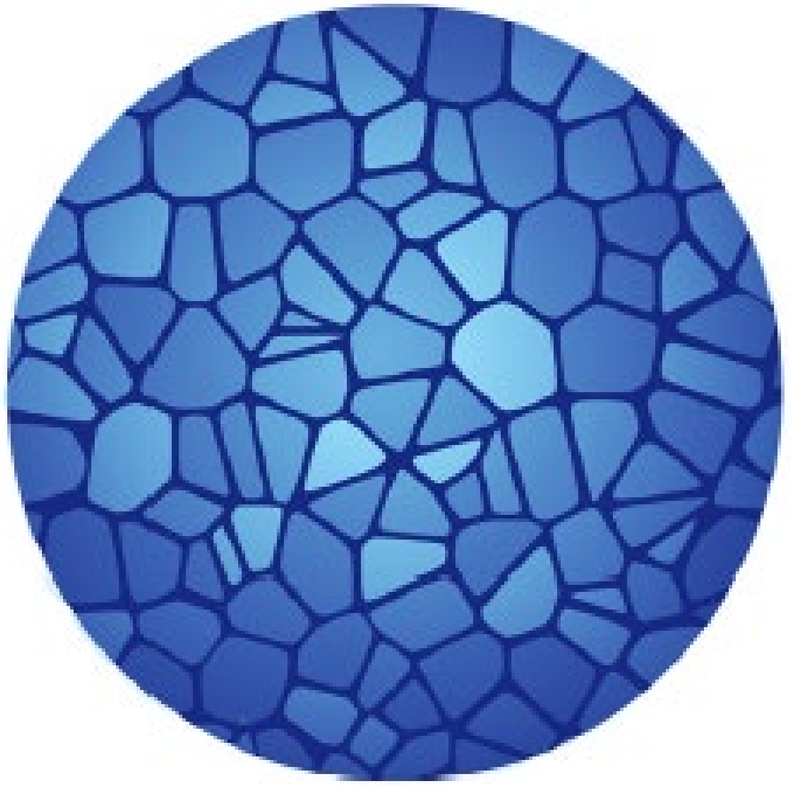
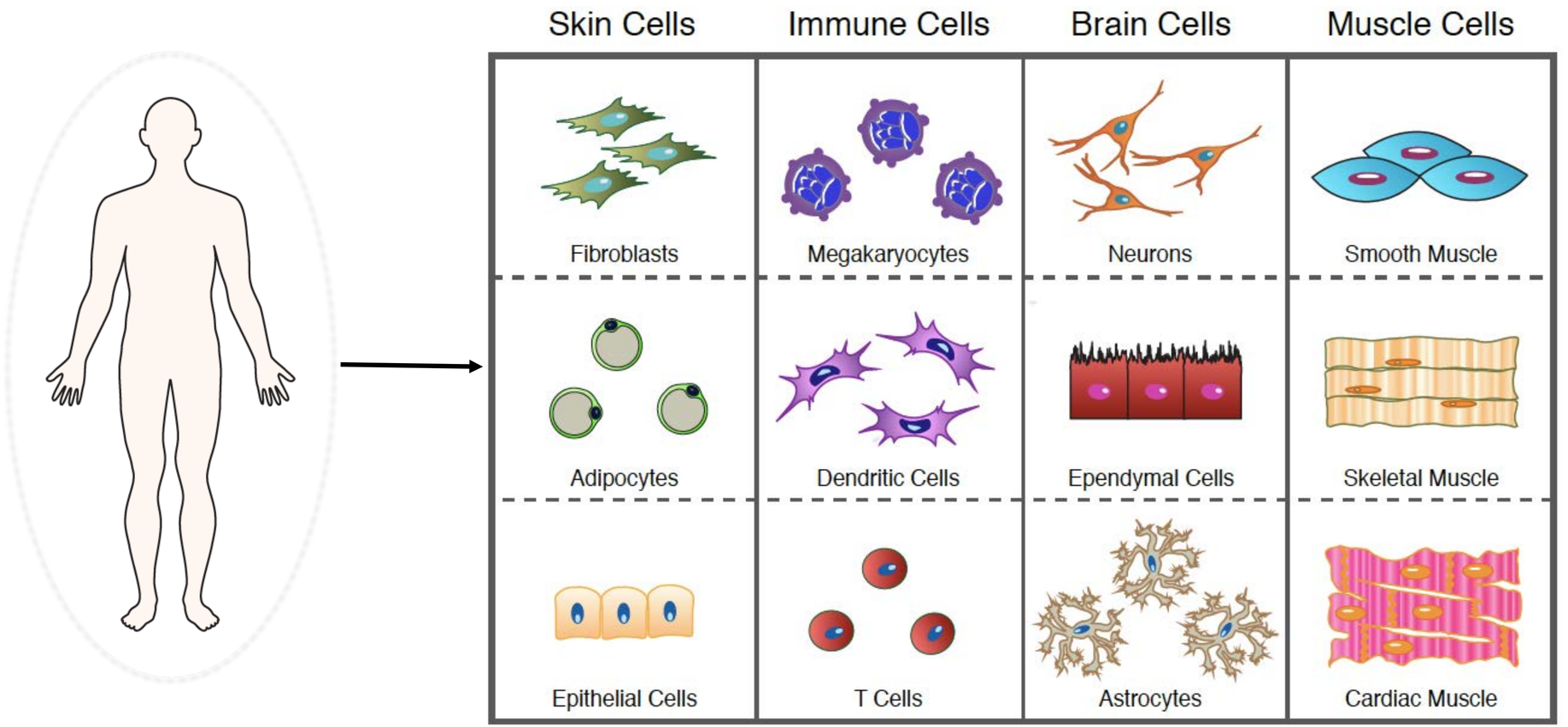
The Human Cell Atlas



Ed Lein, Ph.D
Investigator, Allen Institute for Brain Science
Seattle, Washington USA
Member, Organizing Committee, Human Cell Atlas

Moonshot International Symposium
Tokyo, Japan
December 18, 2019

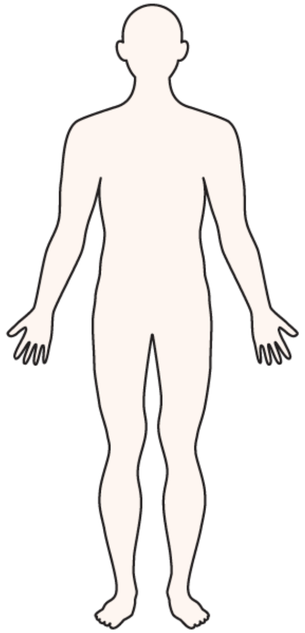
Cells are our basic units



Cells are classified by structure, location, function, molecules

Graphics credit: Aviv Regev and Alex Shalek

Problem: we do not really know our cells



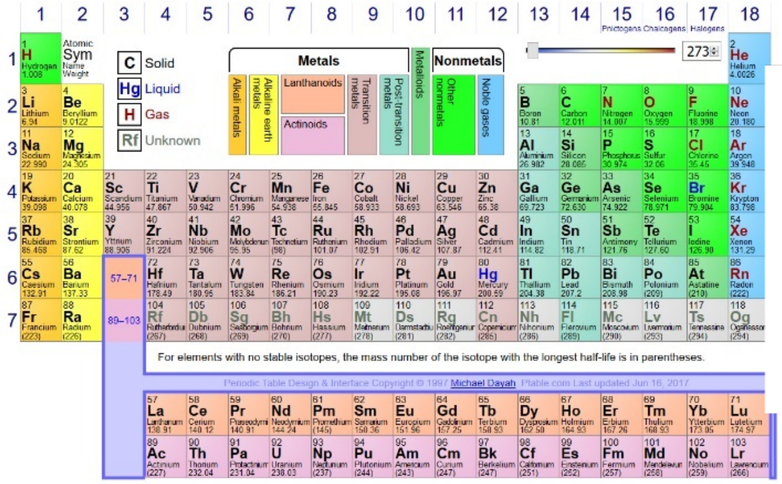
- ~37.2 trillion cells
- Text book: ~300 ‘major’ cell types?
- Science: ~100 sub-sub-types of neurons just in the retina, or a single region of the brain’s neocortex

Graphics credit: Aviv Regev and Alex Shalek

Human Cell Atlas: Mission

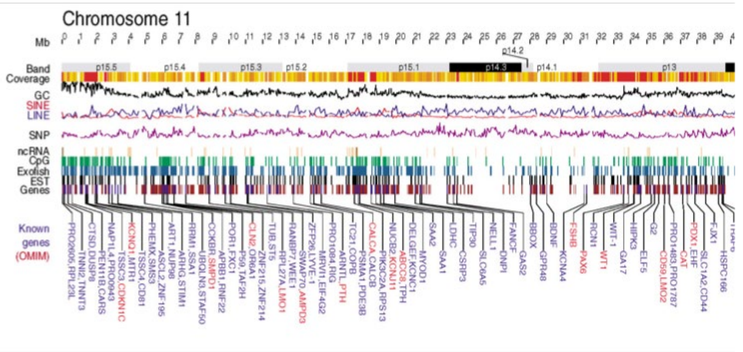
To create a comprehensive reference map of the types and properties of all human cells, the fundamental unit of life, as a basis for understanding, diagnosing, monitoring, and treating health and disease

Chemistry



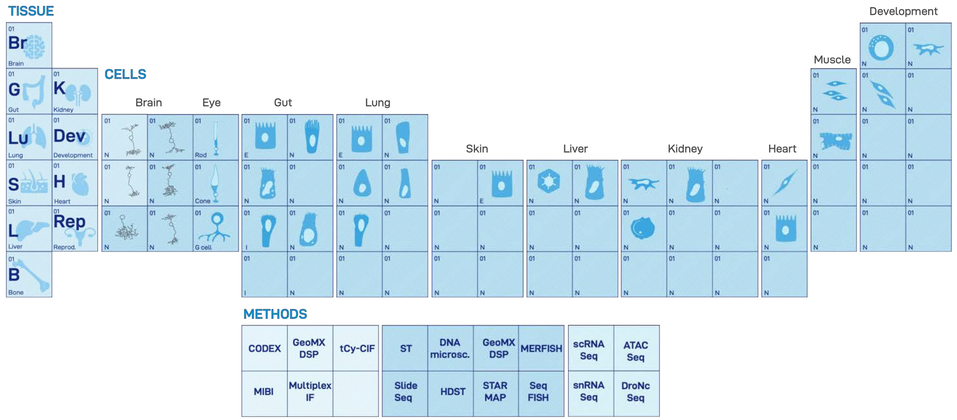
Mendeleev, 1869-1900

Genomics



Human Genome Project, 1990-2003

Biology and Medicine



What gives different cells different properties?
They use different genes

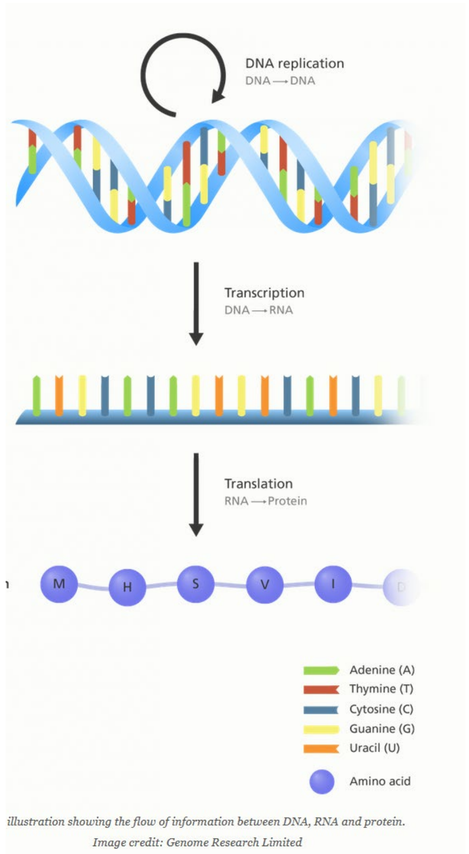
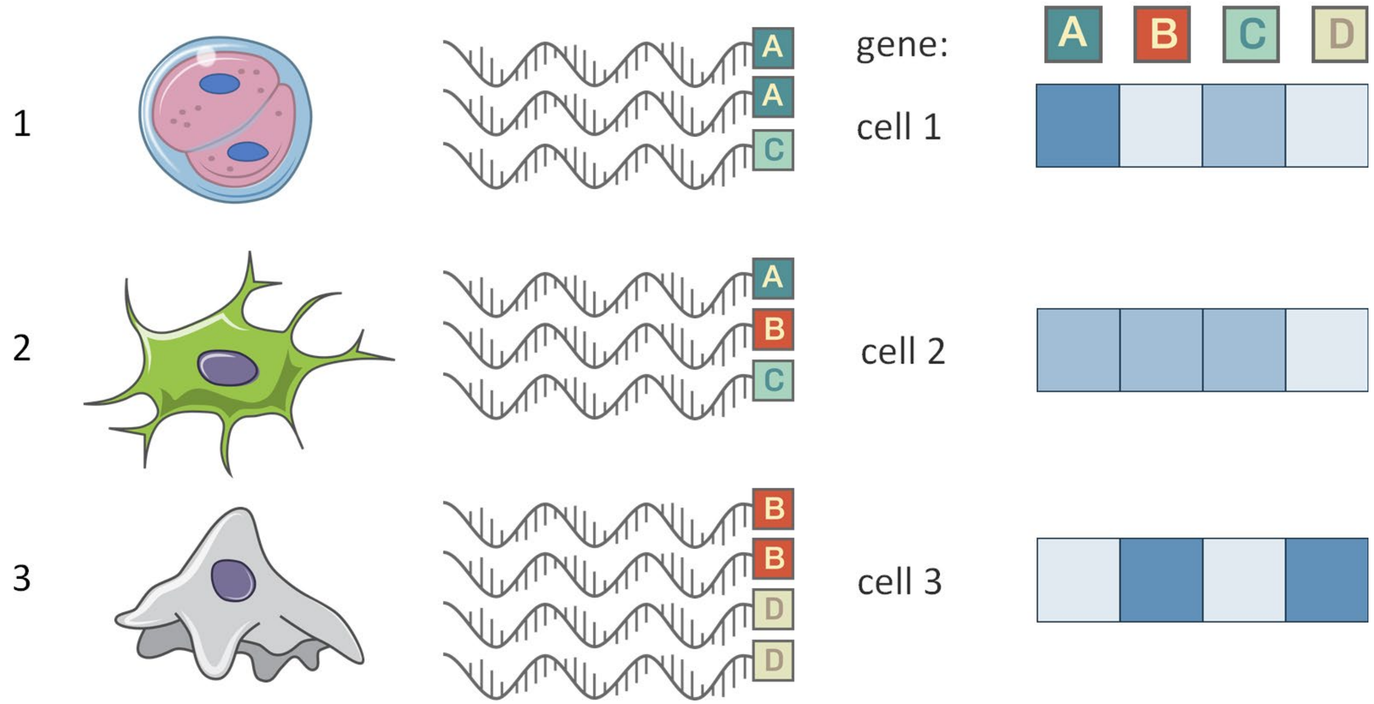


illustration showing the flow of information between DNA, RNA and protein.
Image credit: Genome Research Limited

