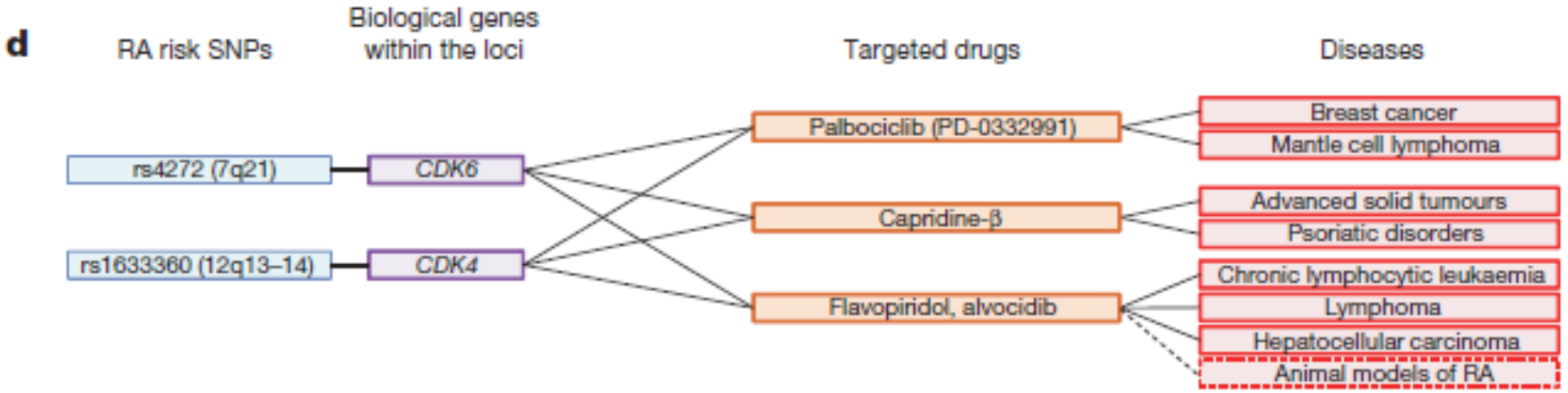
Example: PPI link to known RA drugs



- 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

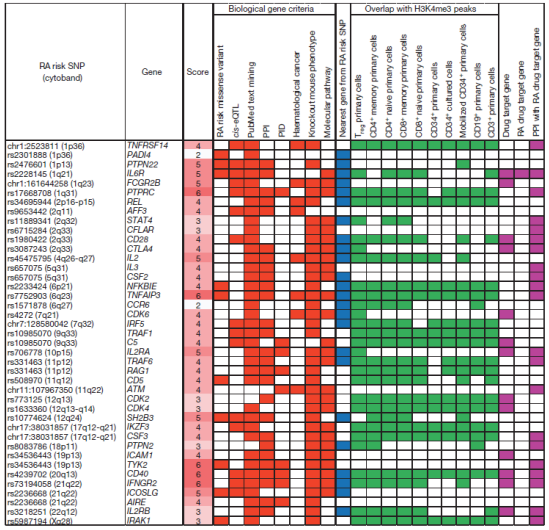
- Systematic approach to derive disease biological insights and novel drug candidates by integrating human genetic data with different layers of orthogonal information

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

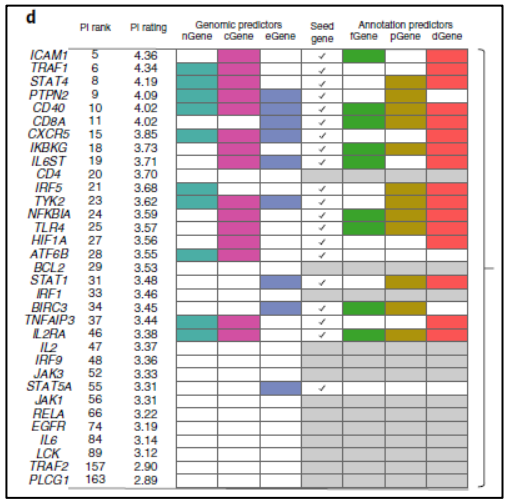
Similar Approaches to Data Integration

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

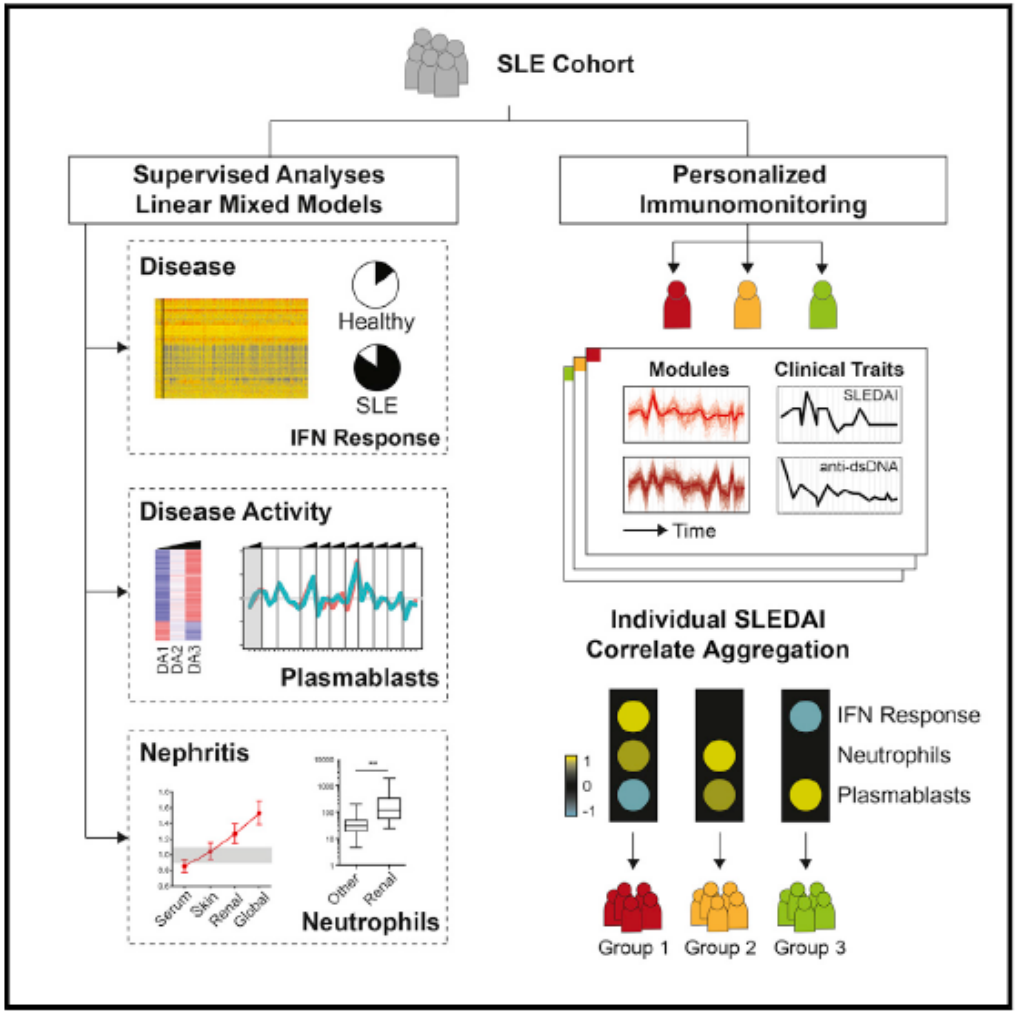
Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients

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,*]

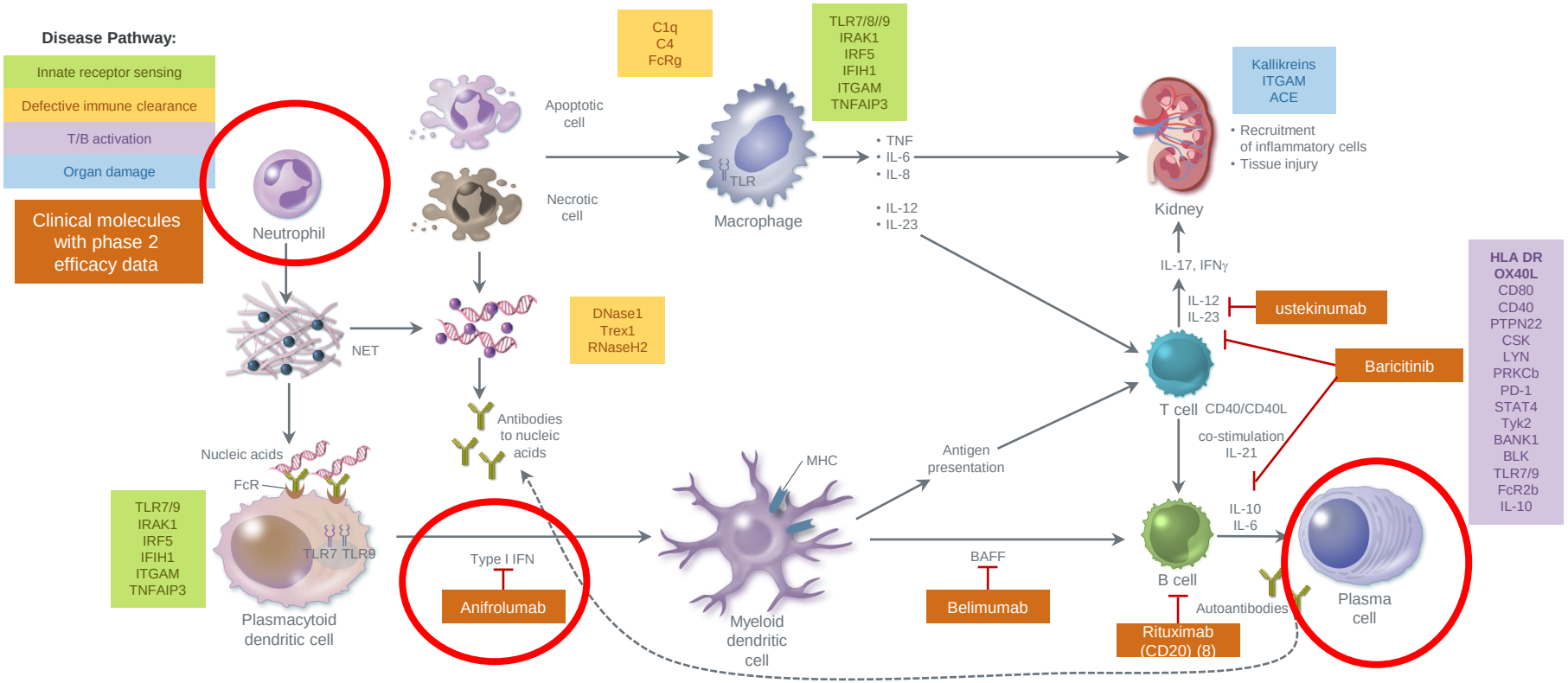
[1]Baylor Institute for Immunology Research, Dallas, TX 75204, USA
[2]UT Southwestern Medical Center, Dallas, TX 75235, USA
[3]Texas Scottish Rite Hospital for Children, Dallas, TX 75219, USA
[4]Vanderbilt University School of Medicine, Nashville, TN 37232, USA
[5]MedImmune, Gaithersburg, MD 20878, USA
[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

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

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



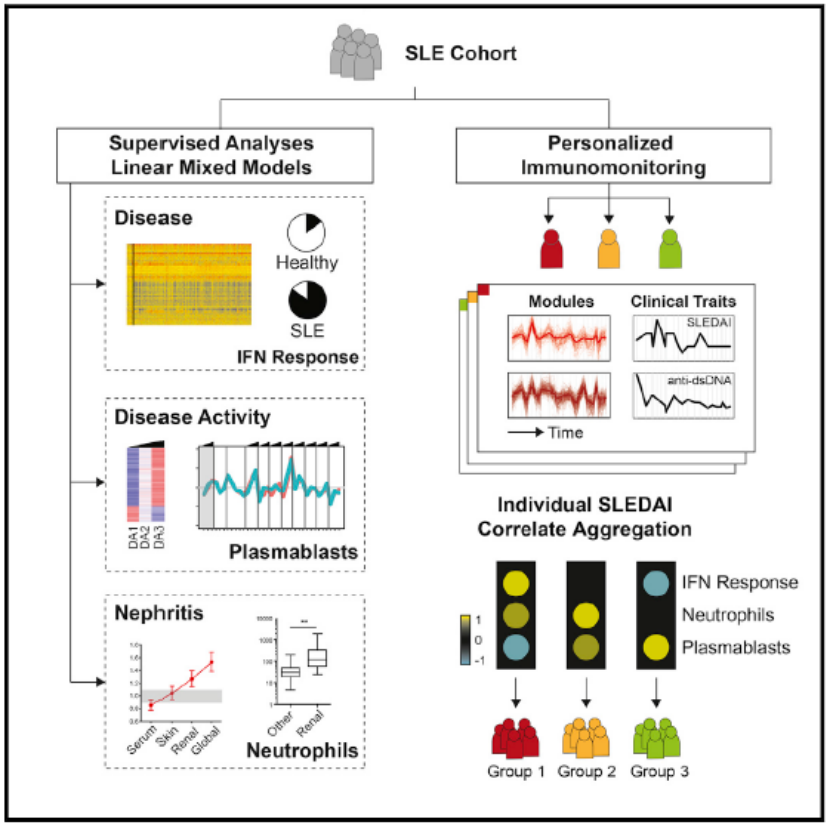
Cells and Pathways Driving SLE



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

SANOFI

Analytical Study Workflow



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”