A single RNA molecule is called a transcript

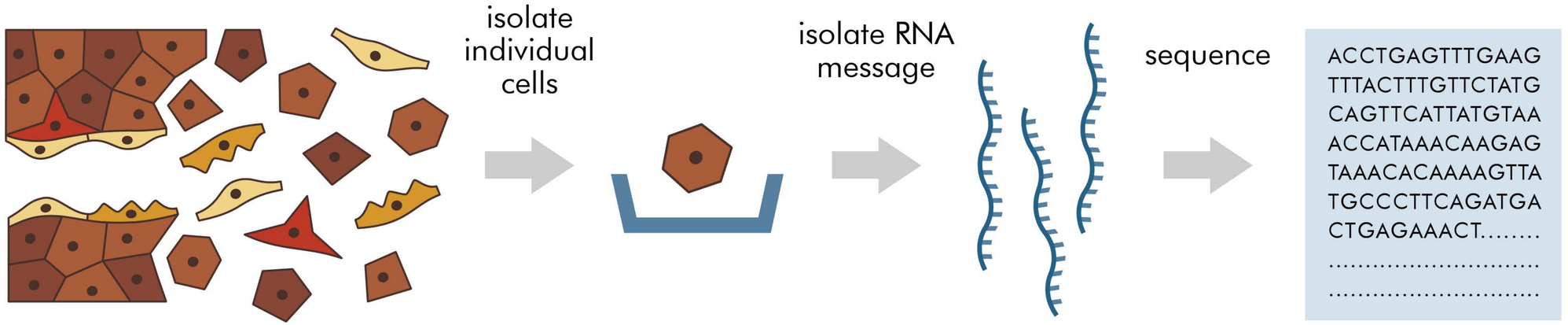
The complete set of RNA molecules in a cell is a "transcriptome"

Gene expression is a fingerprint of a cell



Graphics credit: Anna Hupalowska

“Disruptive” technological advance: Single cell genomics



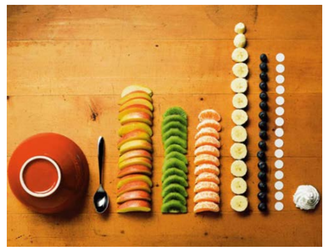
Tissue | Dissociated cells | Single cell | RNA from active genes | Transcriptome active genes in that cell

“Single cell transcriptomics” methods can measure 4,000-12,000 genes per cell, across tens of thousands of cells, in any tissue in any species

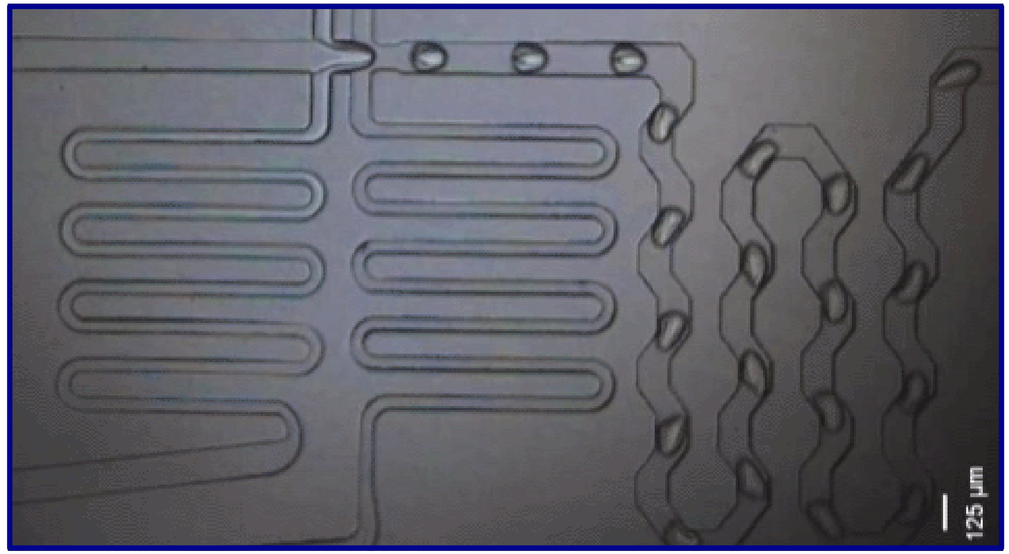
Graphics credit: Sanger Institute/HCA

Massively parallel methods let us profile many cells

Bulk genomics



Single cell genomics

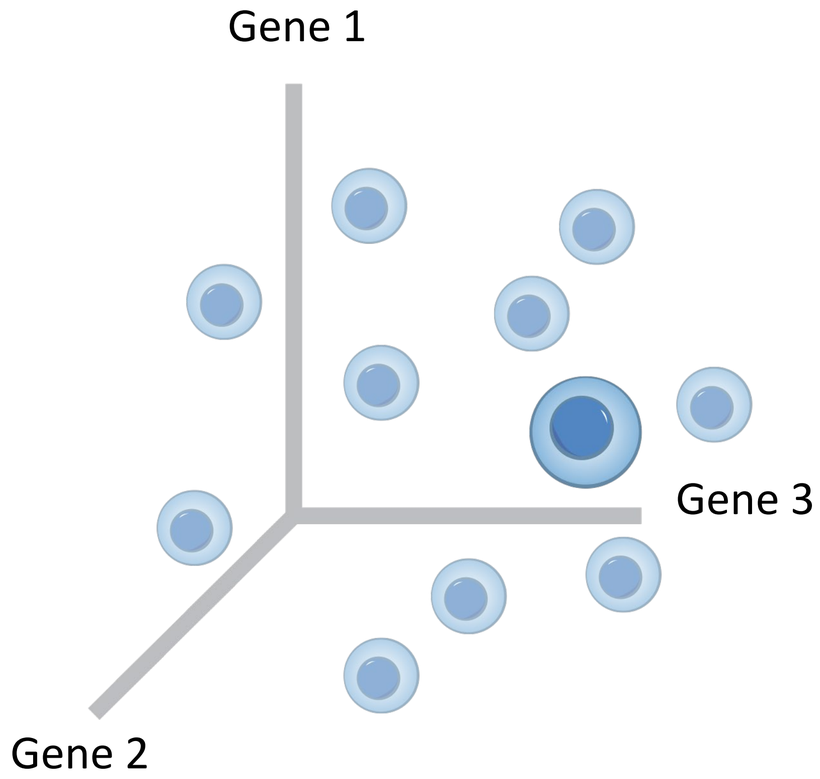


5,000 cells/second

Video courtesy of: Anindita Basu, Broad Institute

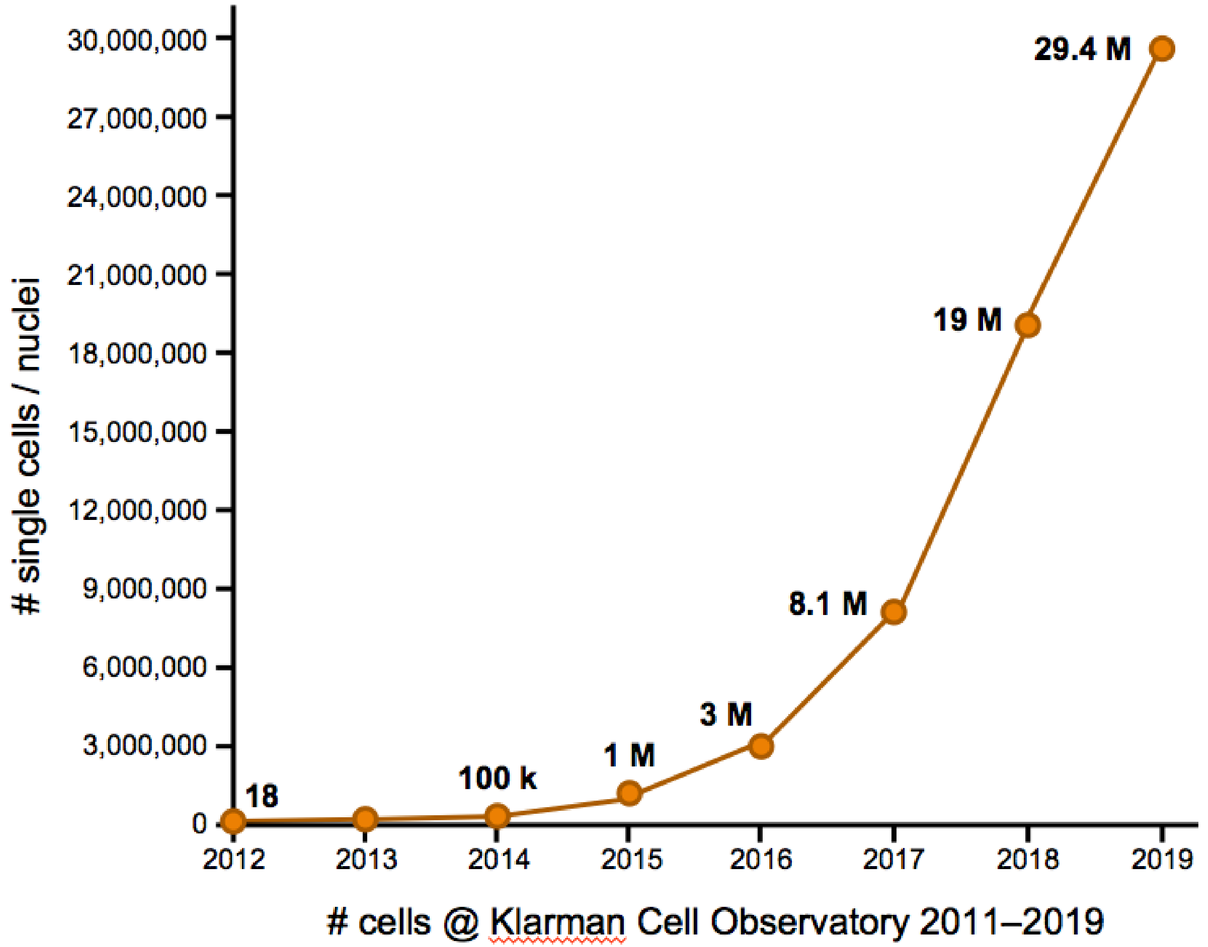
Gene expression provide the coordinates for a map of all human cells

Idea: There are ~20,000 genes in the genome.
We can define a cell as a point in 20,000- dimensional gene expression space

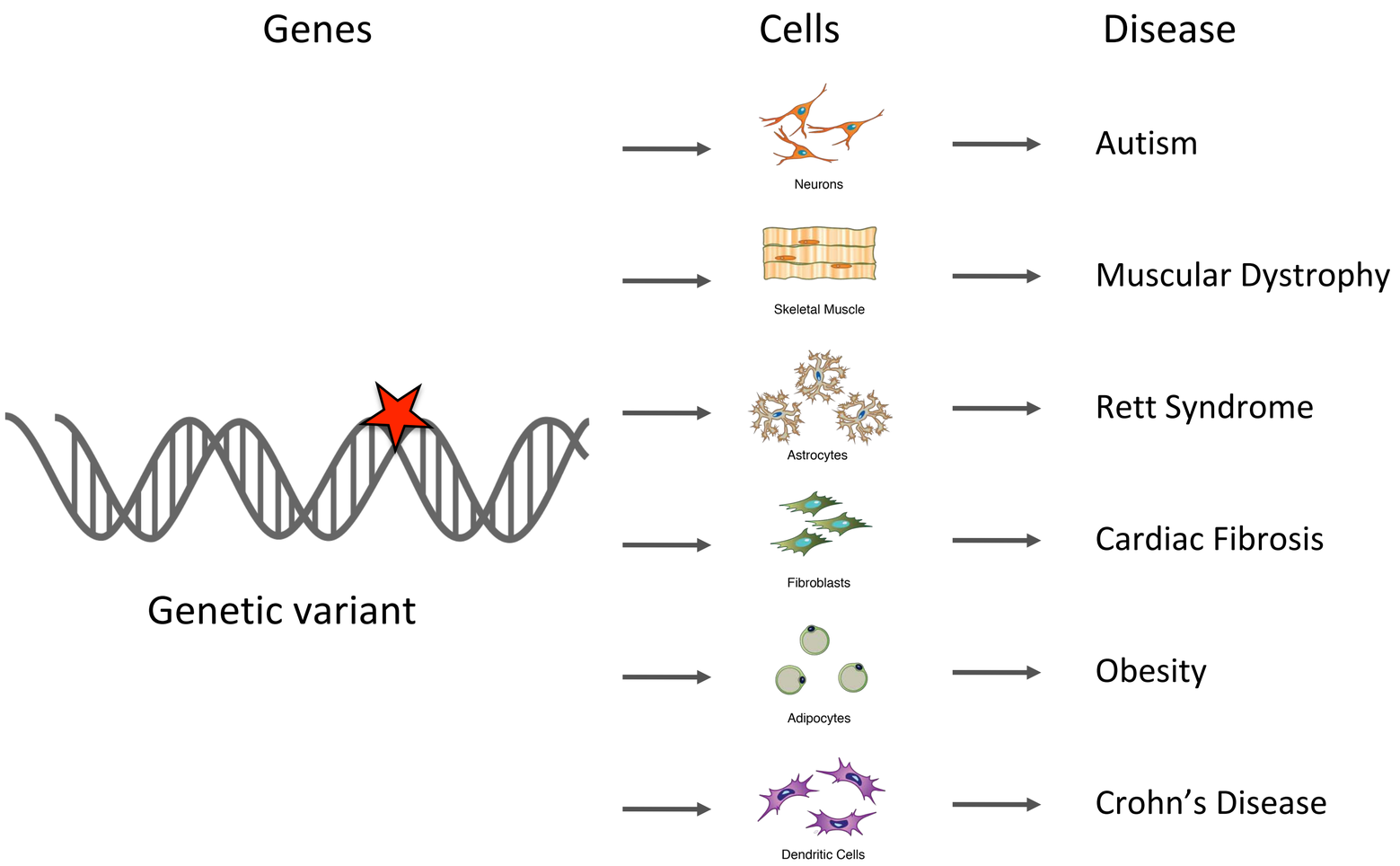


Graphics credit: Aviv Regev, Allon Wagner, Nir Yosef, and Leslie Gaffney

Single cell genomics is growing exponentially, and provides the necessary scale to approach an atlas of human cells

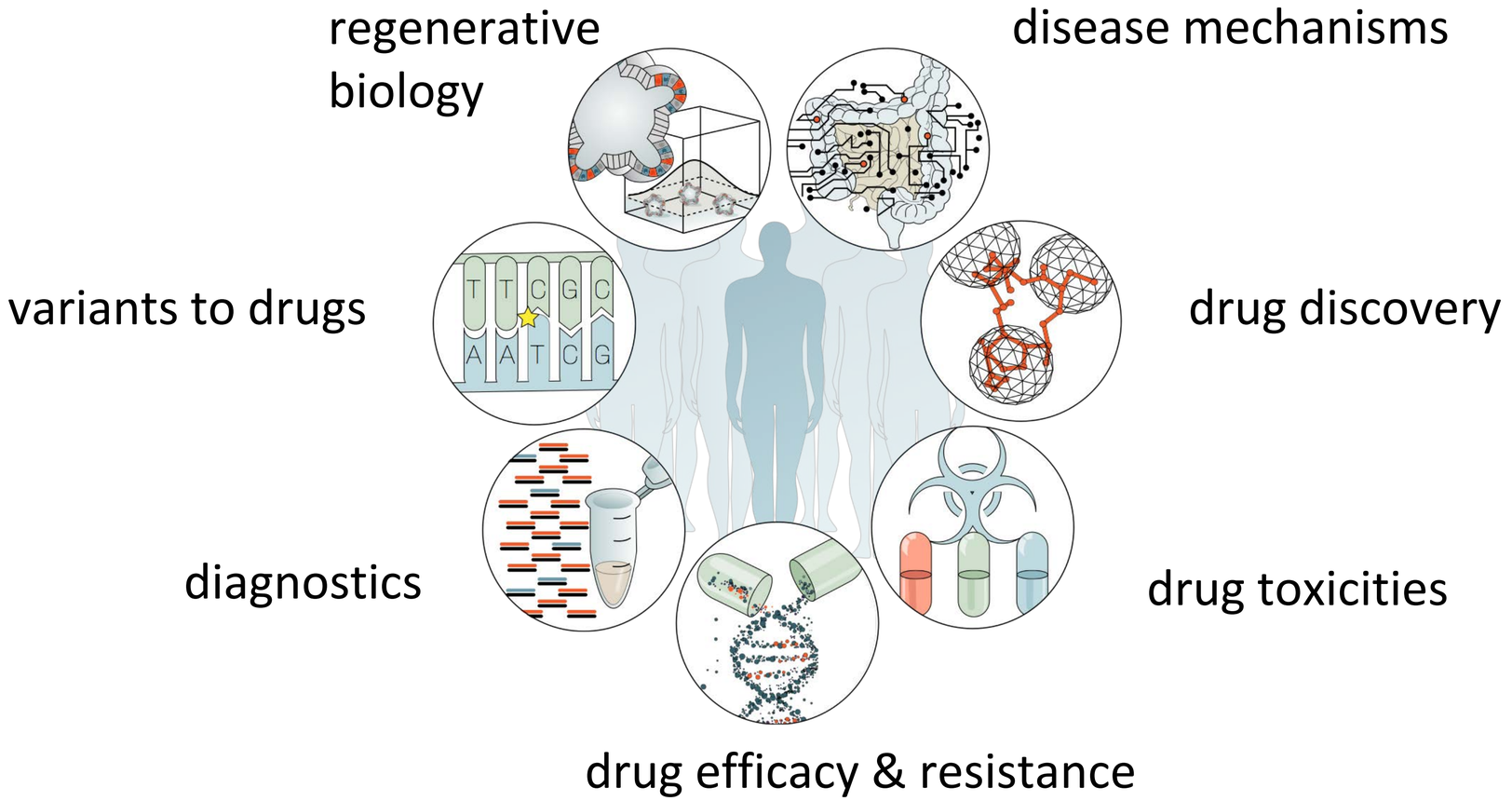


Why a human cell atlas?
Knowing our cells is essential to understand the genes that cause disease

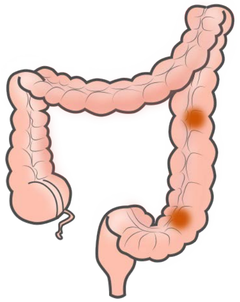


Graphics credit: Aviv Regev and Alex Shalek

Knowing our cells is essential to treating disease

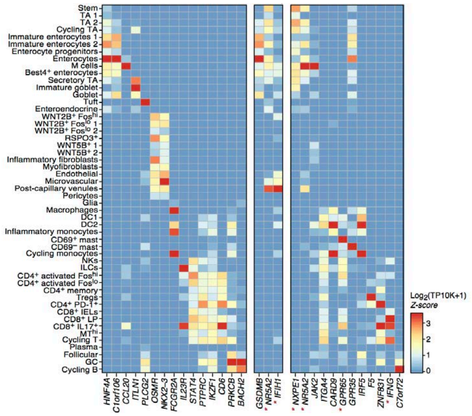


Human Cell Atlas Consortium White Paper, Section 1



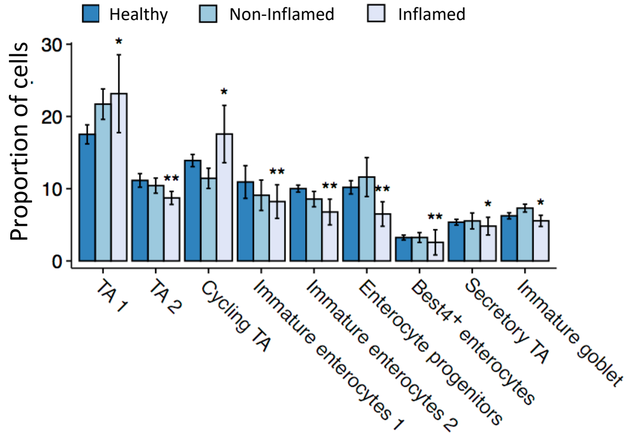
Atlas will provide a refined vocabulary for disease

Where do disease risk genes act?

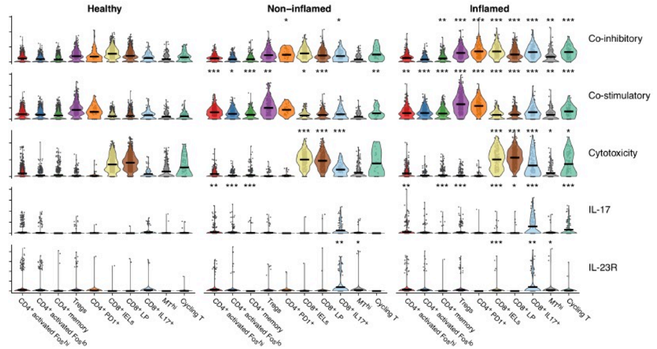


GWAS genes

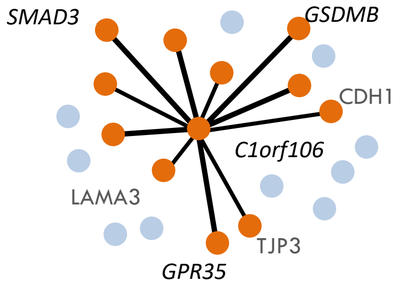
Which cells are disrupted?



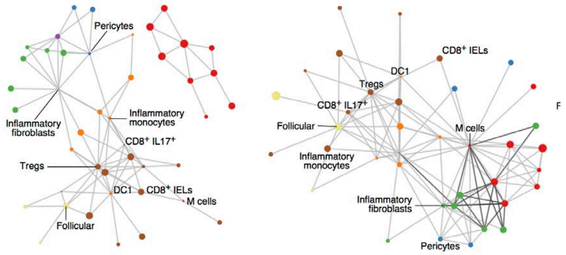
Which cell programs are changed?



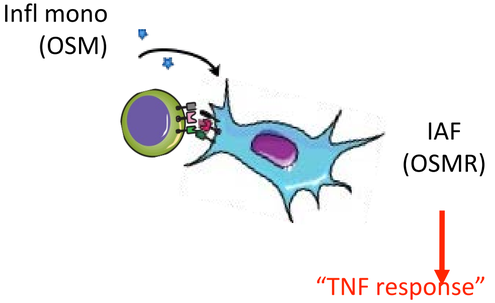
What are their functions & modules?



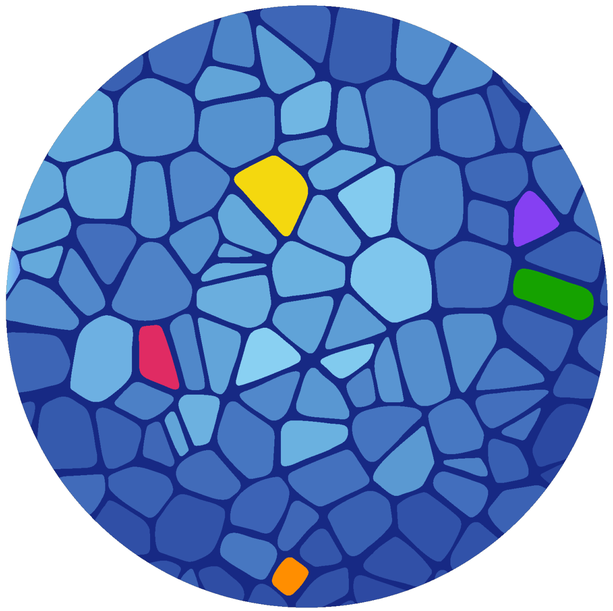
Which communications are disrupted?



What is the effect of drug?



Smilie et al. (2019) Cell

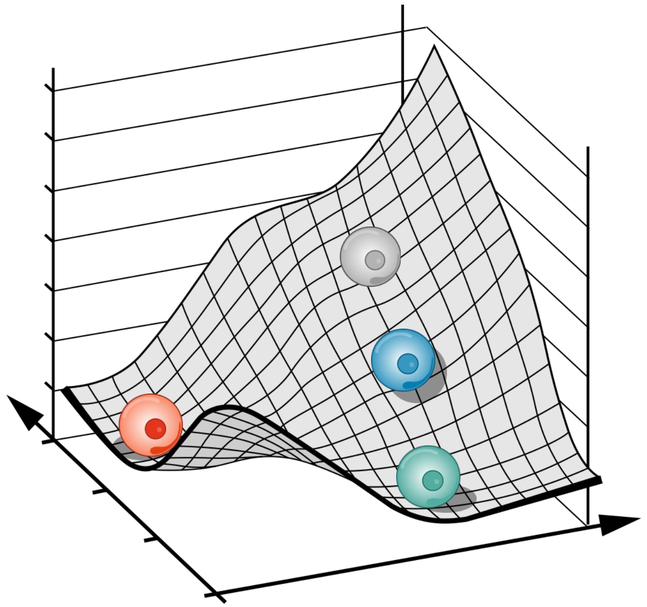


What is the Human Cell Atlas?

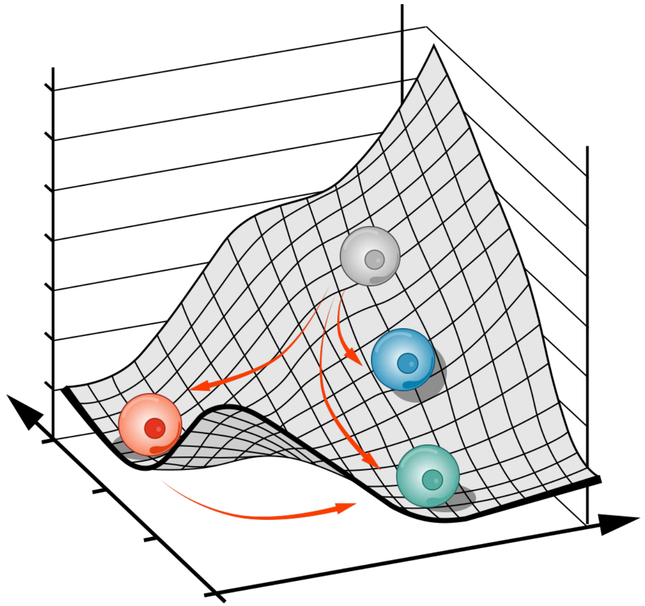
- The HCA Roadmap: concept, practice, and use
- Atlas infrastructure: Tools, data platform