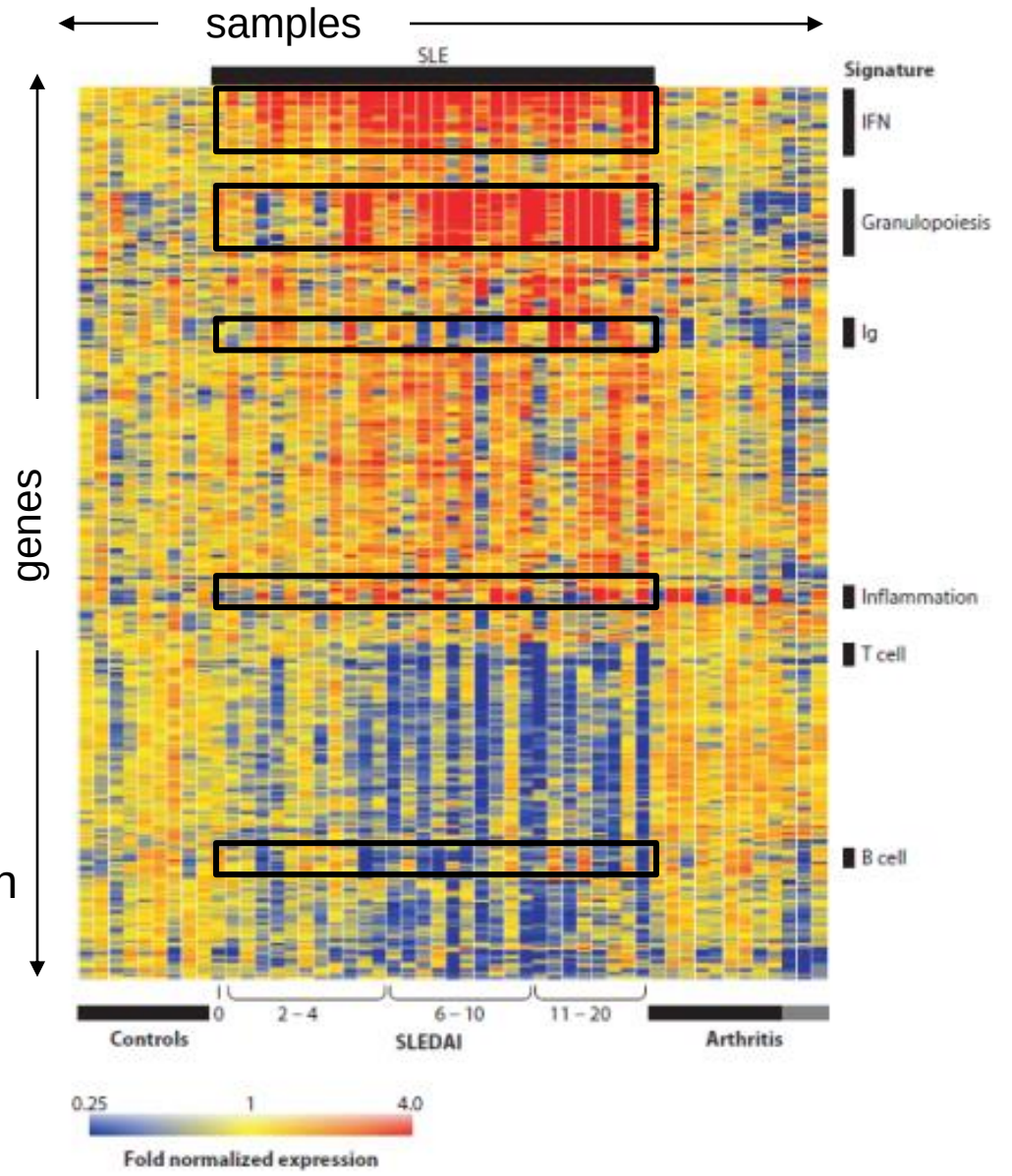
WGCNA modules for each patient and correlation with SLEDAI

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

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

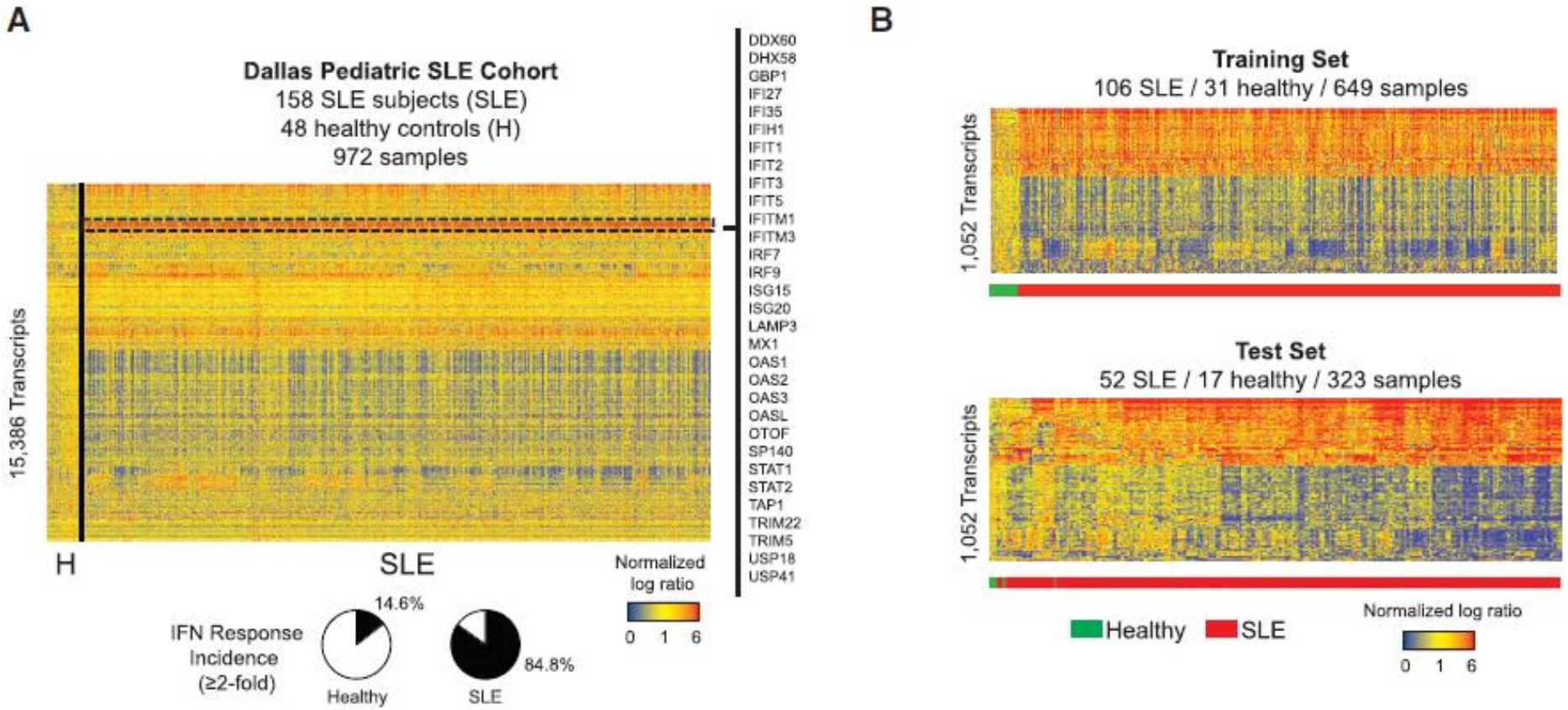
Explanatory Variables (e.g. SLE/Healty, Disease Activity, Treatment etc.)

Expression of gene A

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

Genes Associated with SLE

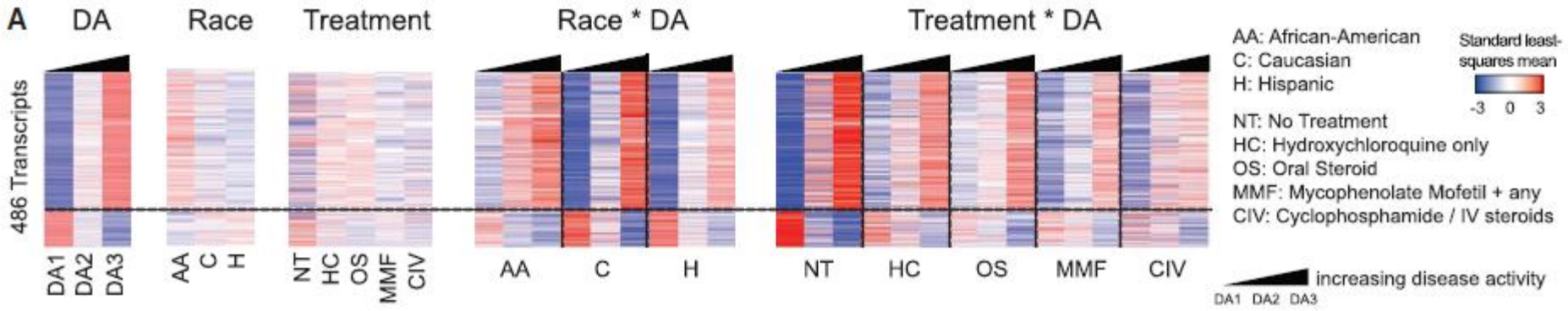
Interferon response



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

➔ 1.052 genes differentially expressed in SLE vs Healthy

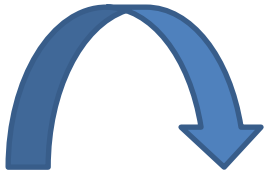
Genes Associated With SLE Disease Activity



- 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

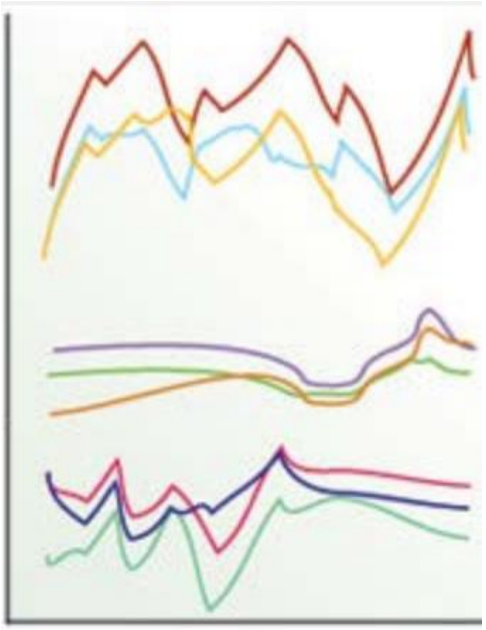
From genes to “Blood Modules”

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

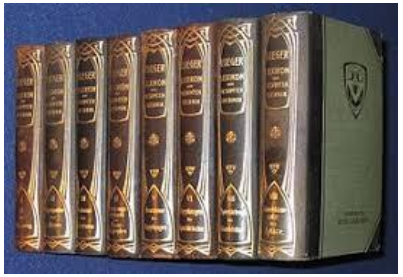


Table 1. Functional Interpretation of Transcriptional Modules

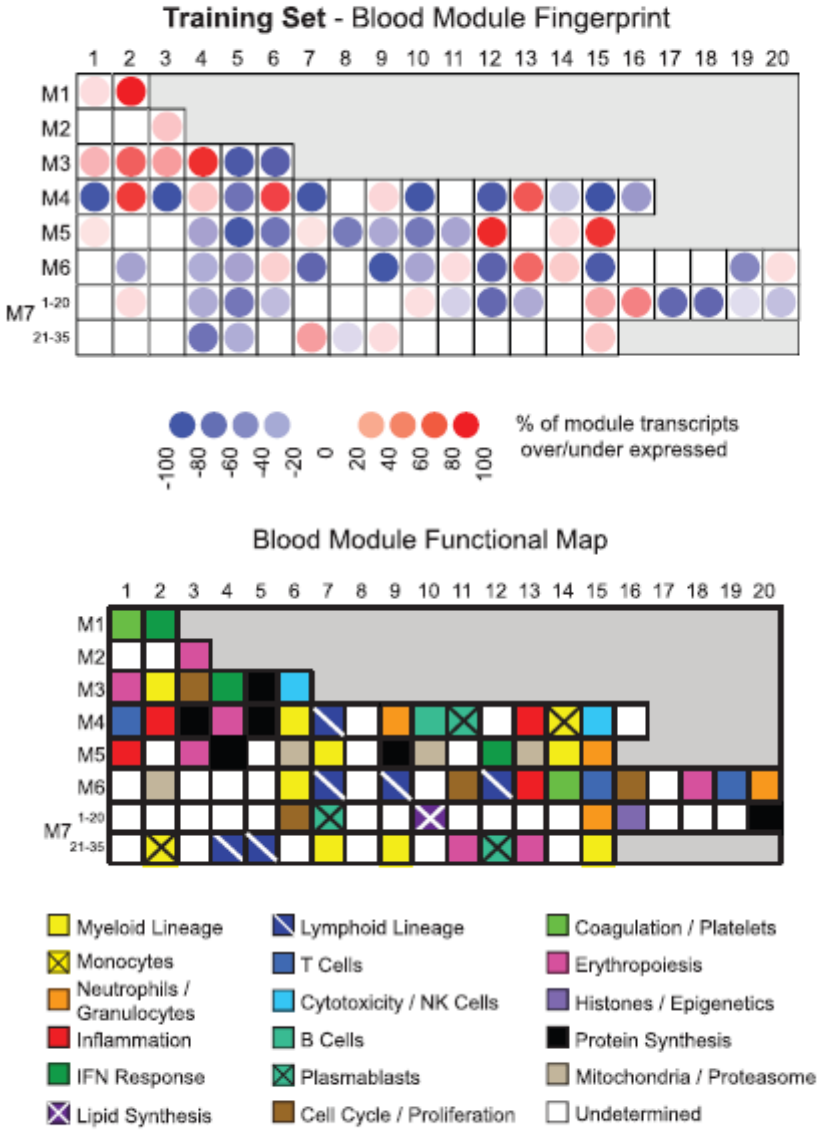
Module I.D.	Number of Probe Sets	Keyword Selection	Interpretation
M 1.1	76	Ig, Immunoglobulin, Bone, Marrow, PreB, IgM,Mu.	Plasma cells. Includes genes coding for Immunoglobulin chains (e.g., IGHM, IGJ, IGLL1, IGKC, IGHD) and the plasma cell marker CD38.
M 1.2	130	Platelet, Adhesion, Aggregation, Endothelial, 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