Concepts

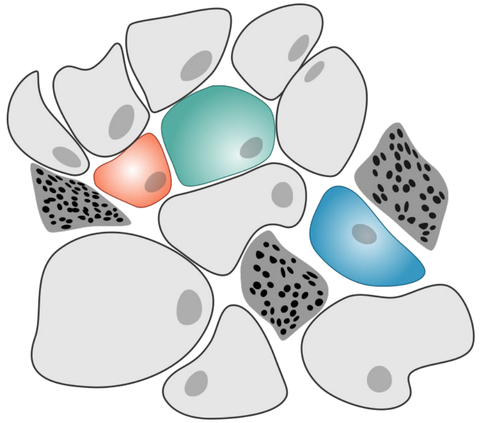
types / states



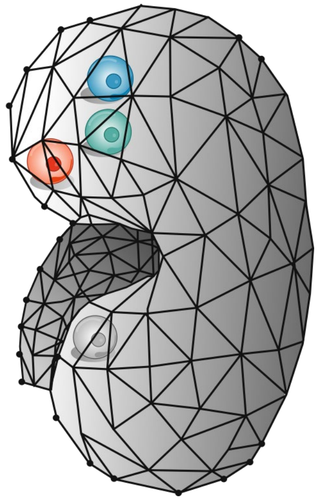
trajectories/transitions



histological modules

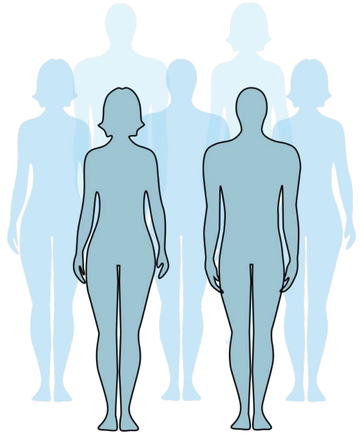


positions

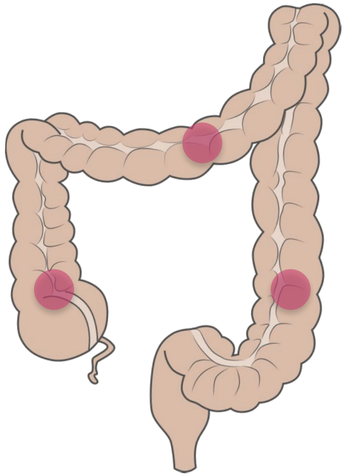


Tissue sampling

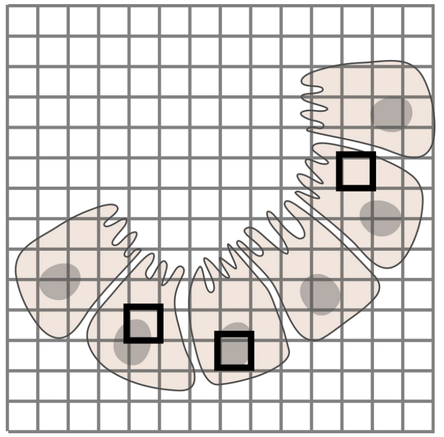
Number of individuals



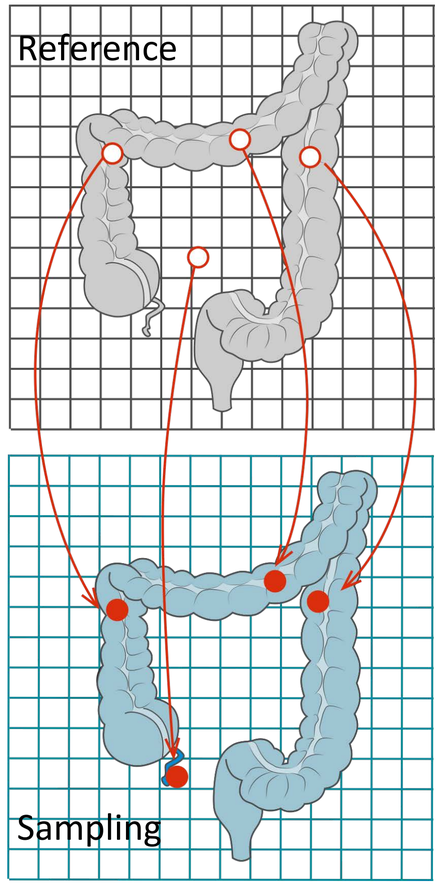
Anatomical sampling



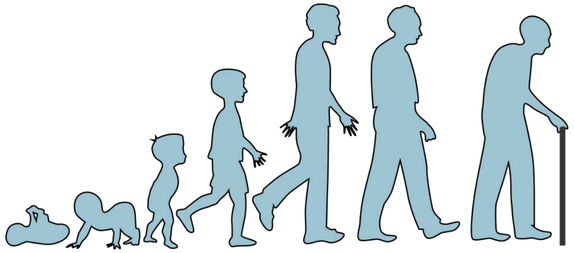
Number of regions



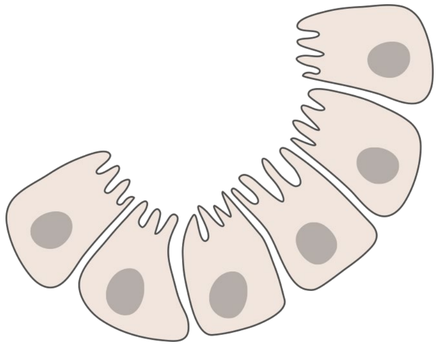
Coordinate framework



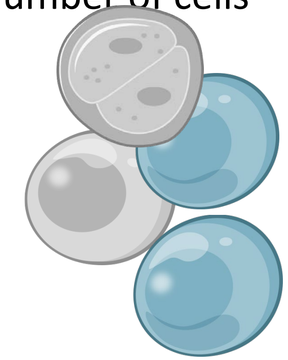
Development and aging



Histological sampling

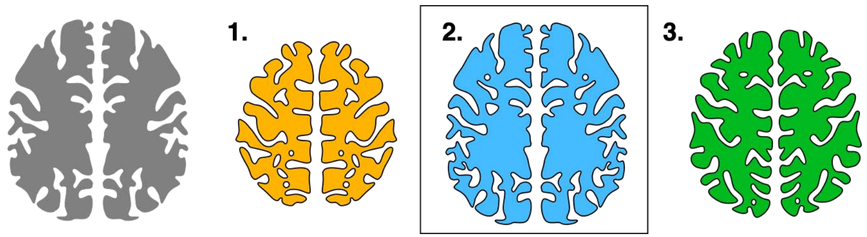


Number of cells

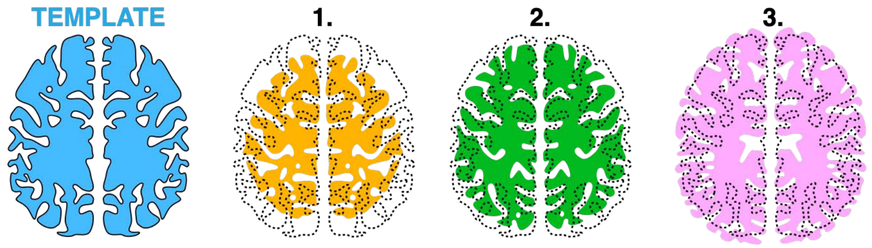


Common Coordinate Frameworks (CCFs) to map and aggregate data

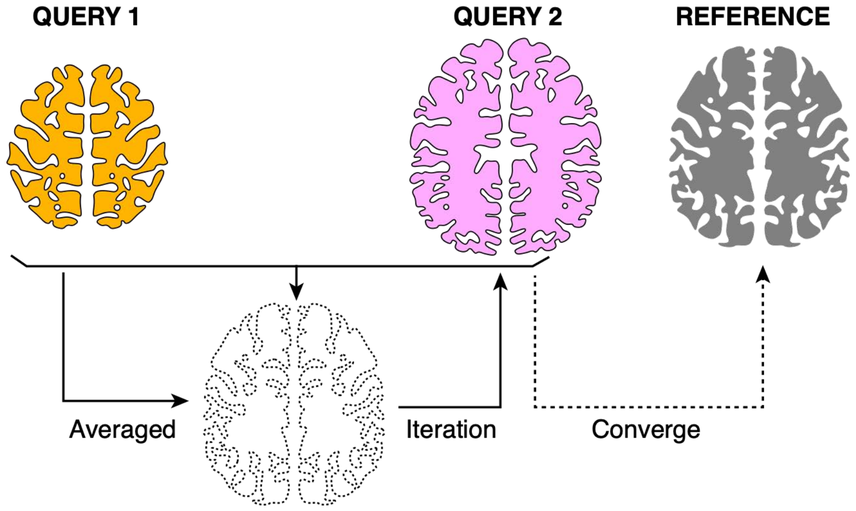
1. Calculate best template



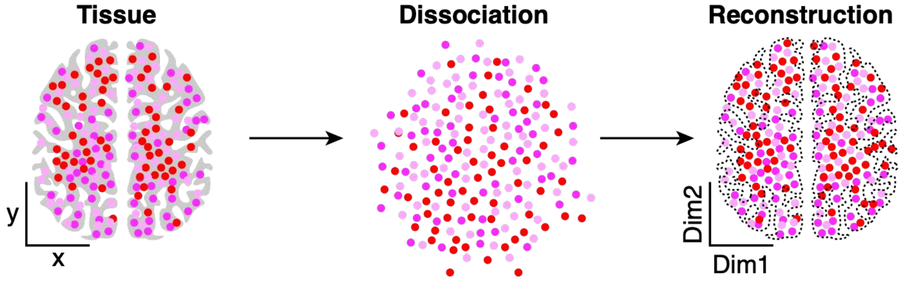
2. Approach 1: Map all to one template



3. Approach 2: Iteratively align and average



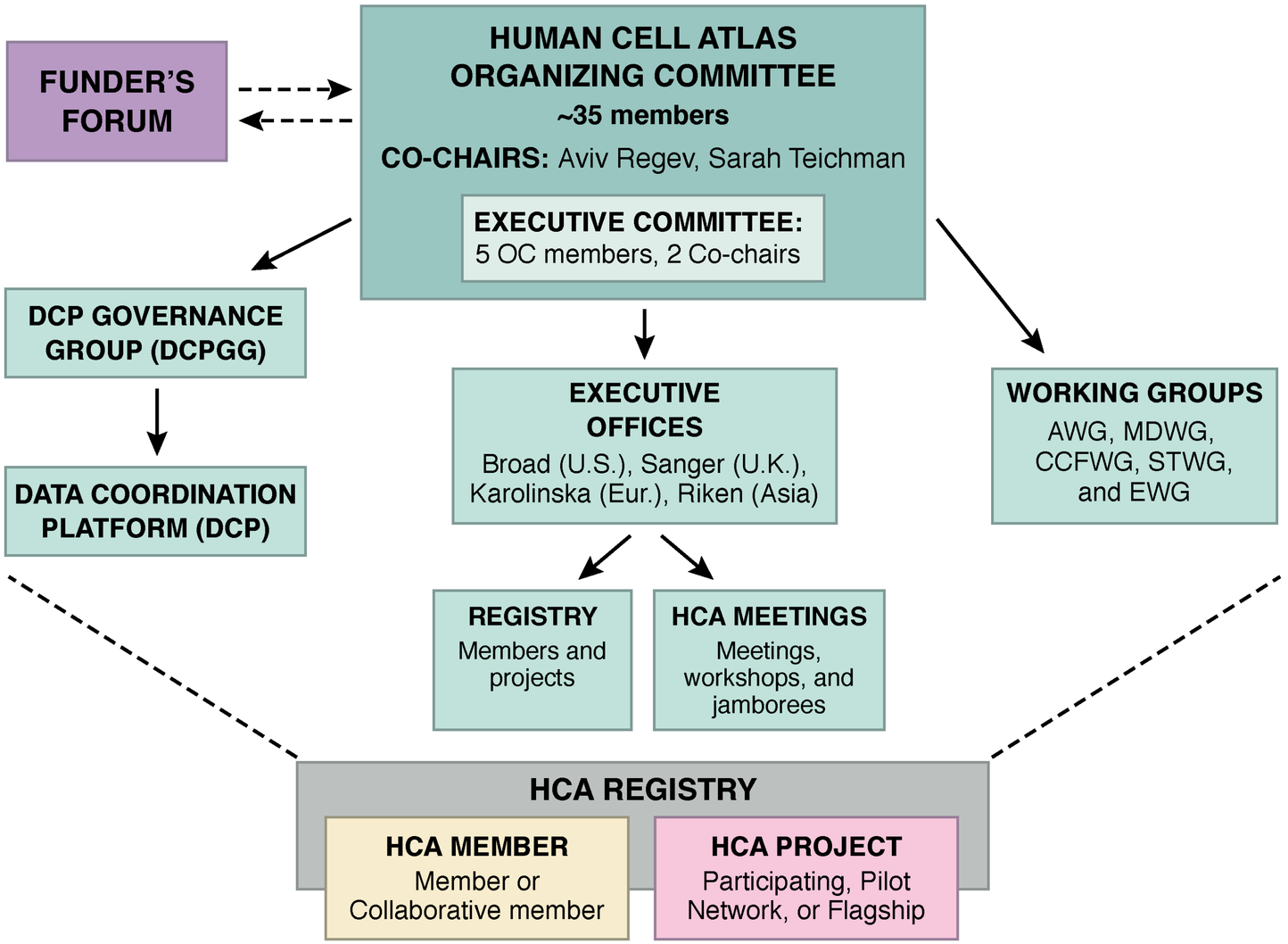
4. Reconstructing an atlas from its features



Support: HubMAP HIVE, BRAIN Initiative

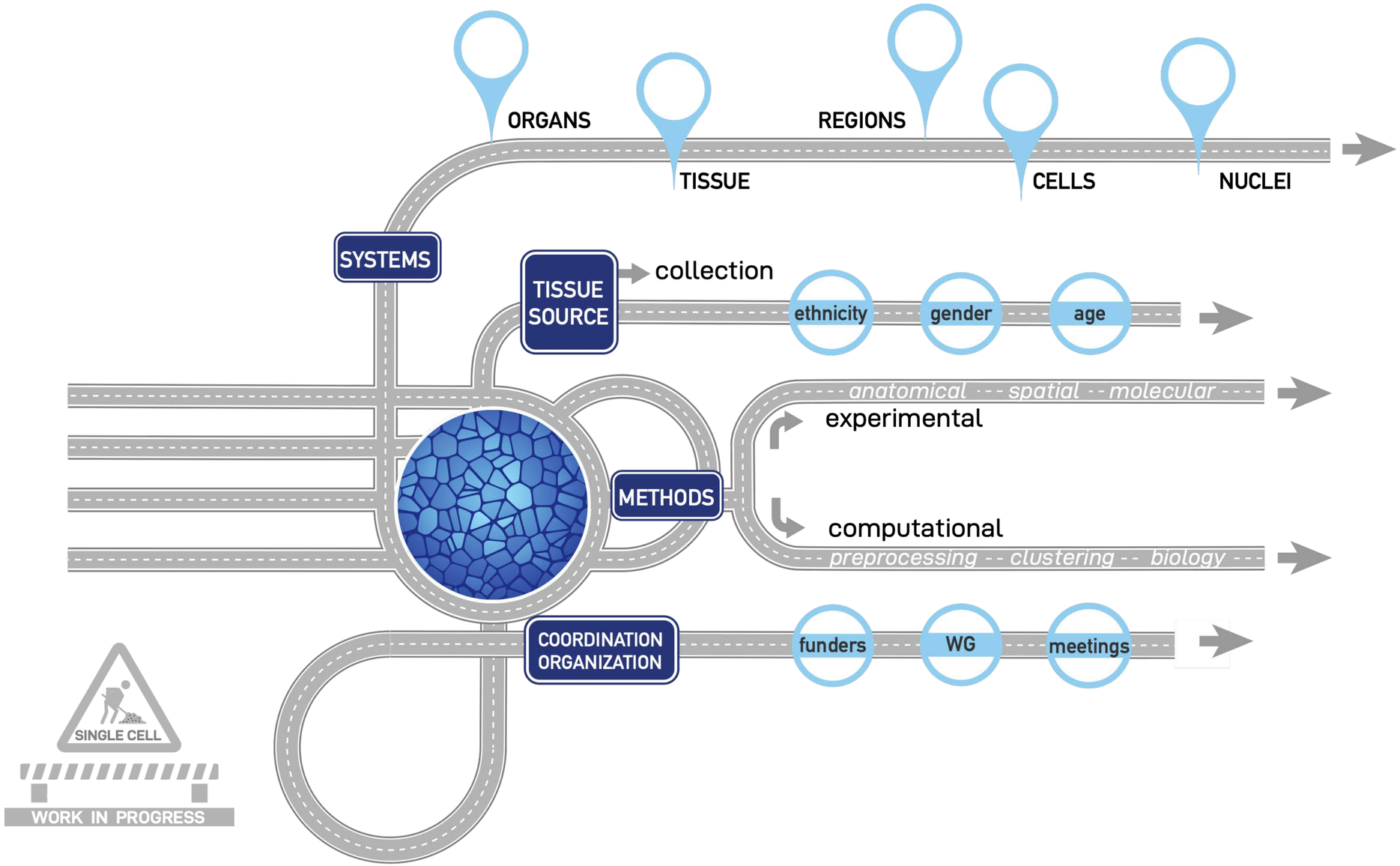
Satija, Regev, Marioni

The HCA Consortium



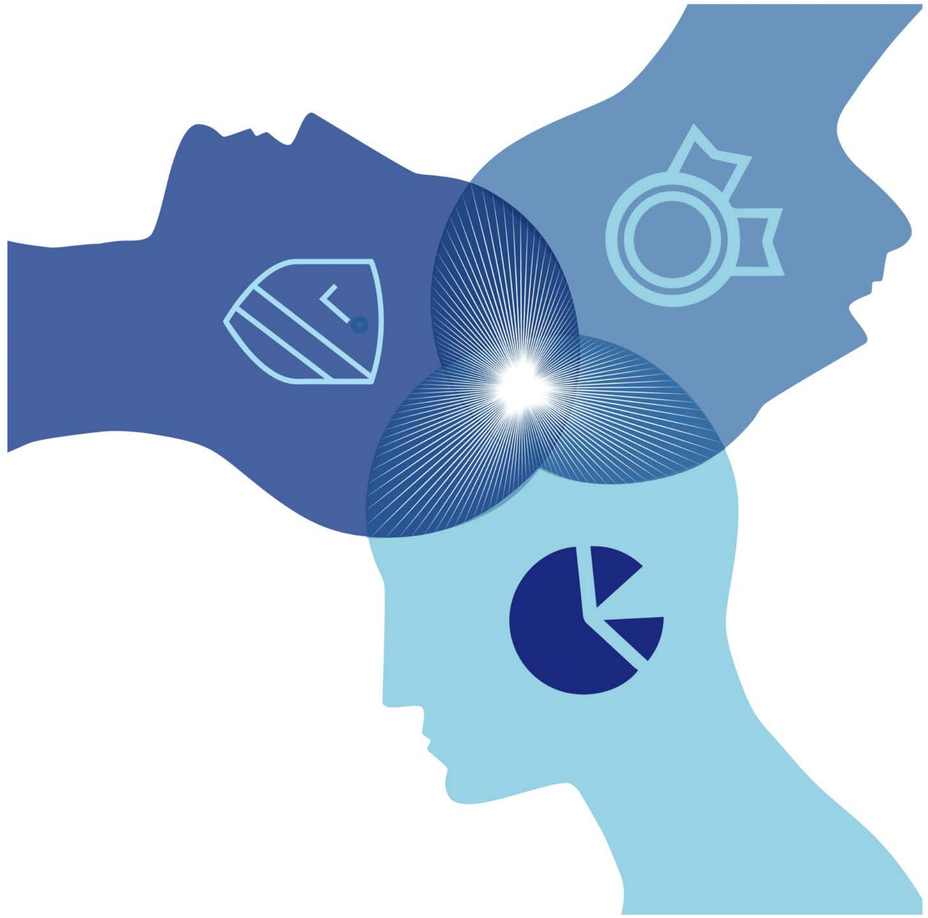
Human Cell Atlas Consortium White Paper, Section 7

The HCA Roadmap



Graphics credit: Ania Hupalowska

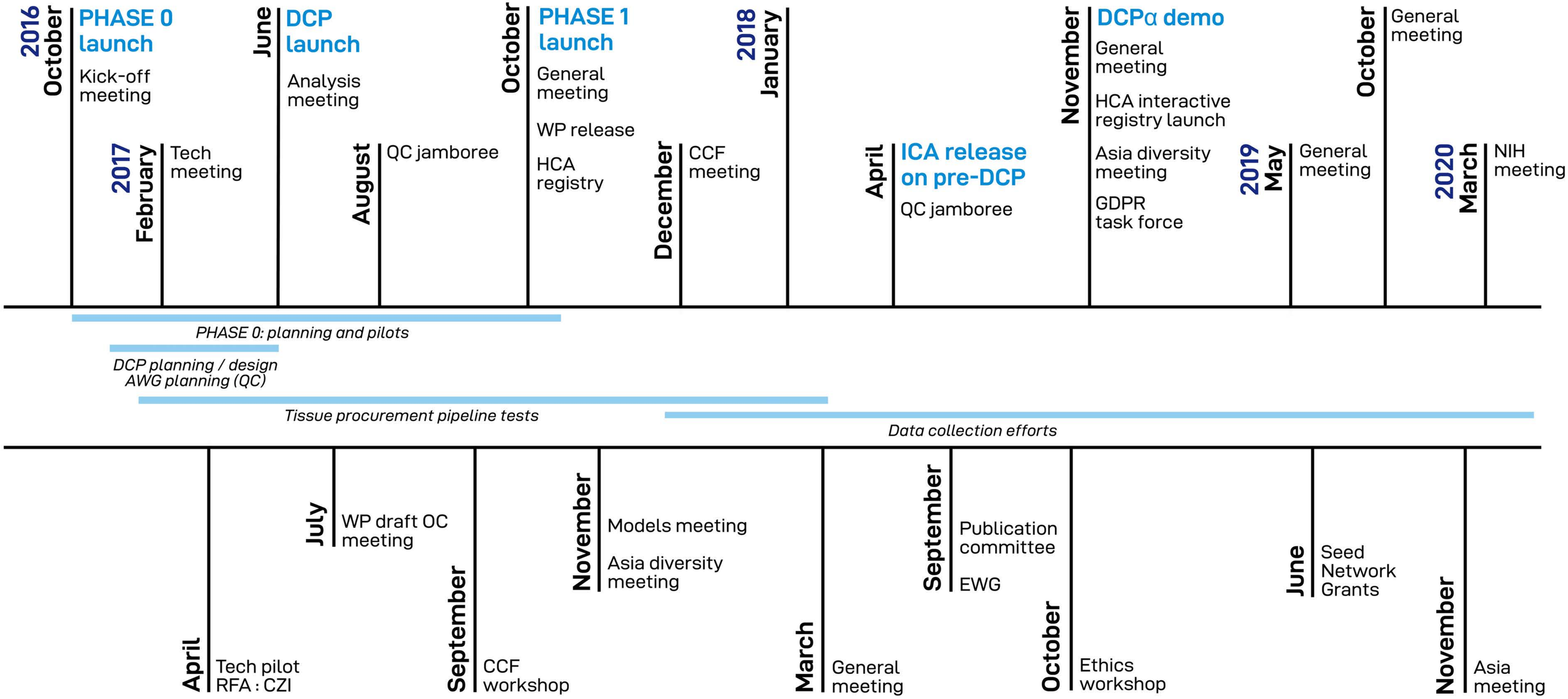
HCA principles and values



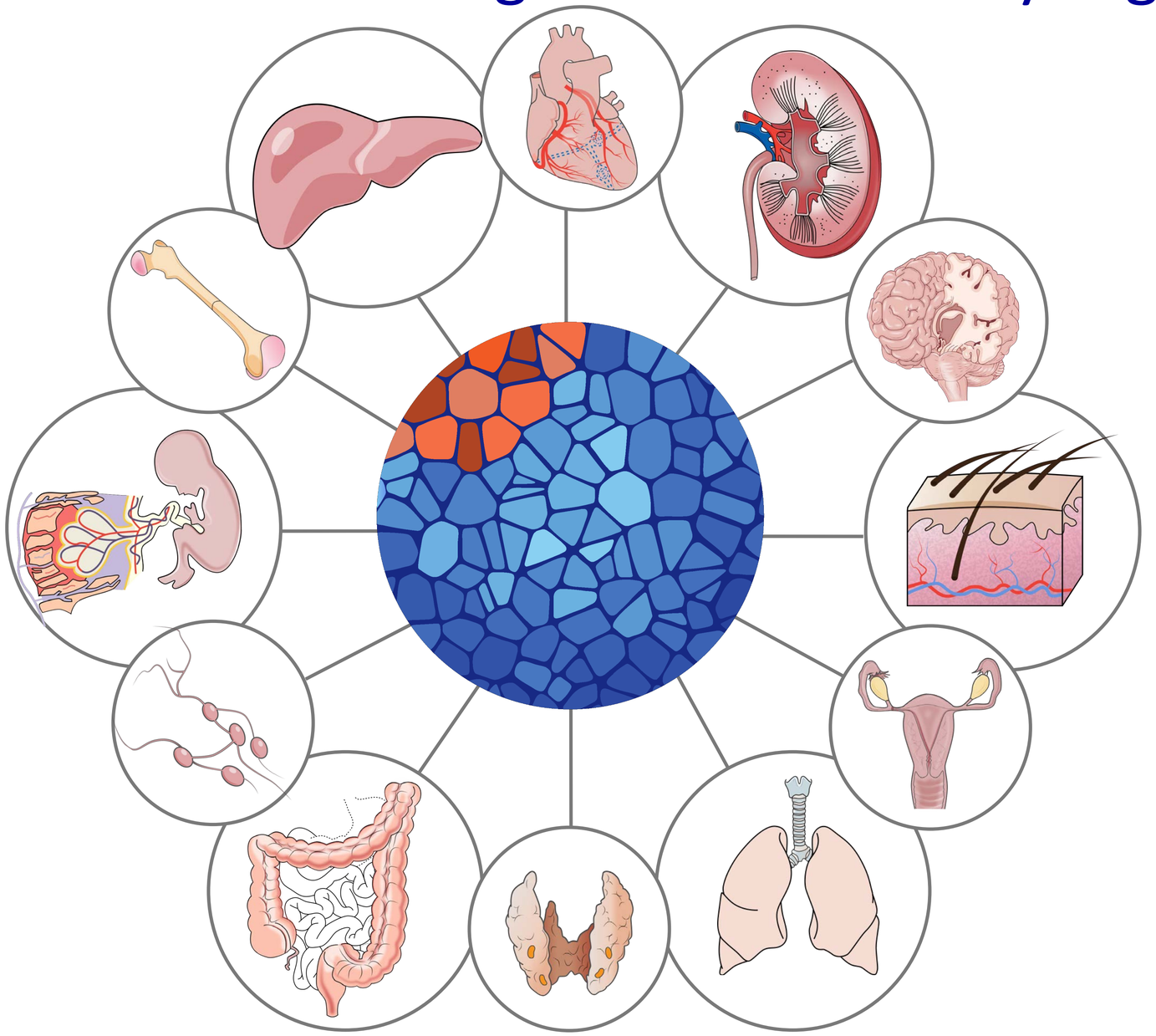
- Quality
- Flexibility
- Community
- Equity: Diversity and inclusion: subjects and scientists
- Transparency and open sharing: data, code, protocols
- Privacy and ethics
- Technological innovation and excellence
- Computational innovation and excellence