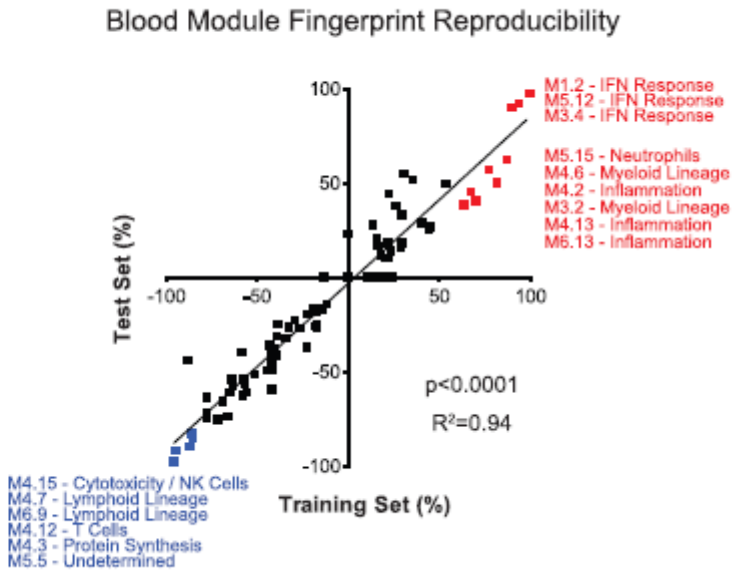
SLE vs Healthy: From Genes to Modules

C

Modules perturbed in SLE vs Healthy



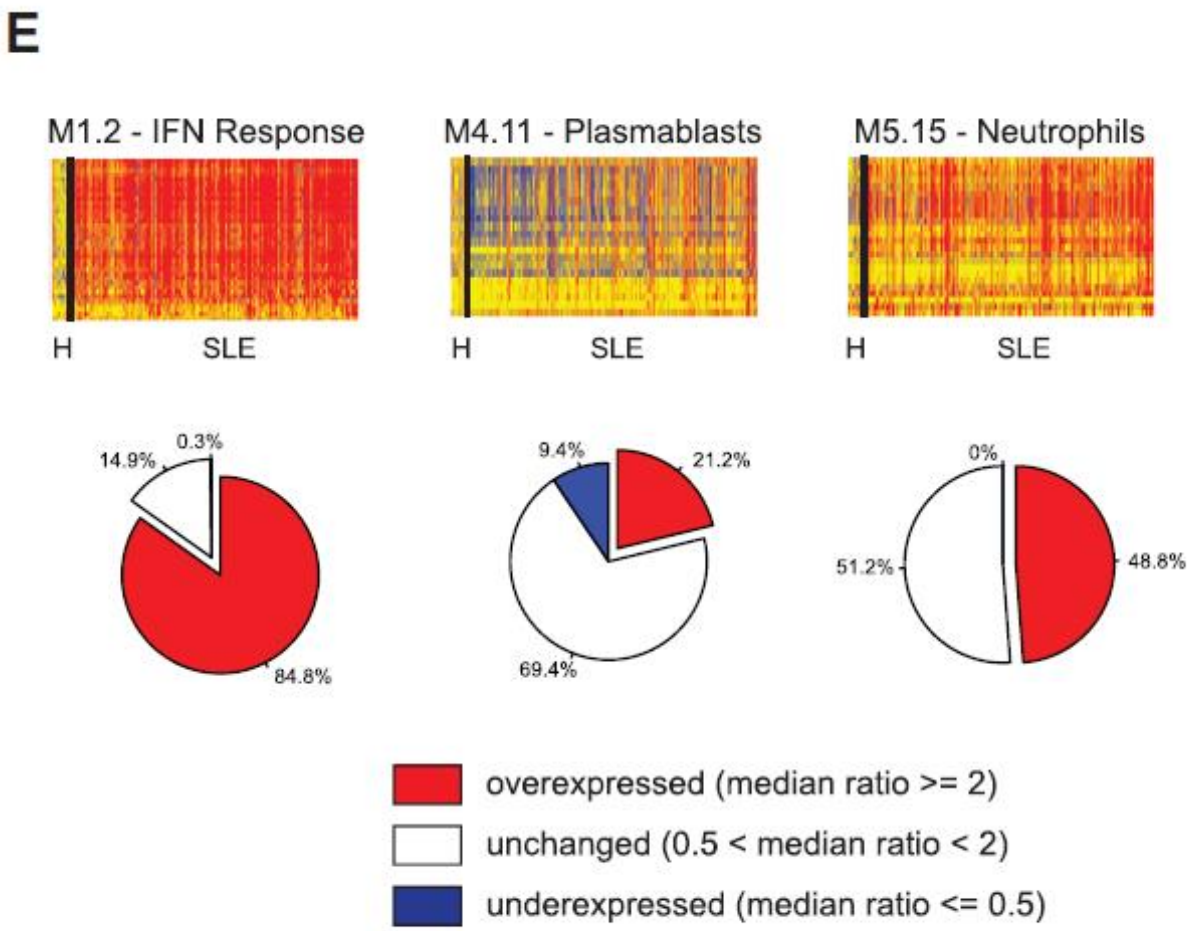
D



- **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**

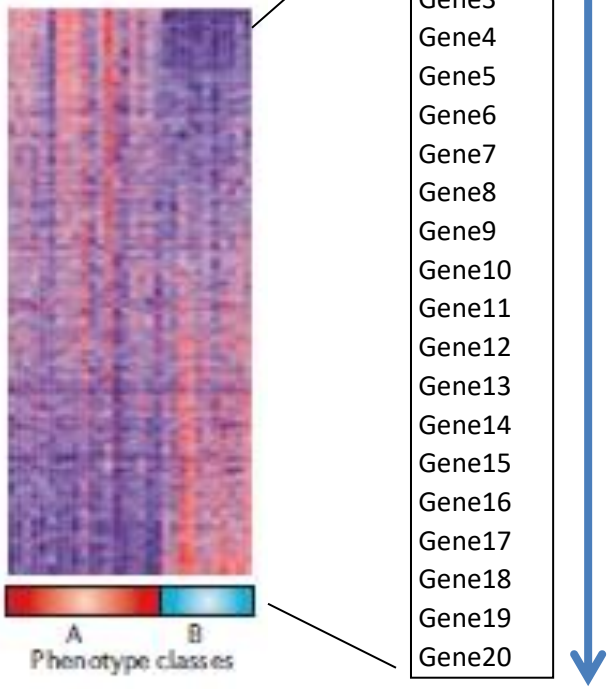
Cell. 2016 Apr 21;165(3):551-65

Over-expression of IFN, plasmablast and neutrophil module genes



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

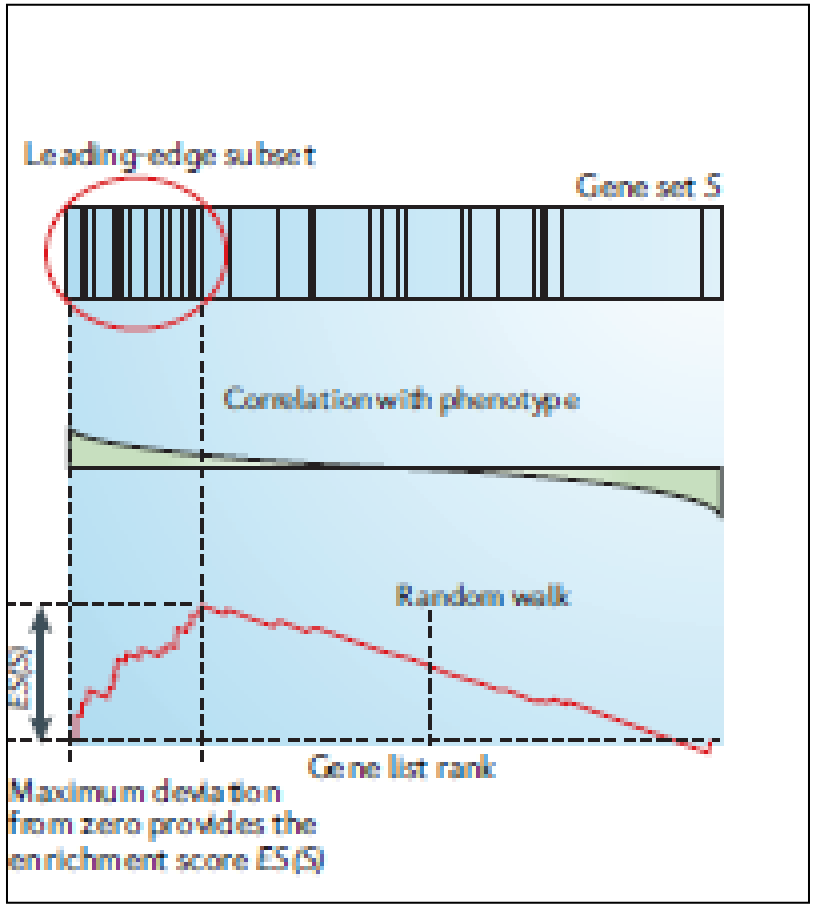
Gene Set Enrichment Analysis (GSEA)



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

Genes are ranked according to their fold-change

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



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

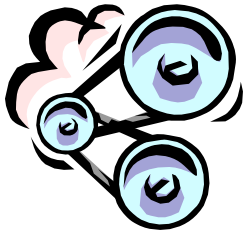
Aravind Subramanian et al, PNAS 2005

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..