Graphics credit: Anna Hupalowska

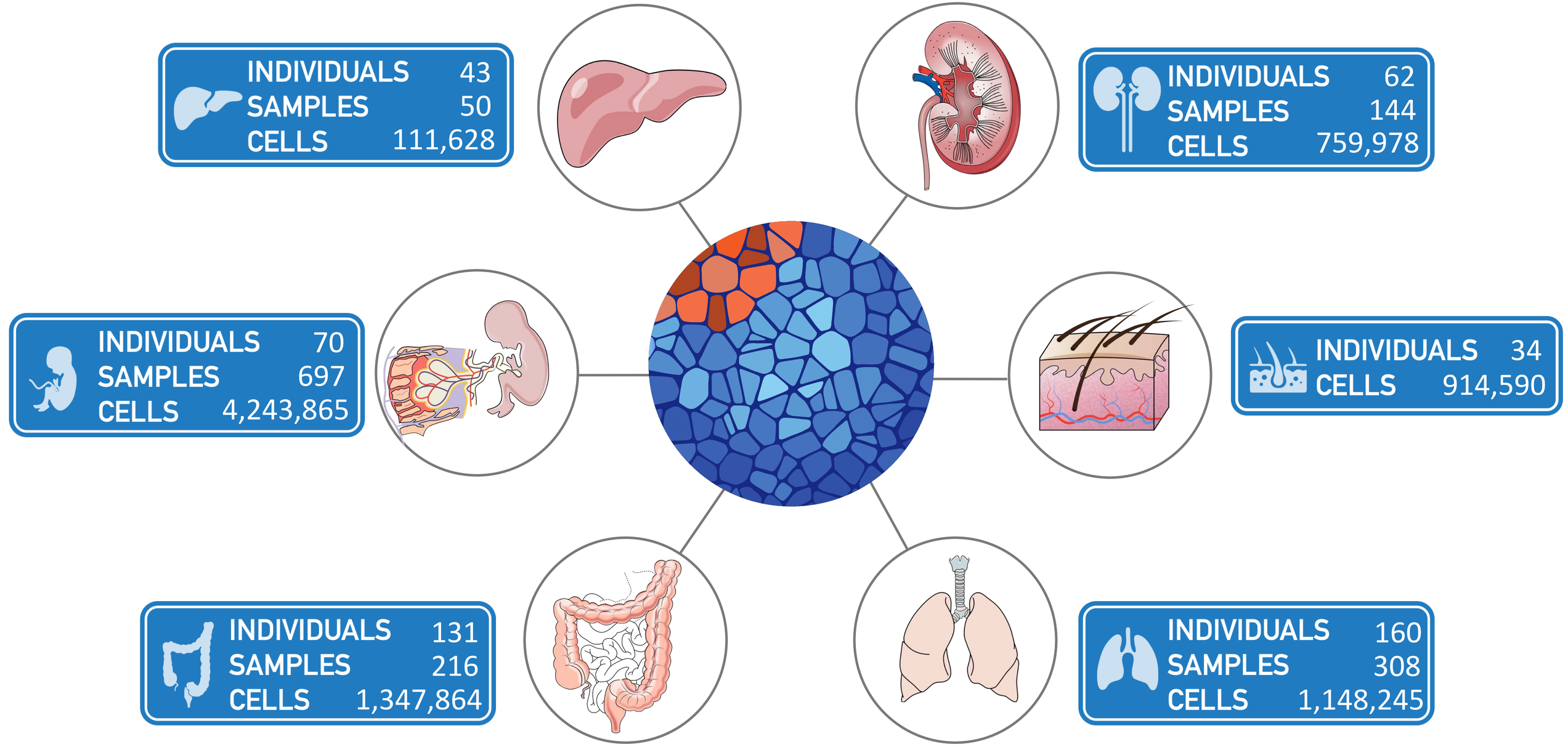
HCA Timeline: 2016 - present



Atlases are now being made from many organs



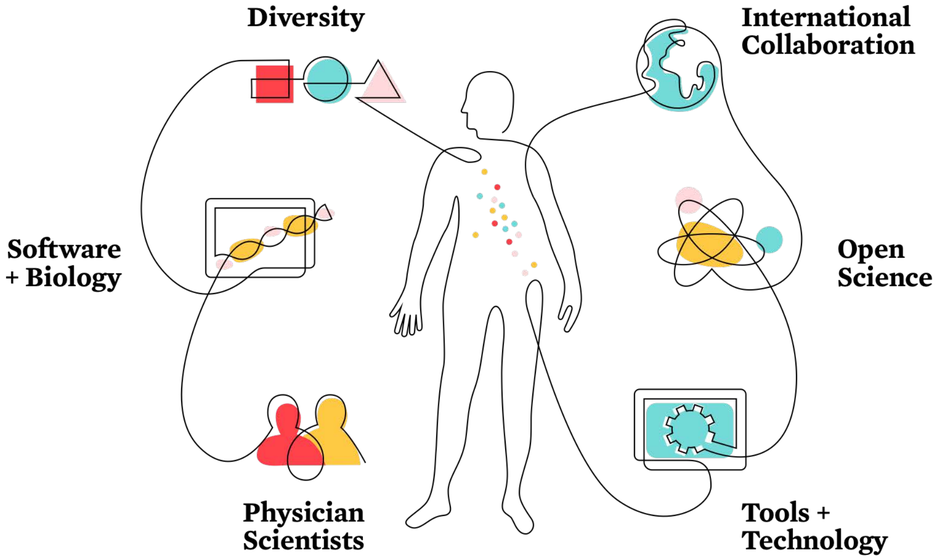
Views from the Atlases



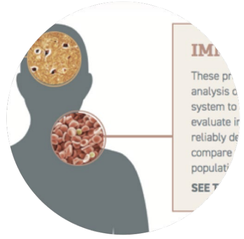
Building our Biological Networks

200+labs in 20 countries

CZI Seed Networks for the Human Cell Atlas



38 projects on 10 organ systems

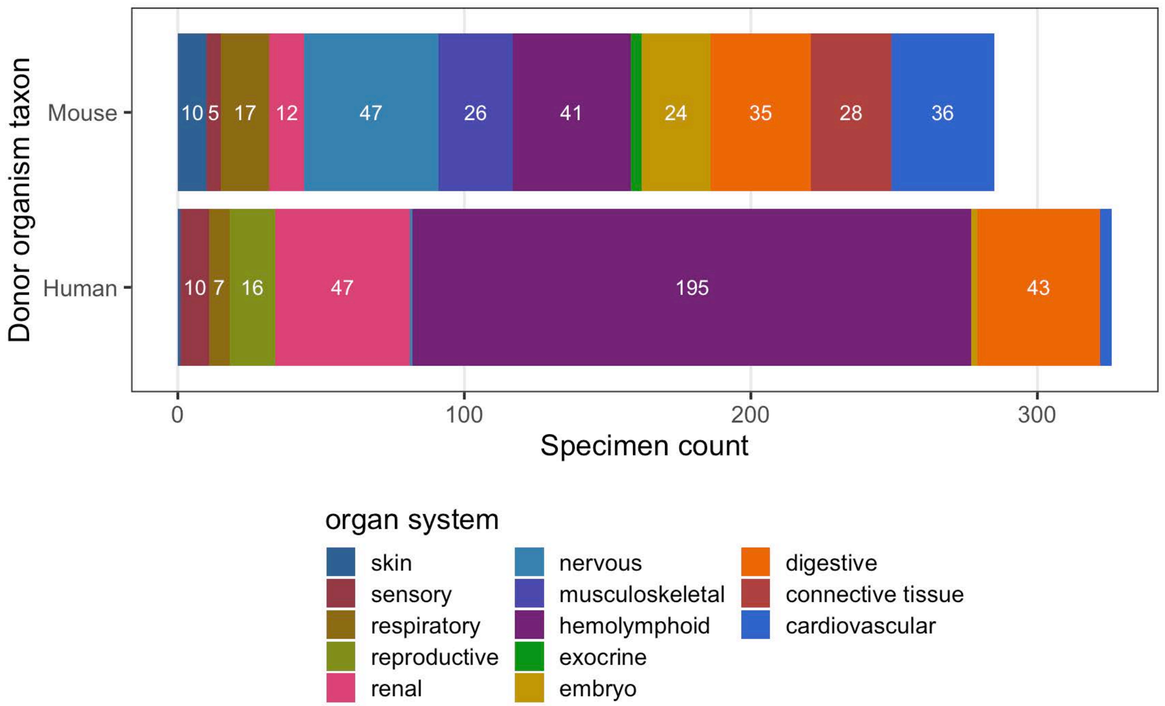


pilot projects

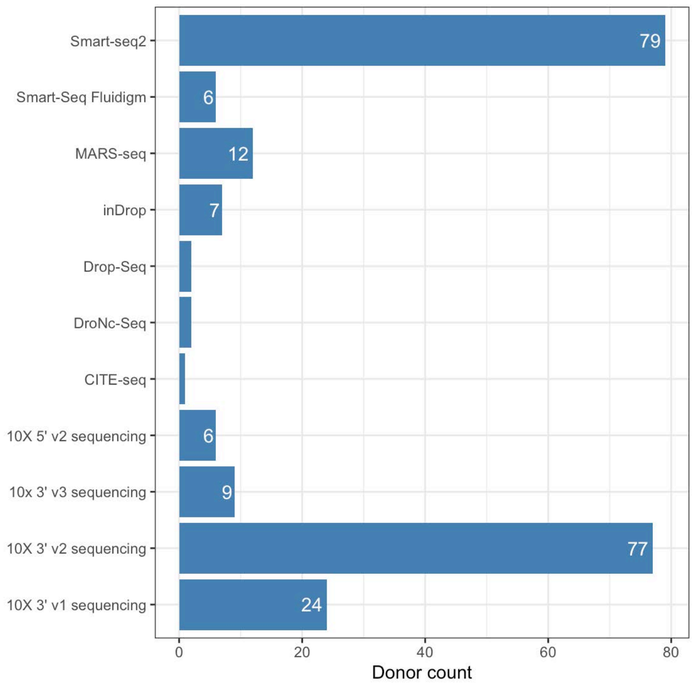
Slide courtesy of: Jonah Cool/ CZI

Building a data coordination platform for HCA

Data from 13 organ systems



Donors per Assay



Pipeline

✔

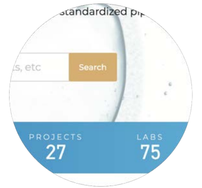
In progress

In progress

✔

In progress

✔



2,809,355 cells processed

8 projects in last month - 6 pre-publication projects for travel awardees

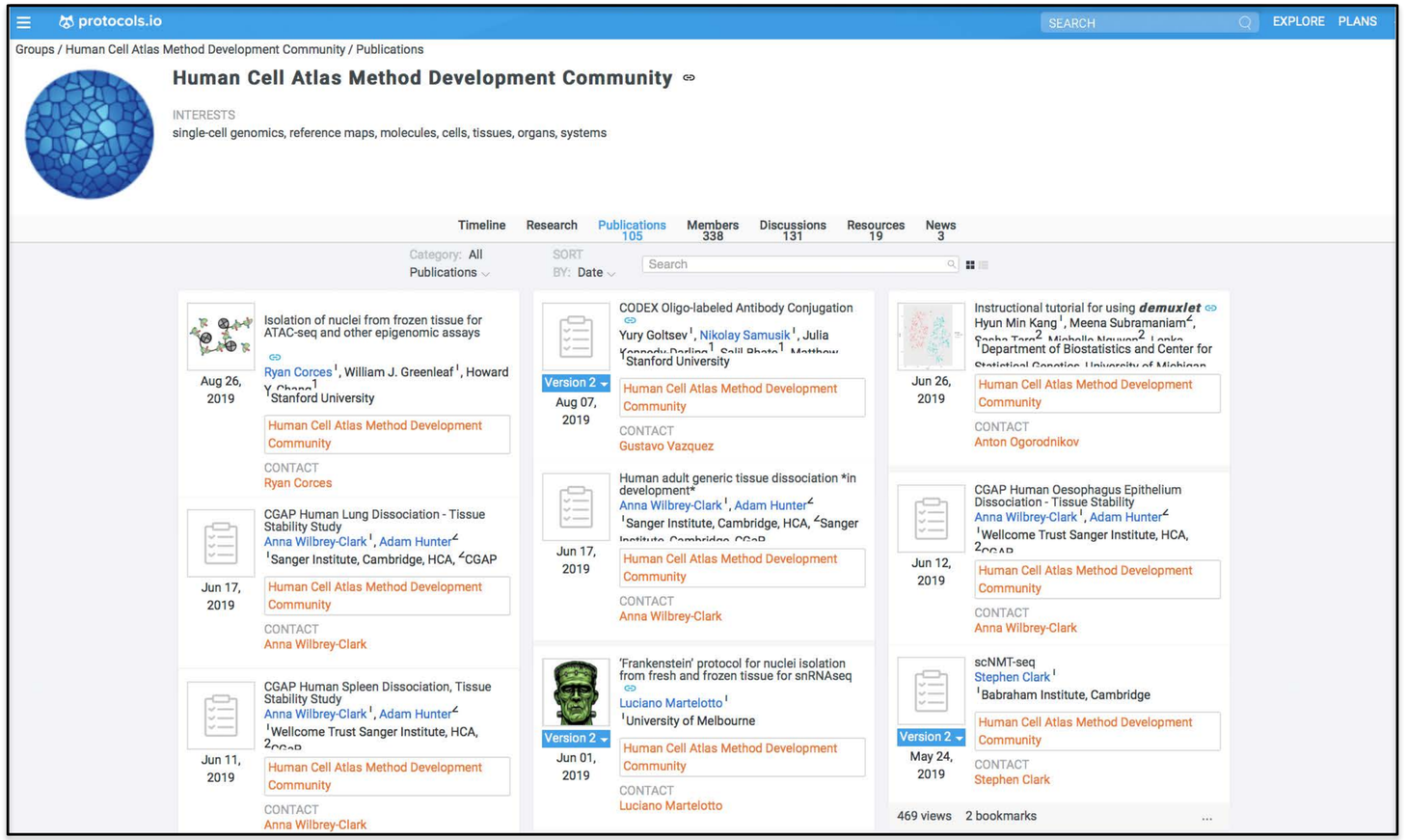
Next up - Single nucleus RNA-Seq pipelines and analysis portals

Data at data.humancellatlas.org

Slide courtesy of: HCA DCP

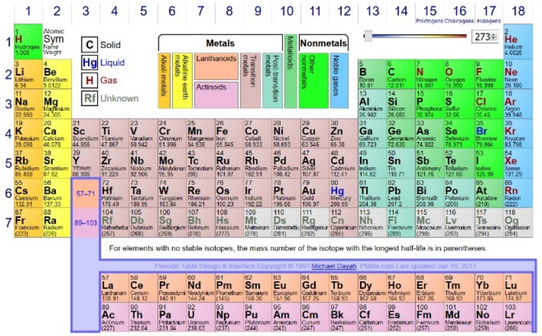
Sharing our protocols

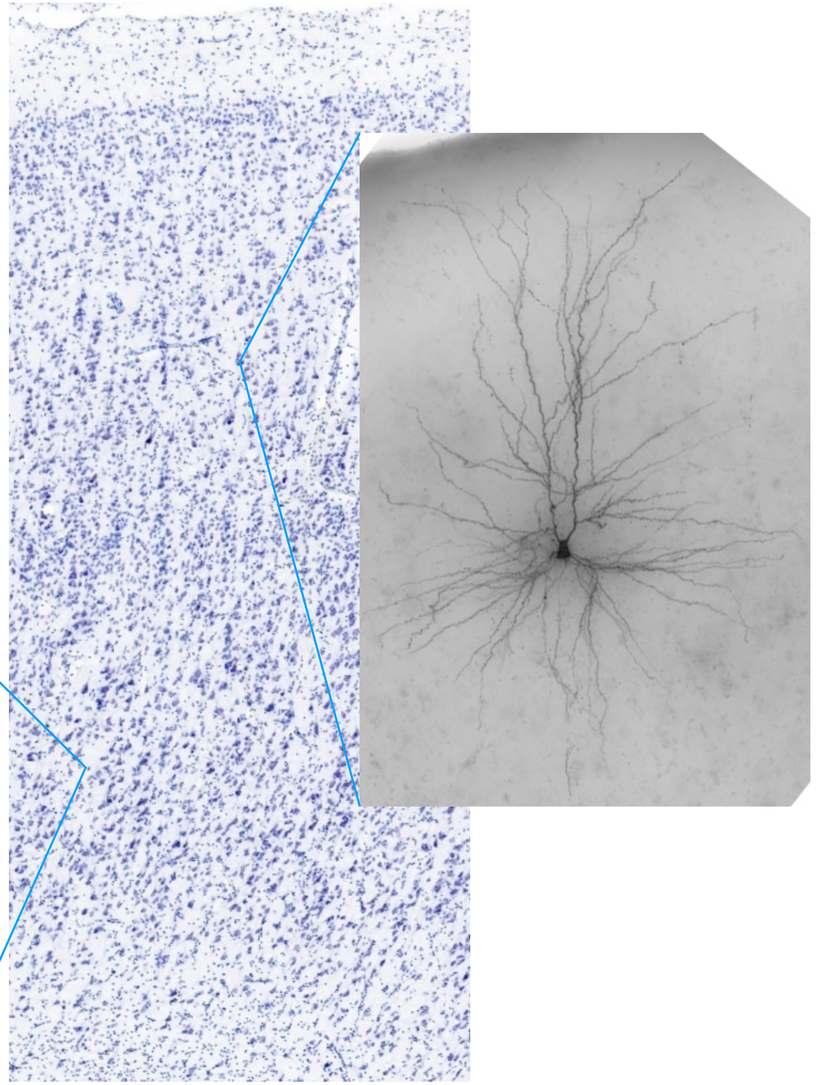
105 protocols, 338 members, 131 Discussions