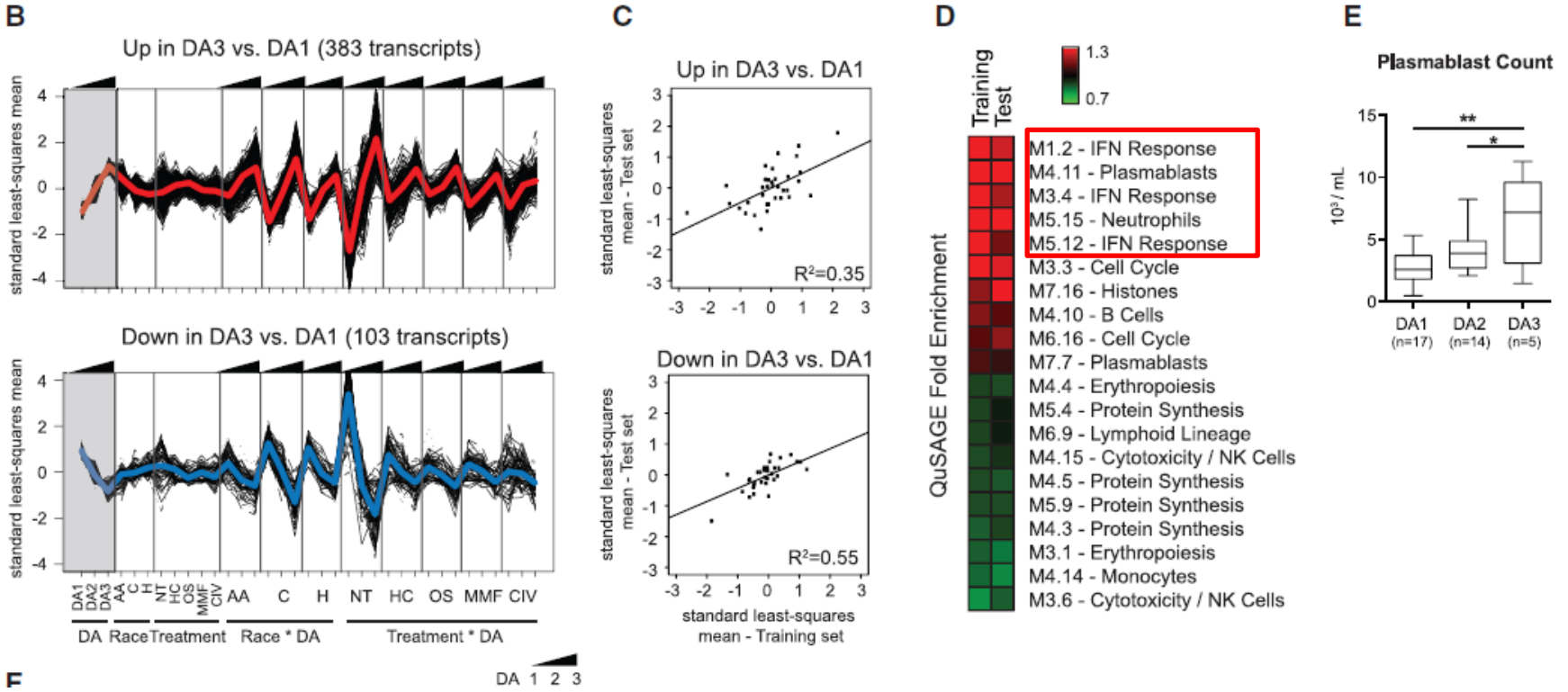


Bioinformatics analysis using pathway DBs

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

List of perturbed pathways

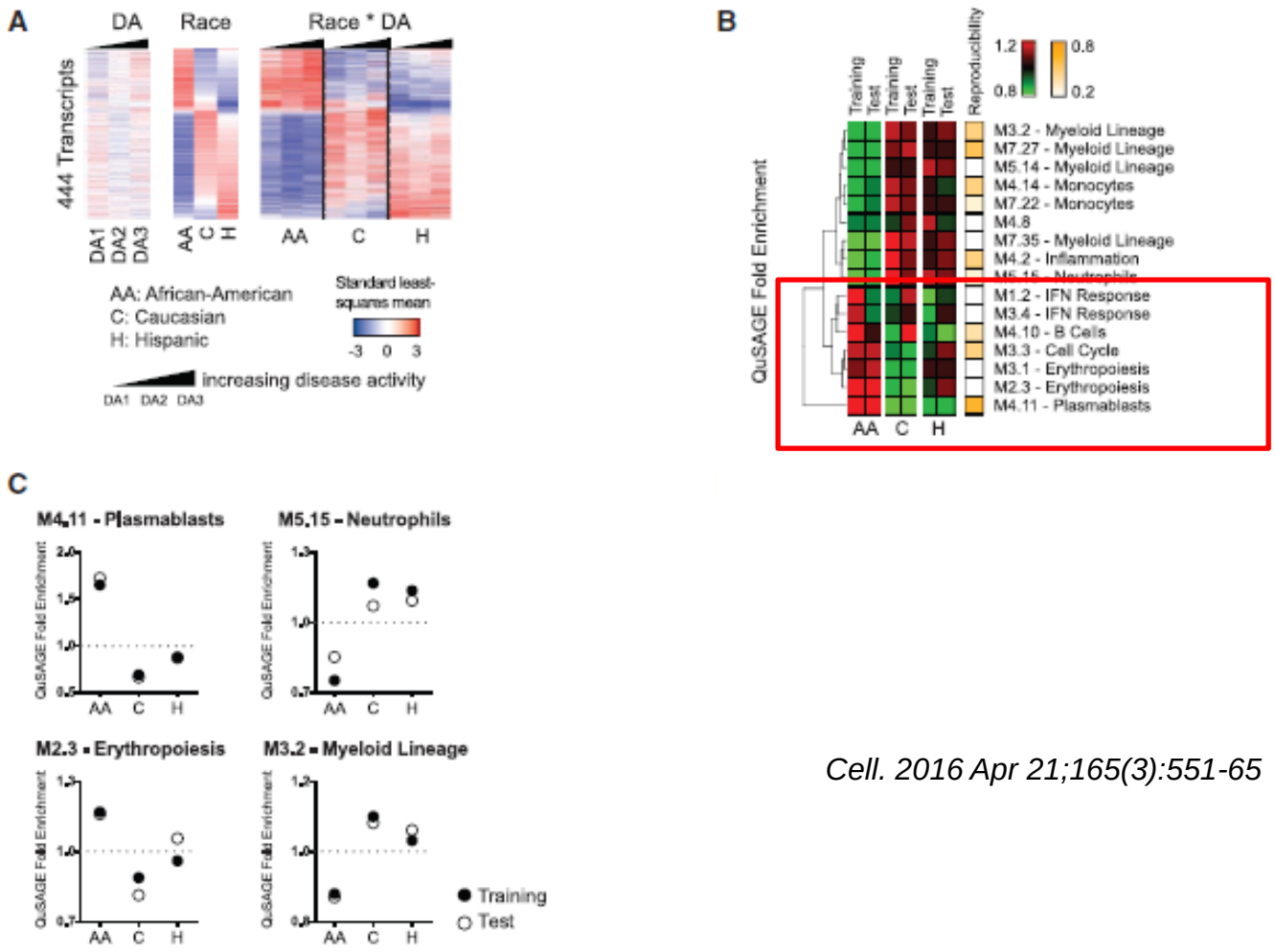
From Genes to Modules Associated with Disease Activity



➔ 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



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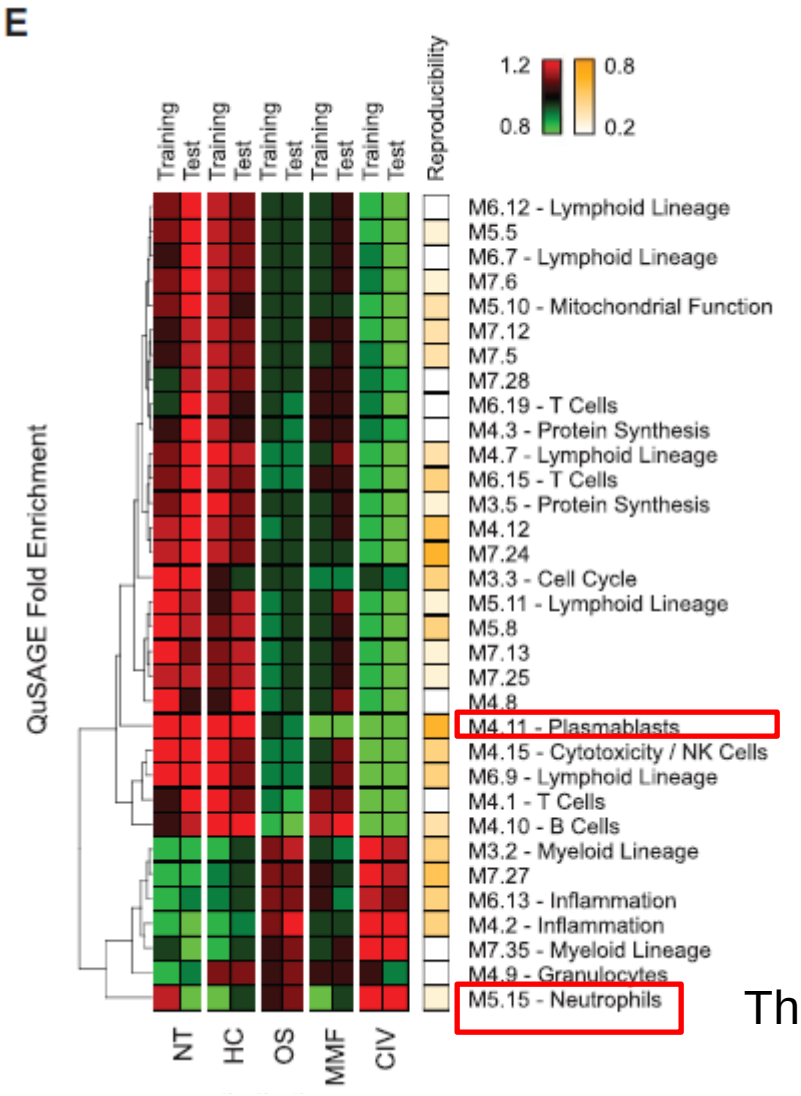
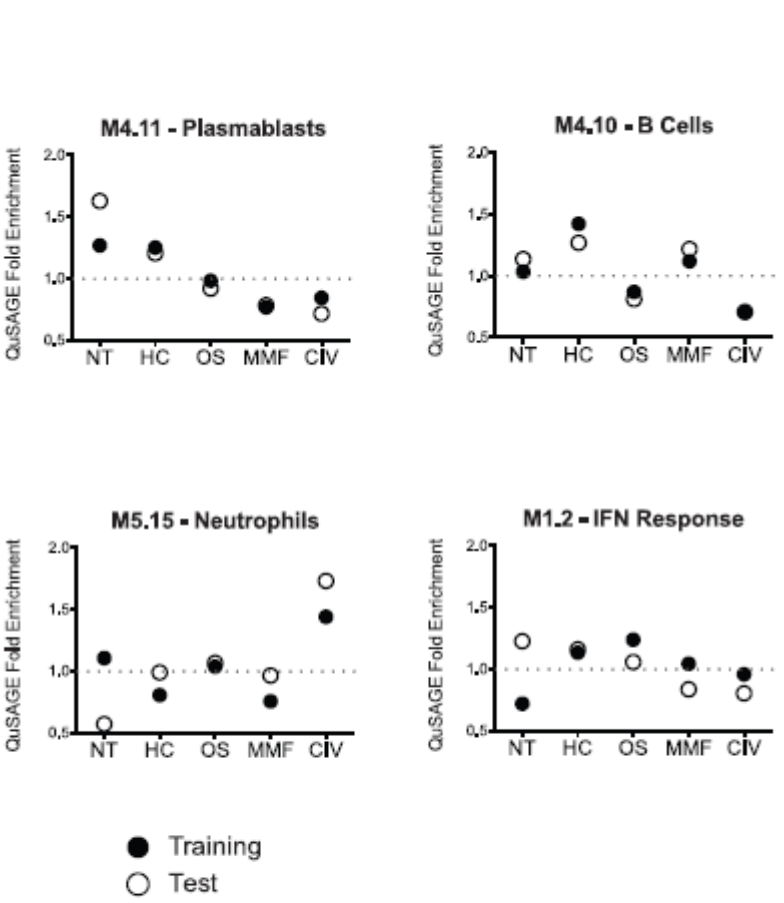
Increased Plasmablast Responses in African-American Patients

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 NT**, but most strongly by (MMF) and CIV, two cytostatic drugs that suppress activated lymphocytes

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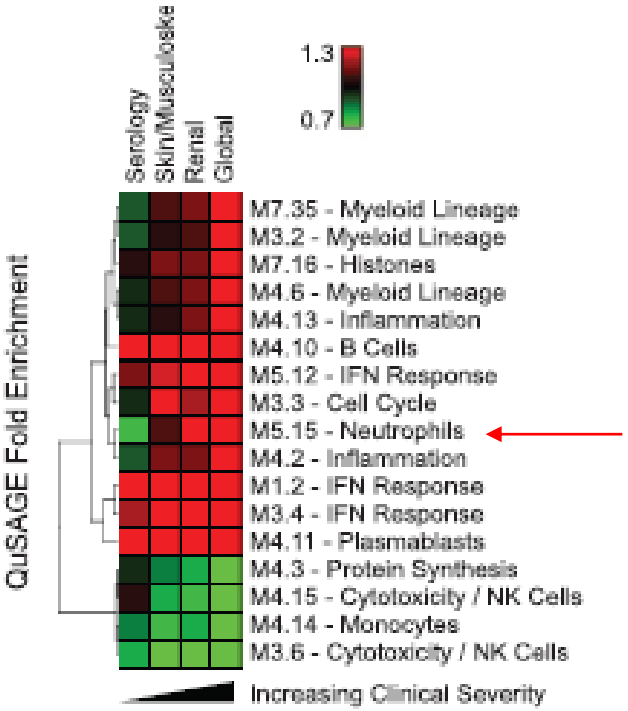
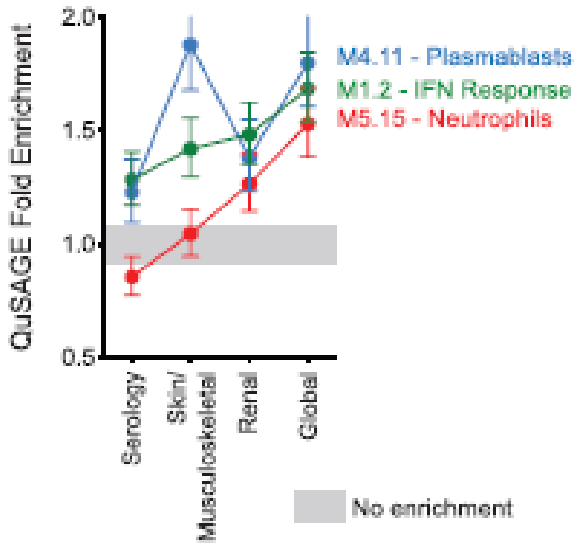
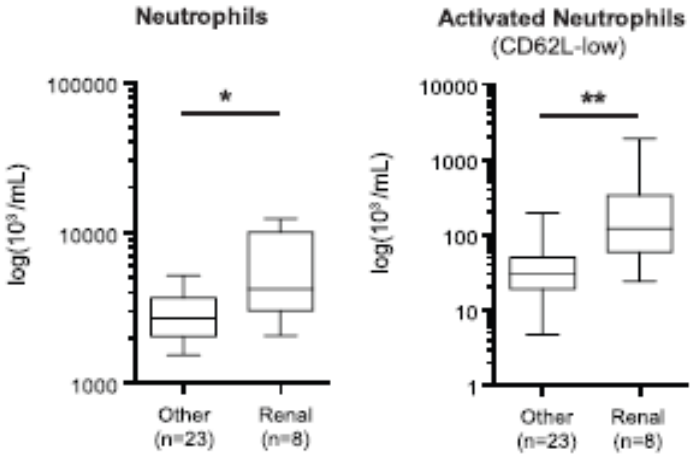
Modules associated with Disease Types





Neutrophil module is associated with Lupus Nephritis



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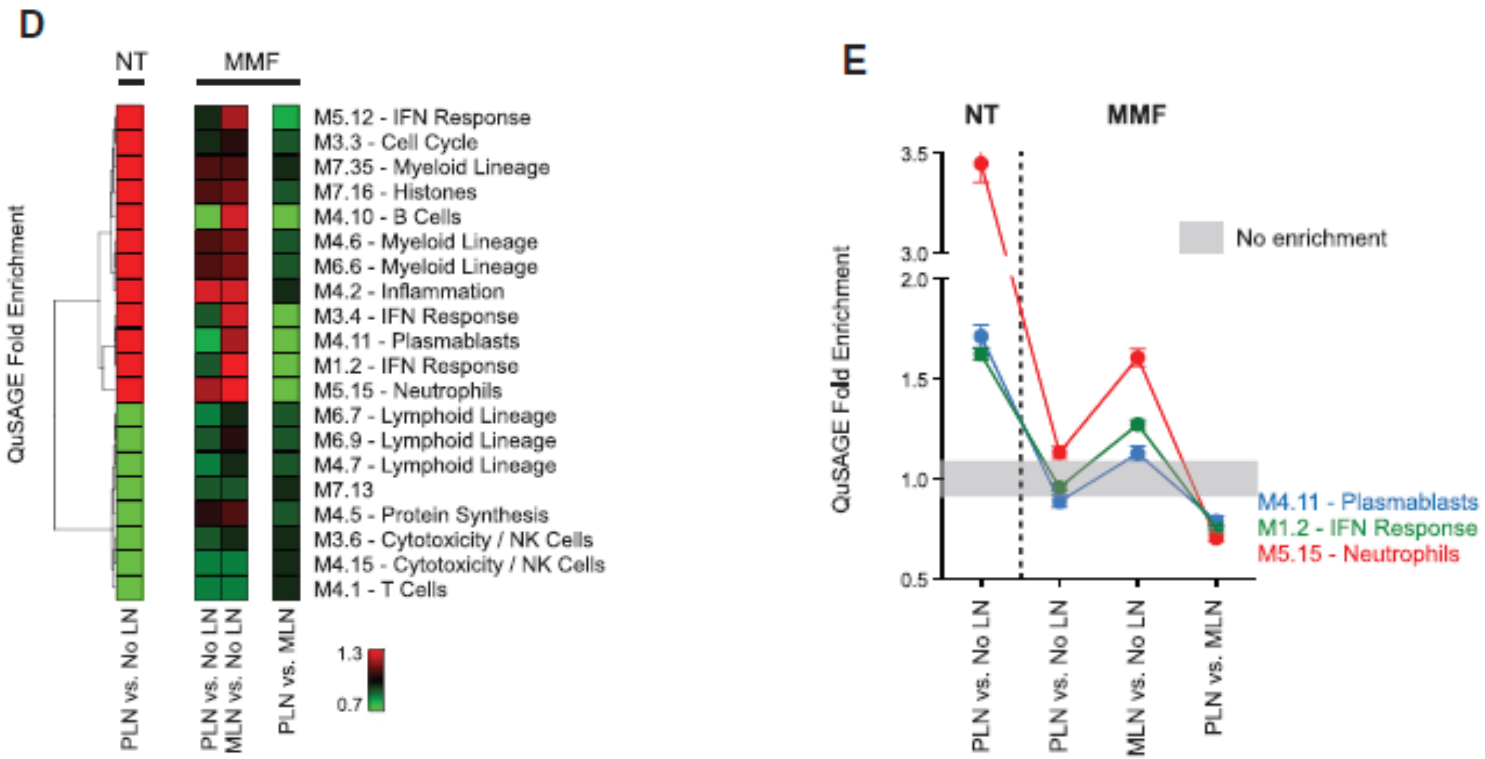
Modules associated with treatment in different nephritis sub-classes



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

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SLE Blood Transcriptional Fingerprint

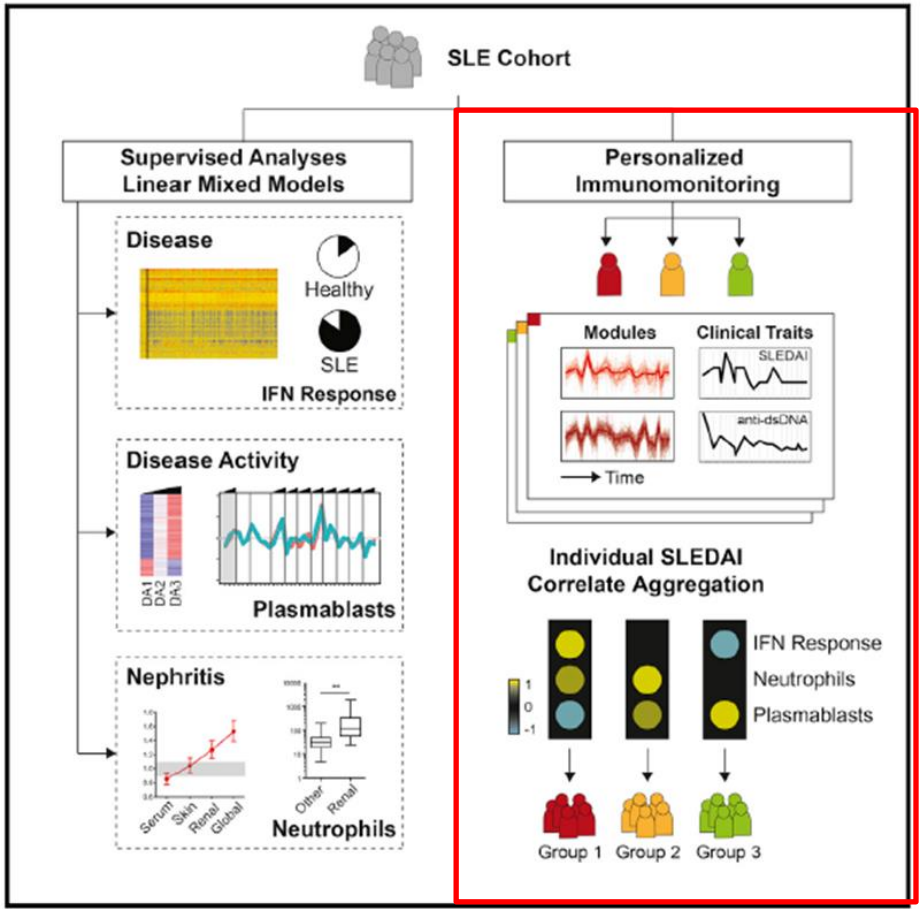
Genes associated to SLE, DA, Race, Treatment, disease subtypes

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

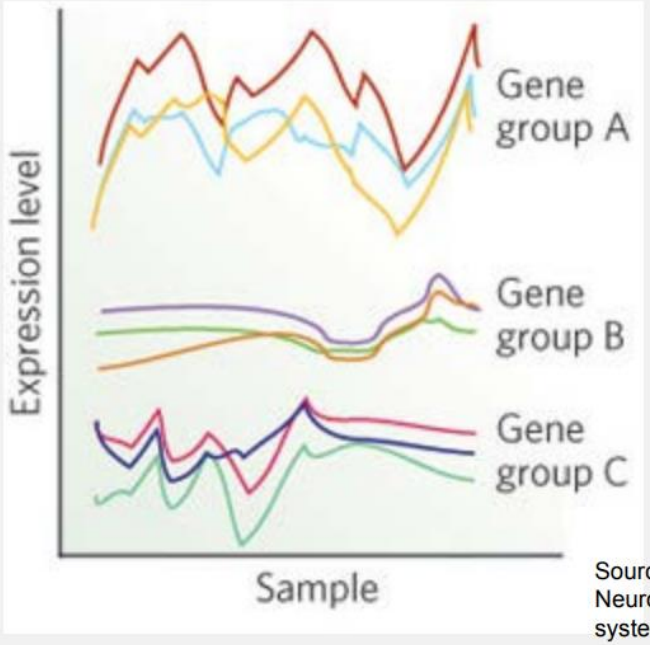


WGCNA – Weighted Gene Correlation Network

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)



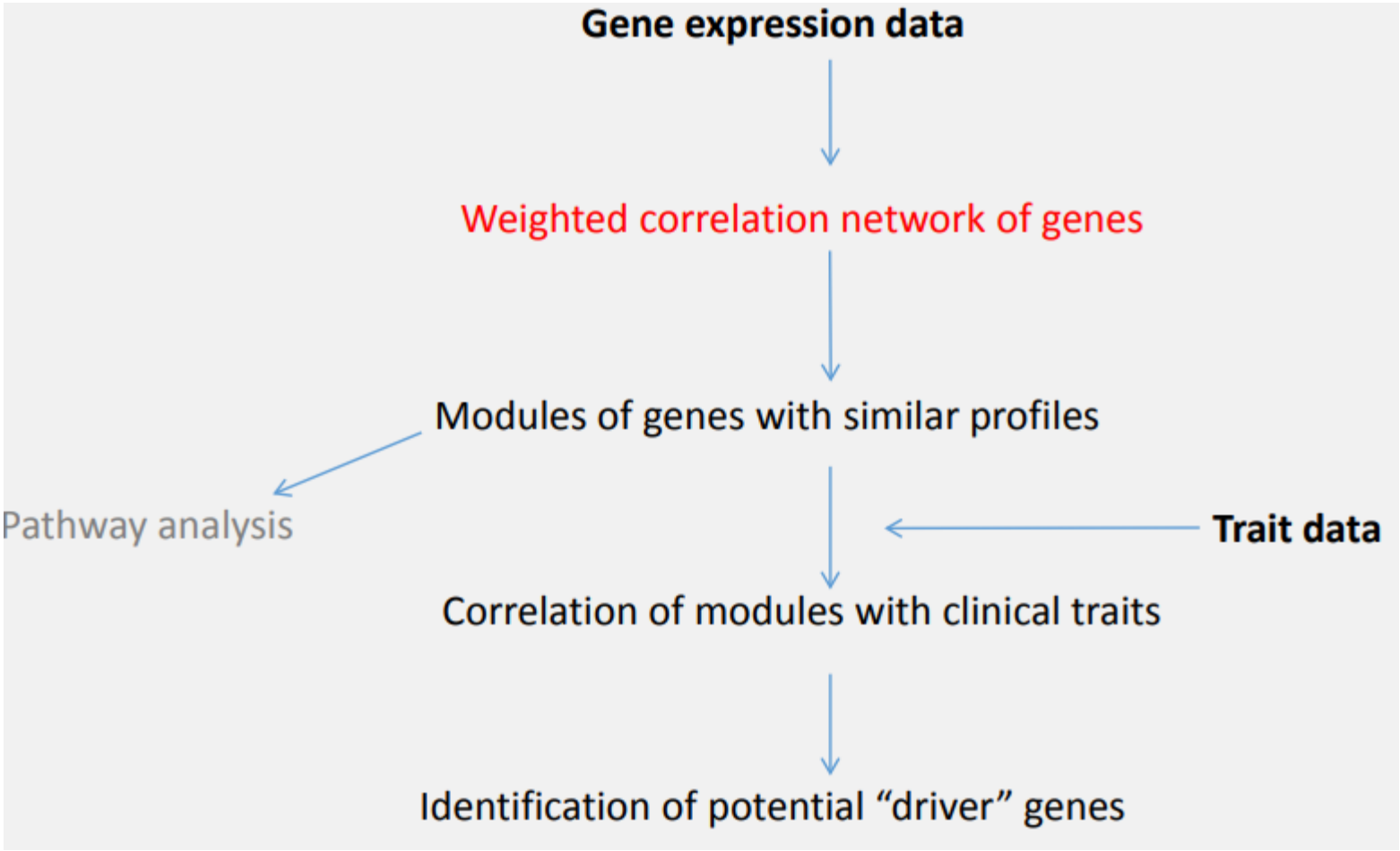
Source: Daniel H. Geschwind & Genevieve Konopka. Neuroscience in the era of functional genomics and systems biology, Nature 461, 908-915

Central Hypothesis:

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

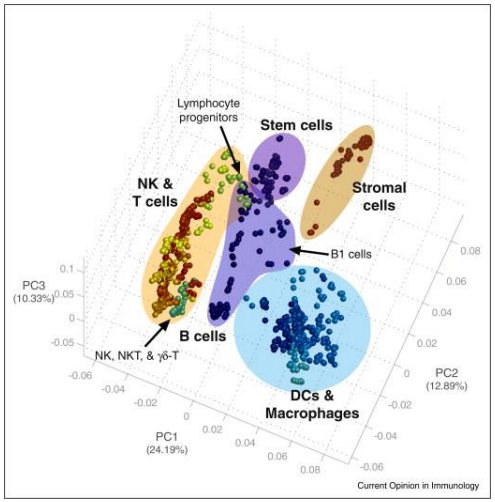
- tightly co-regulated
- functionally related
- members of the same pathway