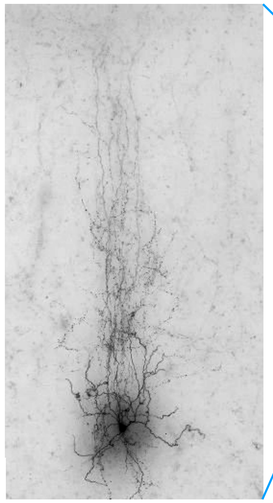


https://www.protocols.io/groups/hca

Defining the census of brain cell types







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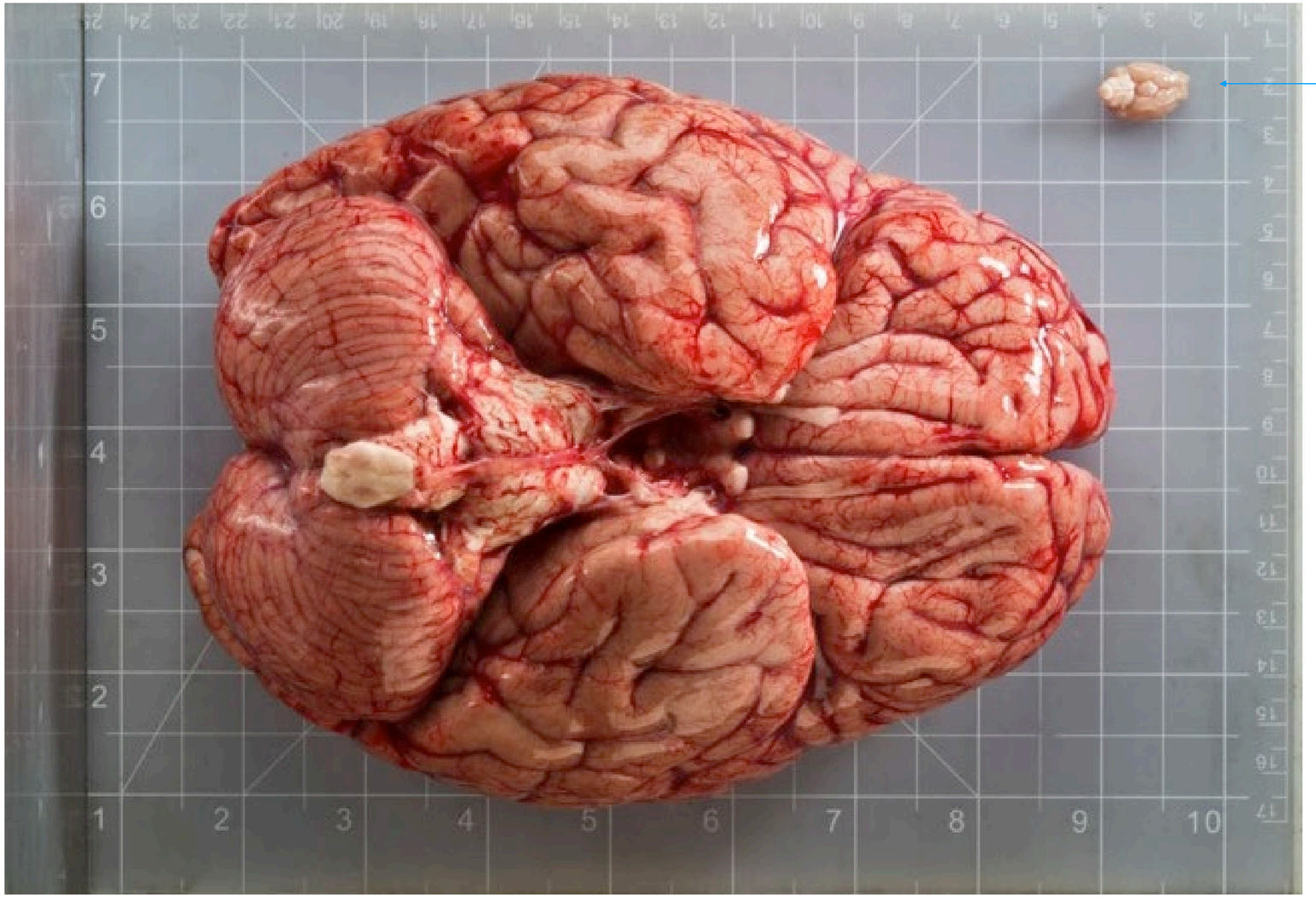
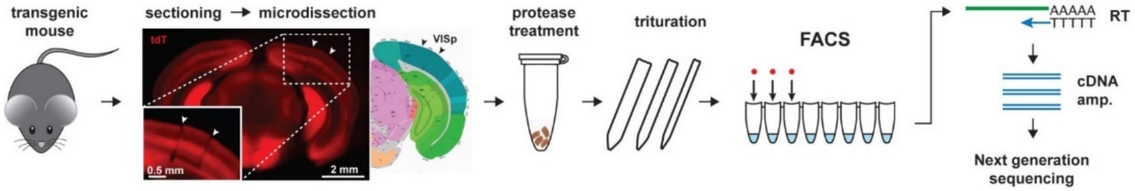
NIMH National Institute of Mental Health

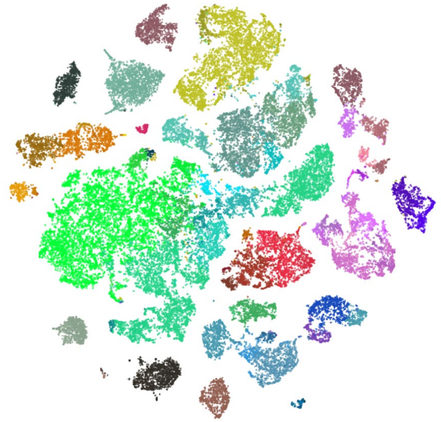
BICCN

Major challenges in characterizing human brain compared to model organisms

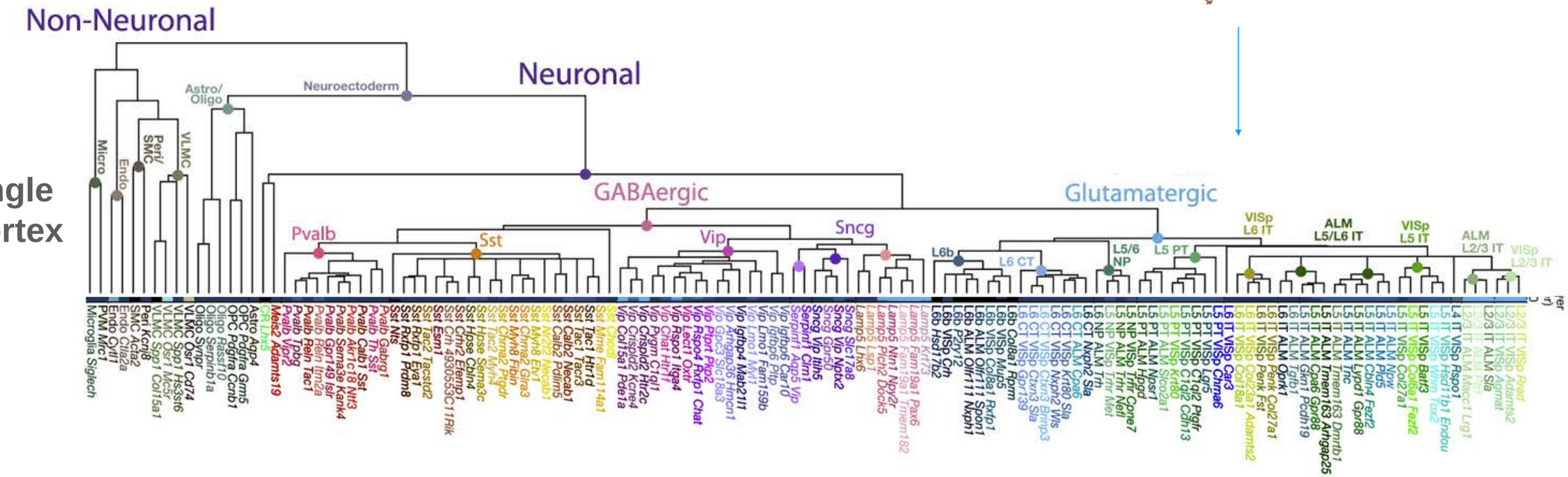


Single cell transcriptomics provides an unbiased and essentially complete molecular classification of mouse cortical cell types





~100 cell types in a single region of the mouse cortex



Tasic, Yao, Smith, Graybuck…Koch, Zeng (2018) Nature

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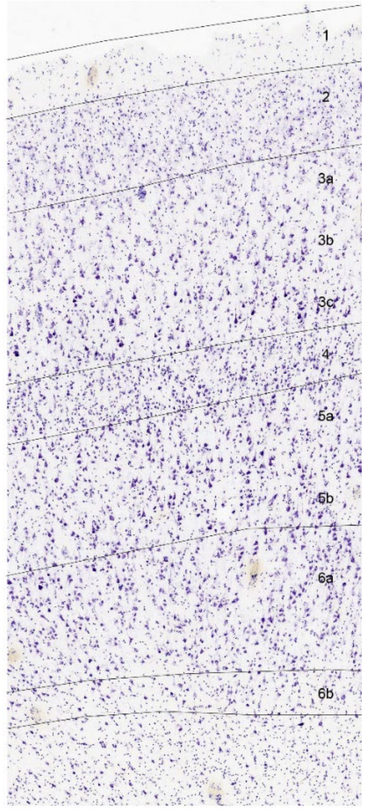
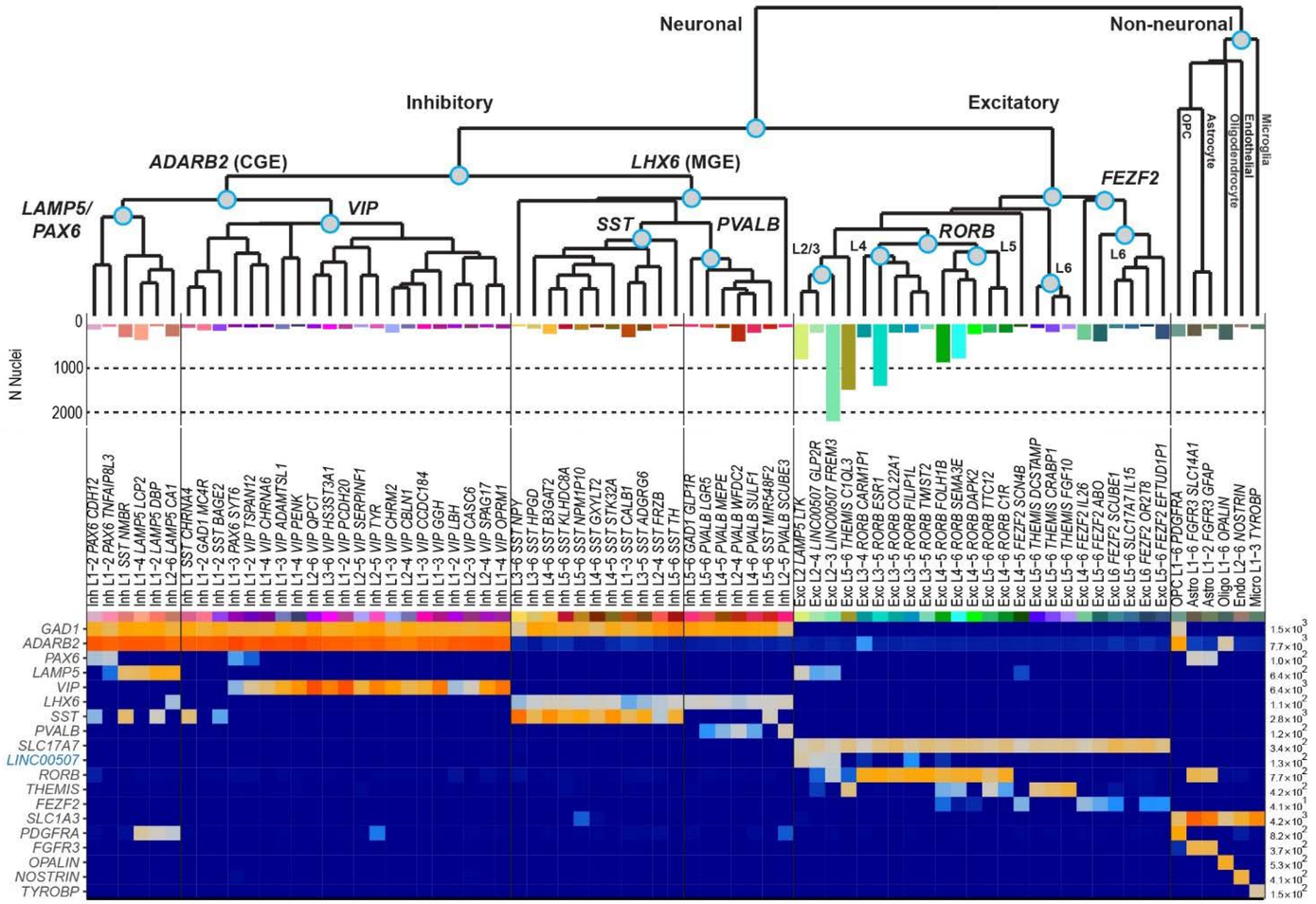
Similarly detailed cellular classifications can be generated in human cortex using single nucleus transcriptomics

Most cell types are rare



Human MTG



Hodge, Bakken, Miller…Lein (2019) Nature

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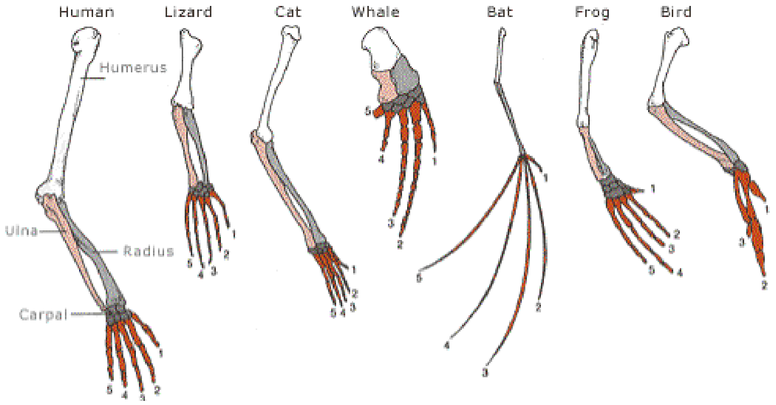
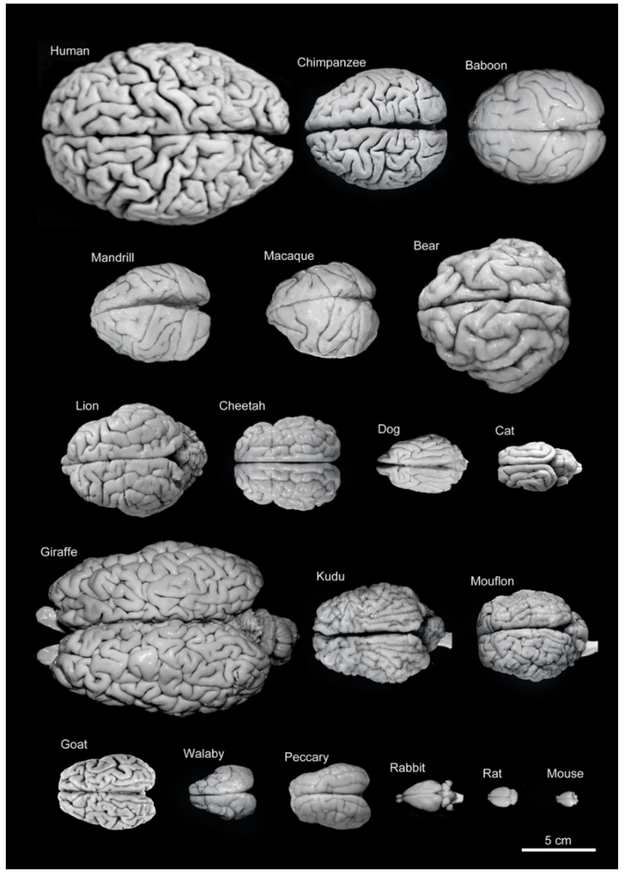
Evolutionary principles explain similarities and differences between species