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

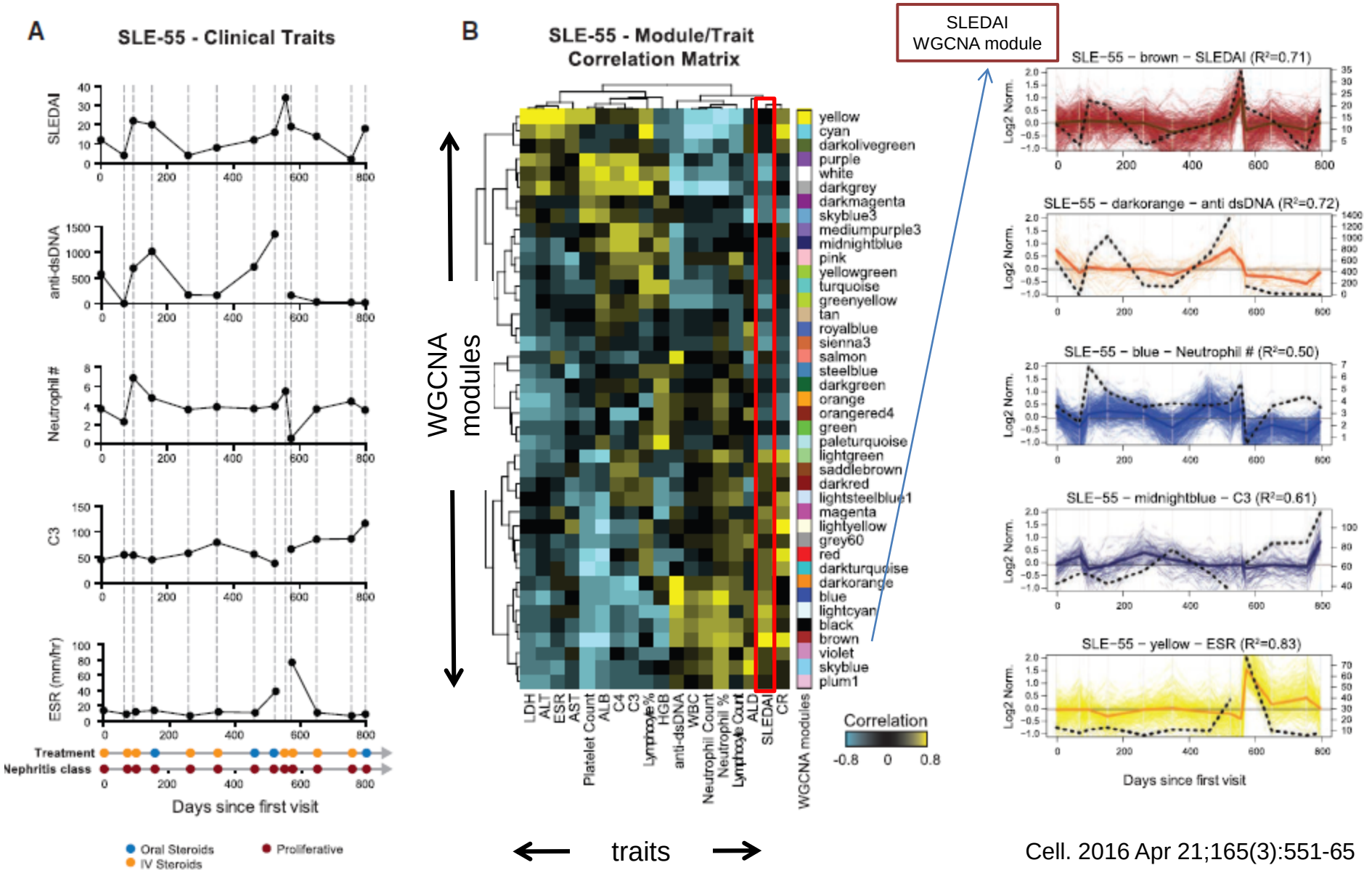


Kim and Lanier – Curr Opin Immun 2013

- 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

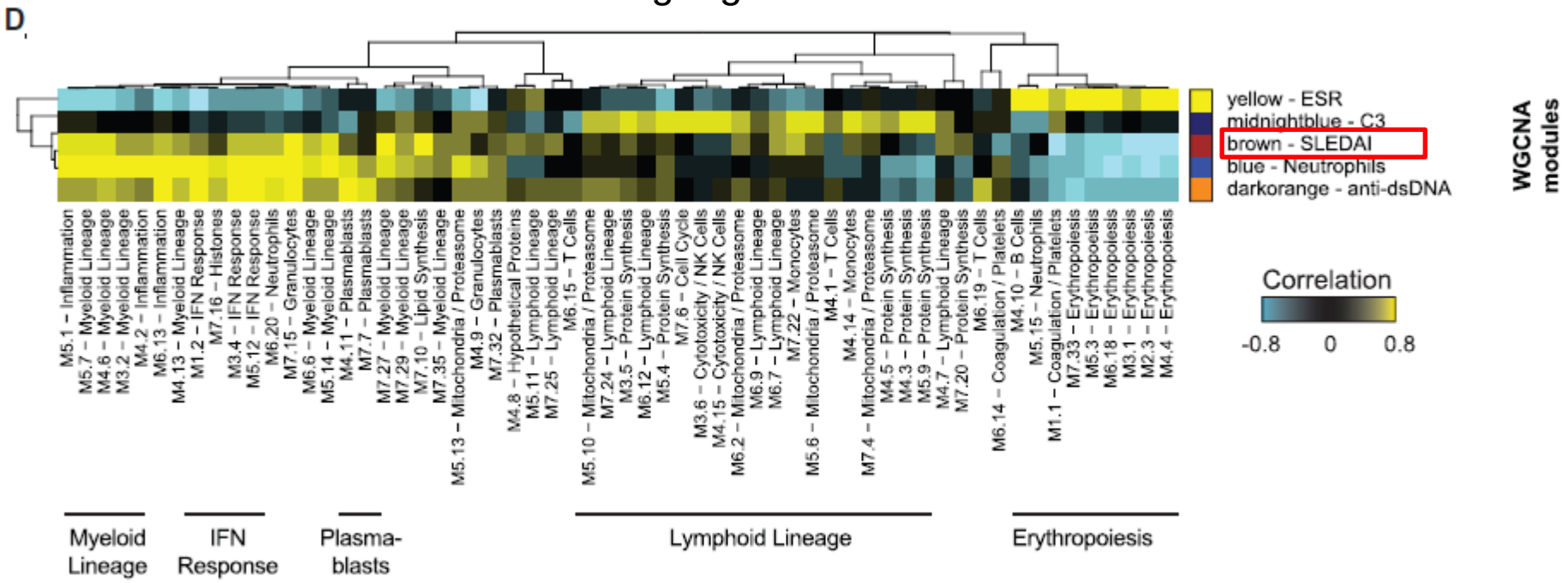
From genes to "blood modules"

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

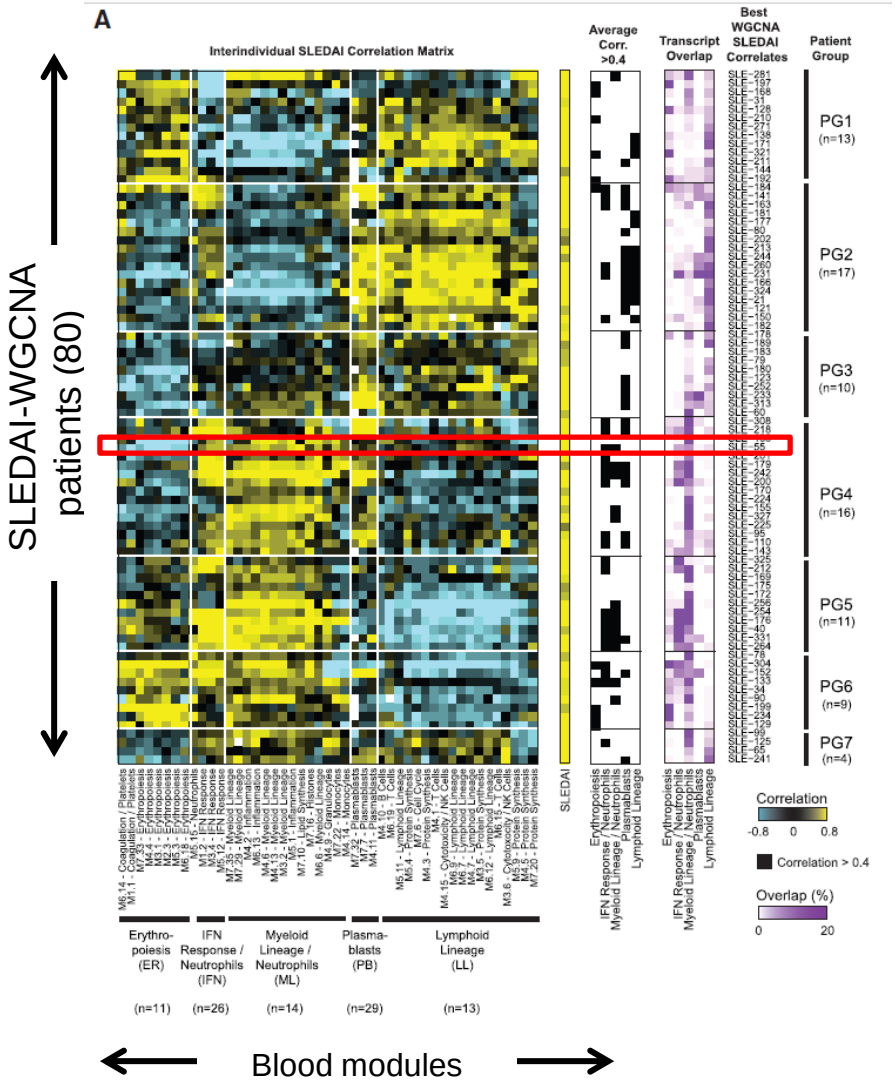
WGCNA modules for each patient and correlation with SLEDAI

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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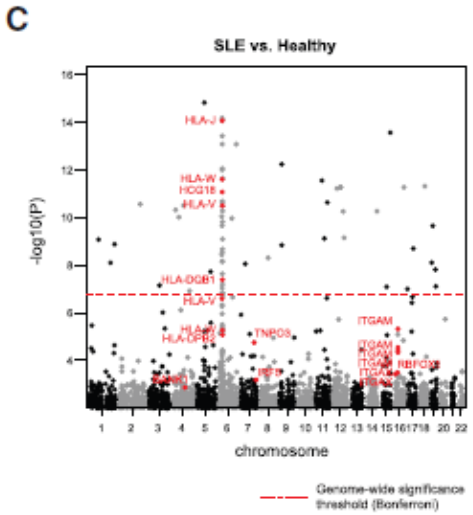
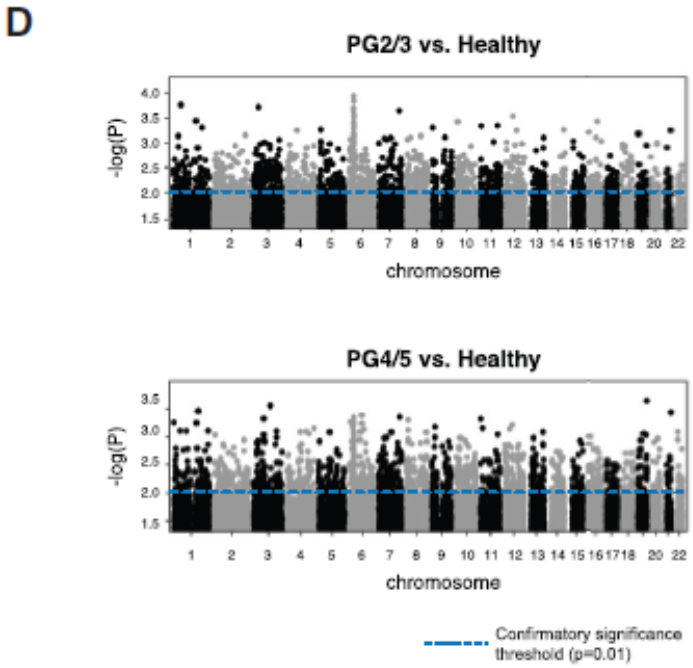
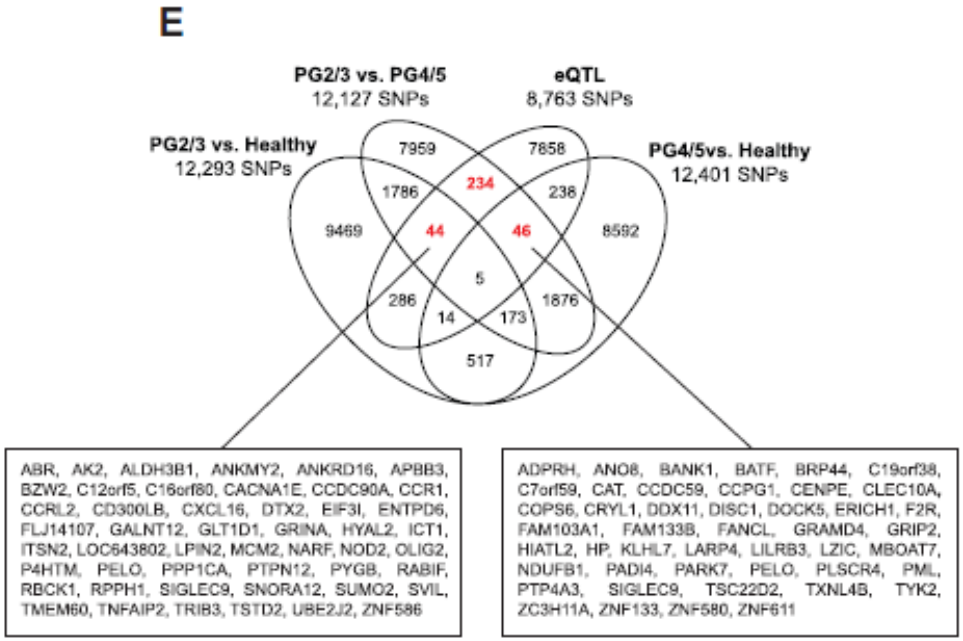
Genetic Analysis of Patients Groups



- 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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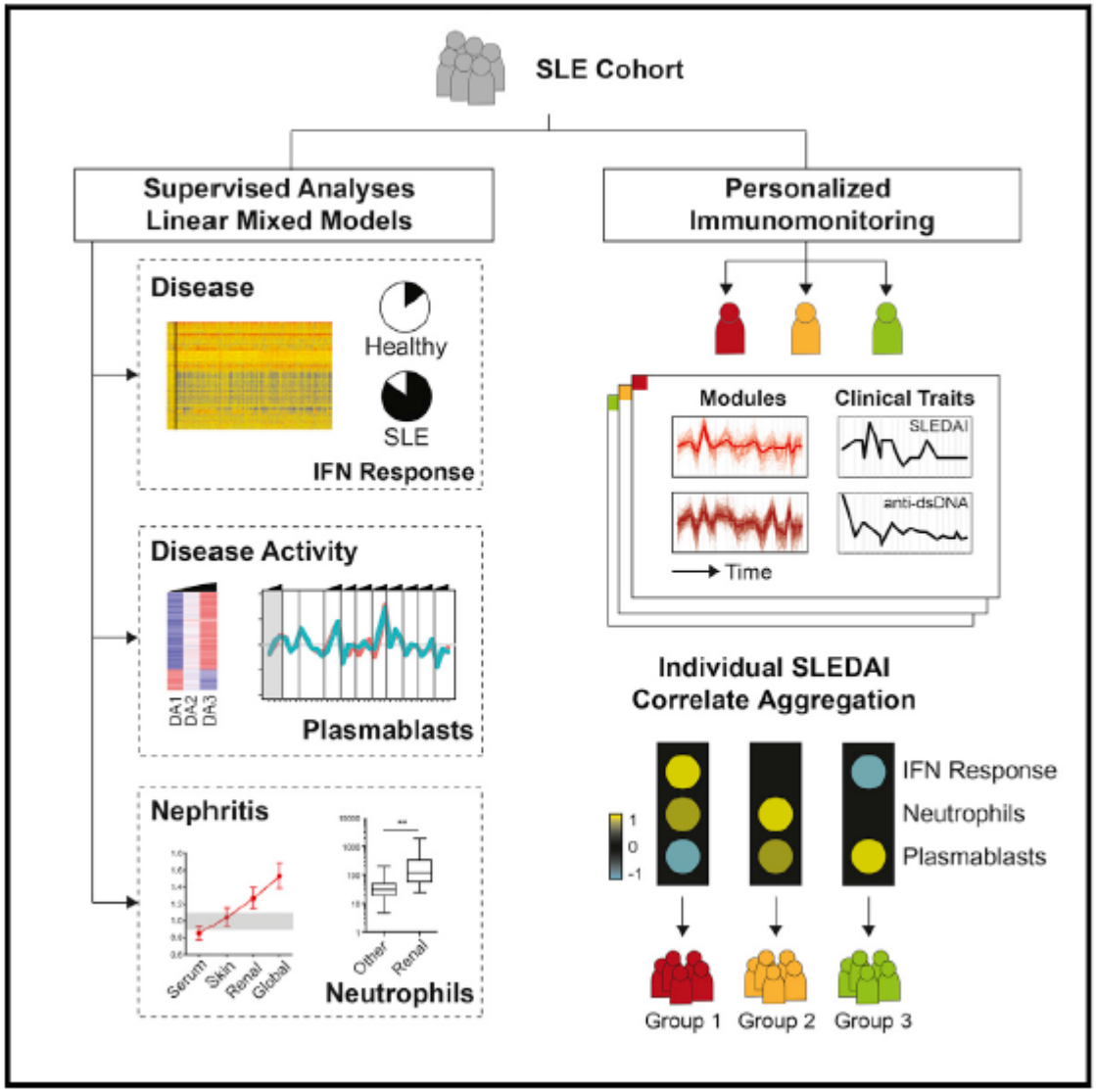
Let's Recap

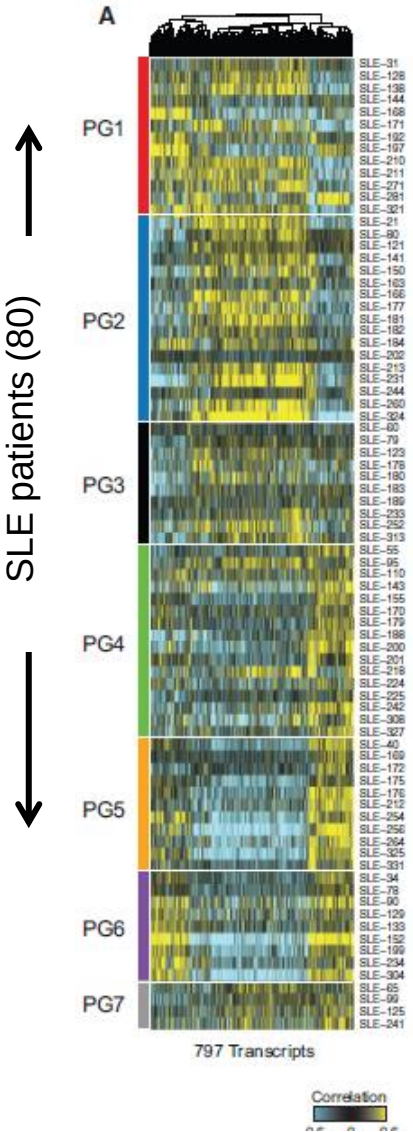


- Supervised analysis, linear models and blood module analyses led to identification of: i) IFN response module in SLE ii) Plasmablasts associated to DA iii) neutrophils associated to nephritis
- Different immune signatures correlating with SLEDAI (7 groups)
- Groups supported by different genotypes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

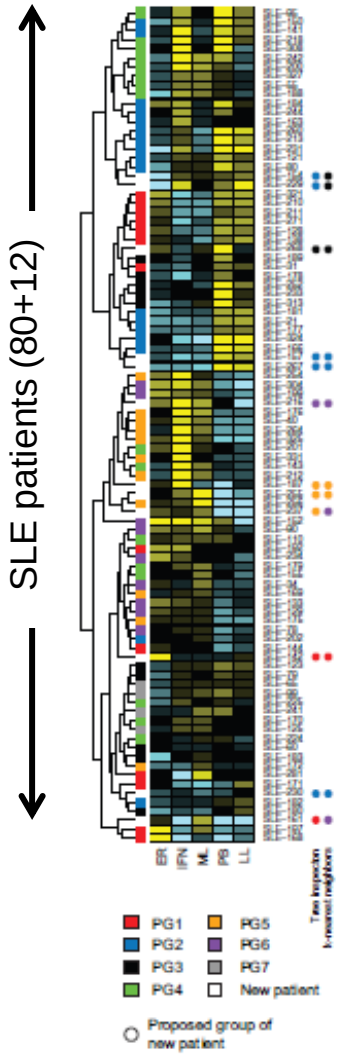
A Targeted Panel for SLE Patient Stratification

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”



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

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

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

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

Q & A

My Best Reference for Systems Immunology: ImmunoScope

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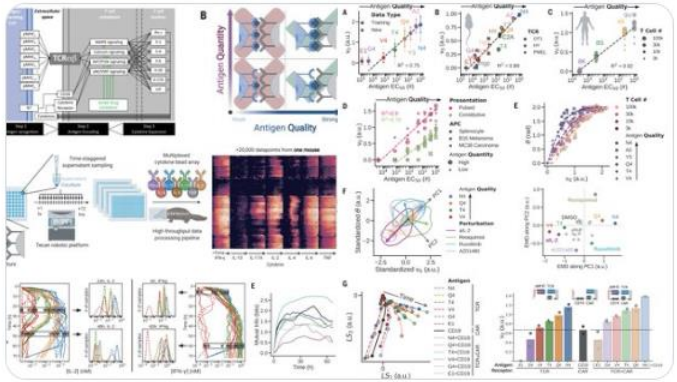
Importance of antigen quality rather than quantity in predicting dynamic T cell responses | #CD8 #TCR #antigen #timecourse | Grégoire Altan-Bonnet @g_altanbonnet @p_r_francois @trademaker17 @McGillUPhysics @NCIResearchCtr | Science @ScienceMagazine bit.ly/3wvUUko



4 36

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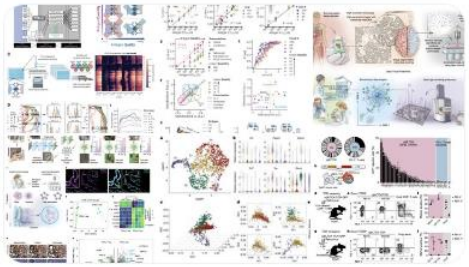

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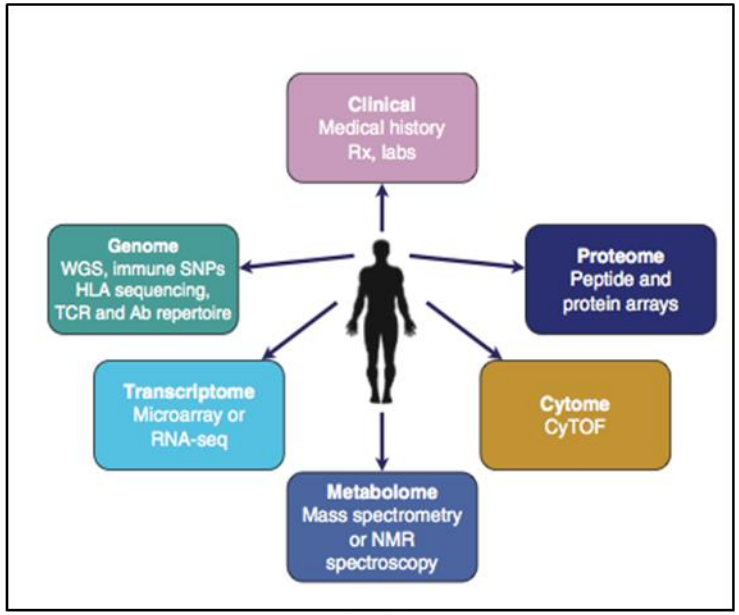
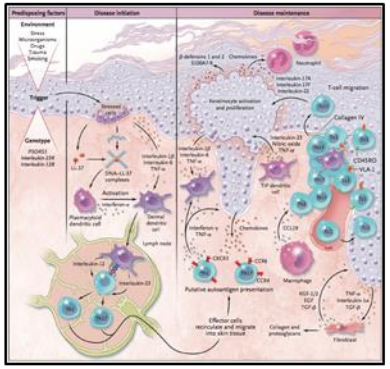
Computational Immunology



Kidd et al. Nat. Immunology 2014



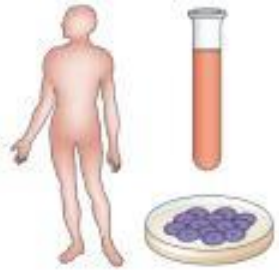
Understanding Molecular Aetiology

P~ f(m)

Explaining clinical phenotype as function of molecular data. Finding biomarkers

Identifying Novel therapeutic targets

Immune Phenotypes

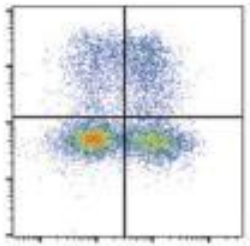


Cell state or material

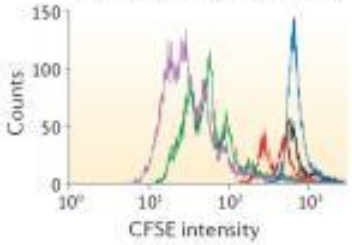
Resting | Cytokine stimulation | Non-antigenic stimulation | Antigenic stimulation | Blood plasma

Technique

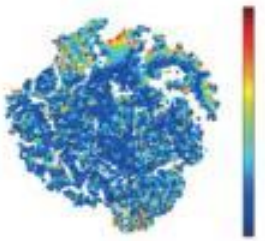
Flow cytometry



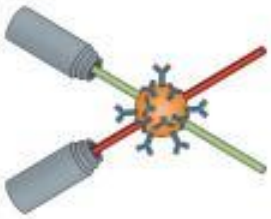
CFSE
(fluorescent cell-staining dye)



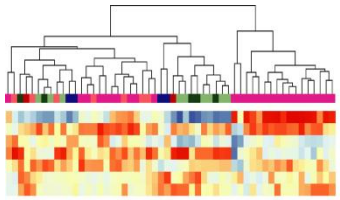
Mass cytometry



Luminex



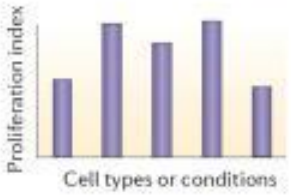
RNA-Seq
Microarrays



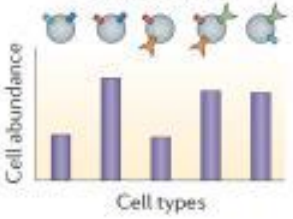
Measurement

Signalling response

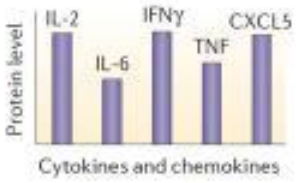
Cell proliferation



Cell frequencies



Serum protein levels



- RNA-levels
- Cell frequencies
- Cell clusters

The last two decades of single-cell

CellPress

Immunity
Perspective

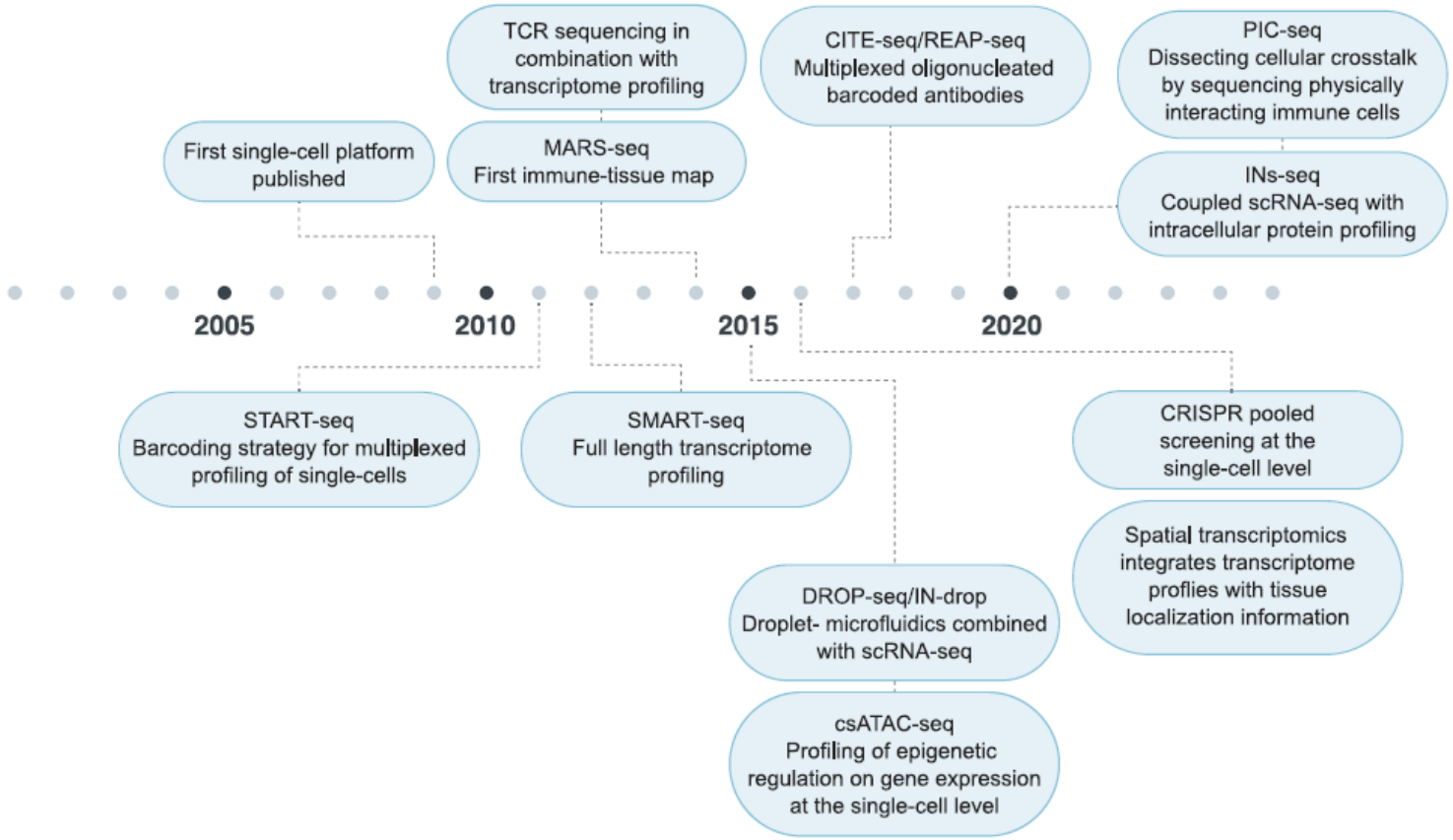
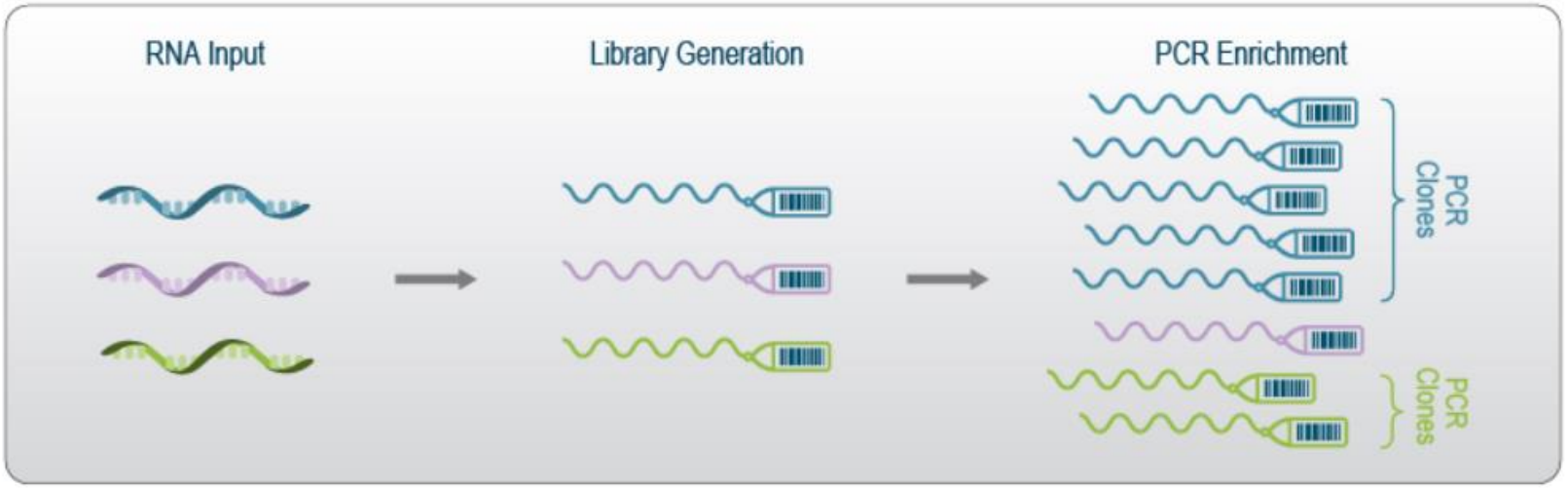
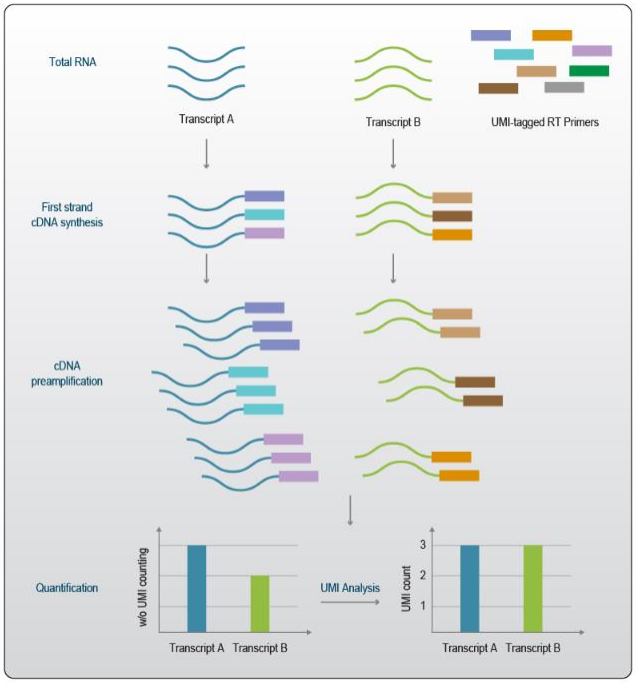


Figure 2. Timeline describing emerging single-cell technologies and their application in immunology research

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

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

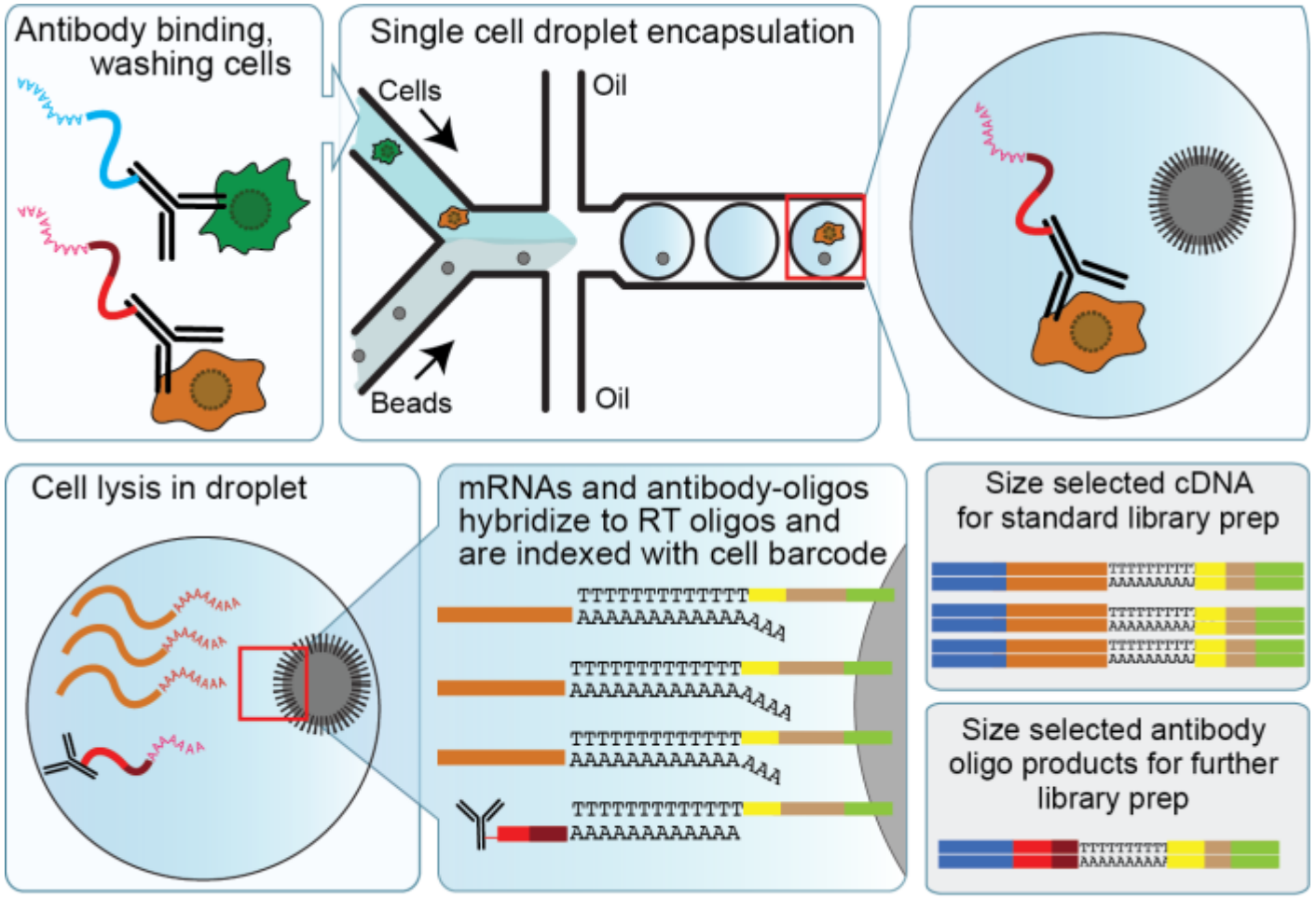




Transcript level quantification with UMIs. Transcripts or cDNAs are tagged with UMIs in an early step of library generation. The UMI sequences can then be used for quantification of the number of molecules that were originally present in the sample. UMIs can thus control for amplification biases associated 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	30
	MARS-seq	Whole transcriptome	100–300	3
	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	16
	sci-MET	Whole genome	1,000–5,000	19
	scRRBS	Reduced representation genome	1–10	18
Histone modifications	scChIP-seq	Whole genome + single modification	1,000–10,000	24
Chromosome conformation	scHi-C-seq	Whole genome	1–10	26
Multimodal				
Histone modifications + spatial	NA	Single locus + single modification	10–100	23
mRNA + lineage	scGESTALT	Whole transcriptome	1,000–10,000	32
	ScarTrace	Whole transcriptome	1,000–10,000	33
	LINNAEUS	Whole transcriptome	1,000–10,000	34
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	29
mRNA + cell surface protein	CITE-seq	Whole transcriptome + proteins	1,000–10,000	20
	REAP-seq	Whole transcriptome + proteins	1,000–10,000	21
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	11

Stuart T, Satija R.Nat Rev Genet. 2019