Limb homology

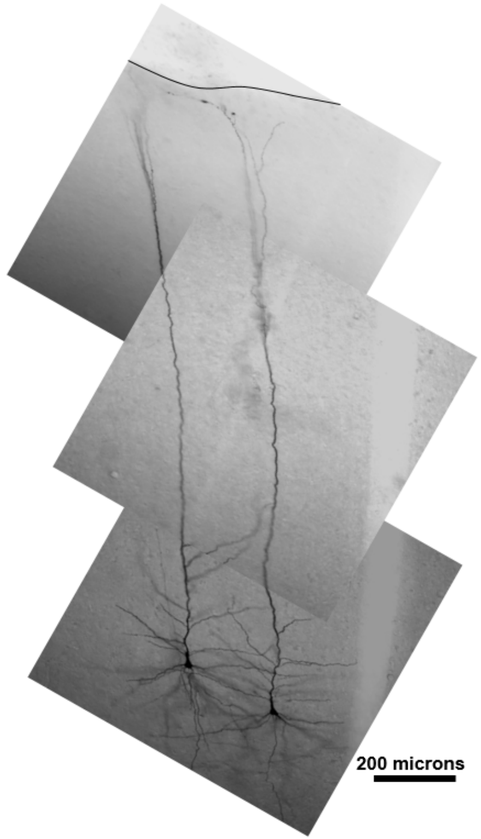


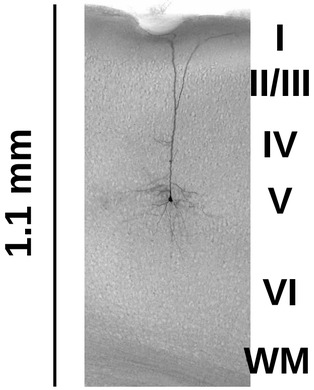
Cortical size and gyrification



DeFelipe 2011

Cell Types

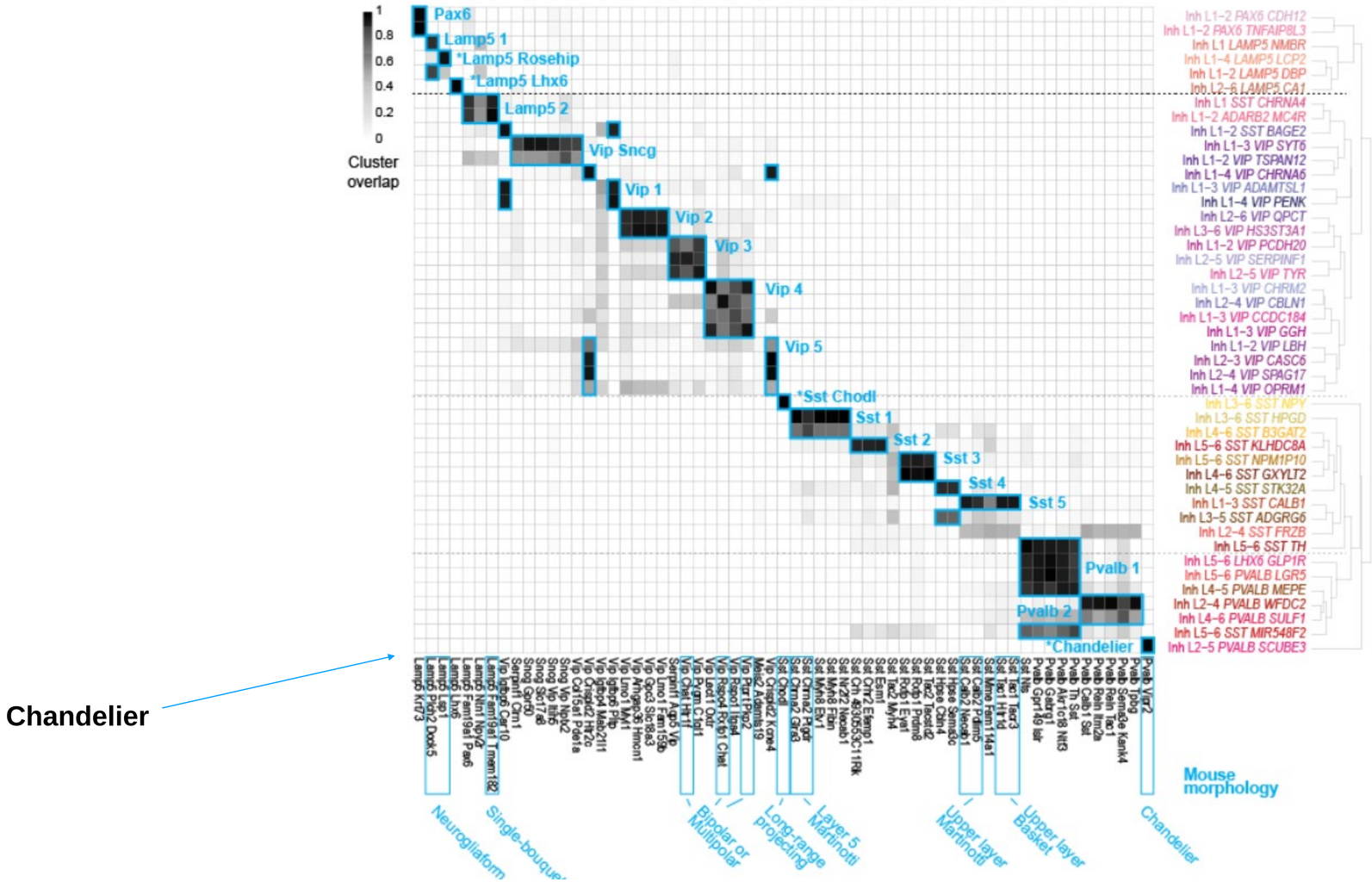




Human Mouse

The makeup of cortical cell types is very similar between human and mouse

“Homology mapping” to align cell types across species



Chandelier

Human

Mouse

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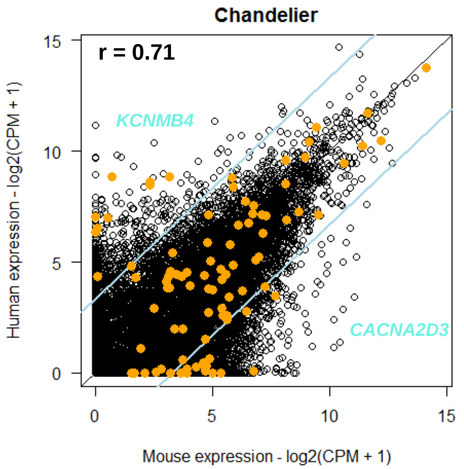
Homologous cell types have many differences in gene usage across species

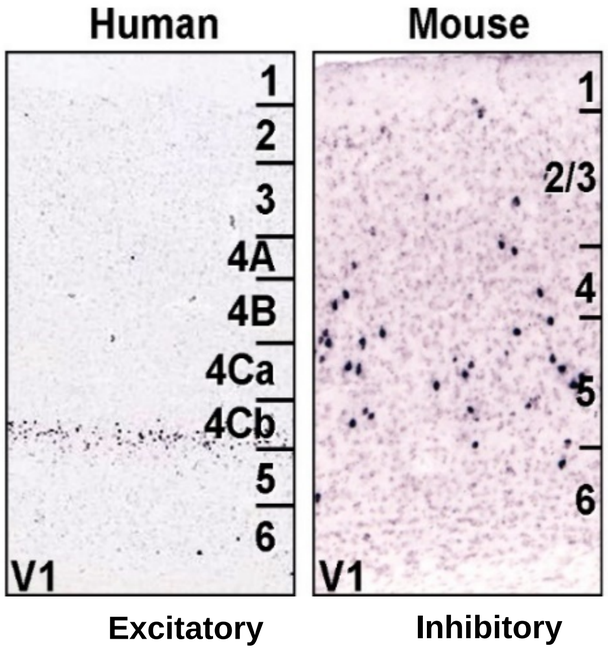
Gene expression in cell types is highly conserved overall



But 12-20% of the ~8000 genes detected have >10x difference

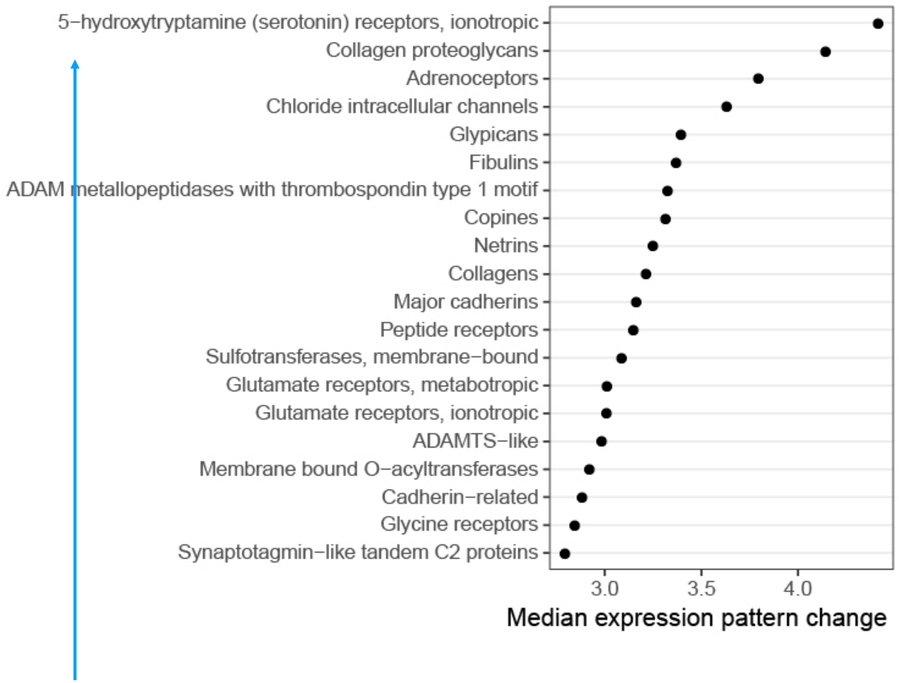
The genes are highly conserved but their cellular usage often changes

Neuropeptide Prodynorphin (PDYN)



Genes with different are functionally important

Top 20 most divergent functional gene classes

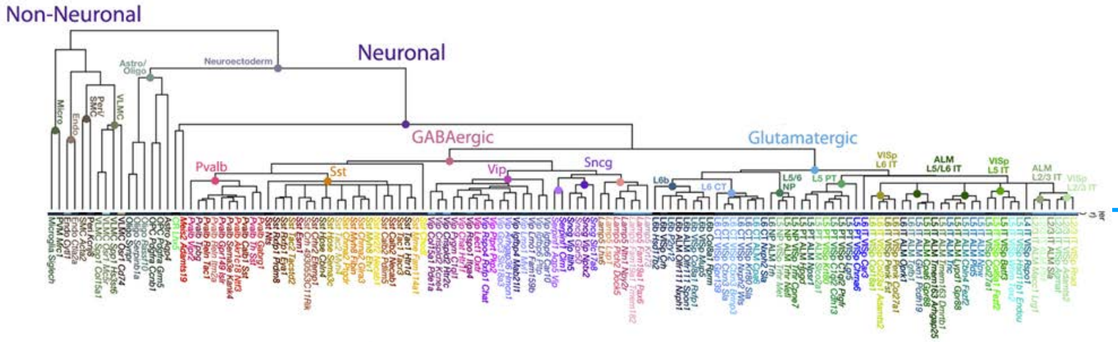


The gene family showing the most differences between human and mouse is involved in serotonin signaling, which is associated with major depression, mood disorders, schizophrenia, addiction, ADHD and autism.

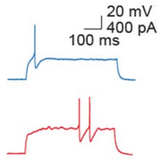
The genetically-based Cell Atlas is only the beginning

Understand the properties of cell types

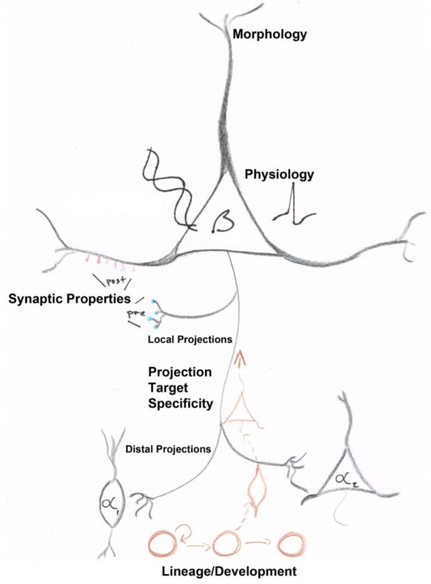
Cell type classification based on Single Cell Transcriptomics



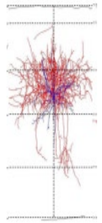
Electrophysiology



Connectivity, function



Anatomy



Understand the cellular basis of brain diseases

Develop viral genetic tools for selective genetic manipulation and gene therapy

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First single cell transcriptomic studies of Alzheimer's Disease are revealing cell type-selective phenotypes

Cell

Article

A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease

Hadas Keren-Shaul,[1,8] Amit Spinrad,[1,2,8] Assaf Weiner,[1,3,8,*] Orit Matcovitch-Natan,[1,2,8] Raz Dvir-Szternfeld,[2] Tyler K. Ulland,[4] Eyal David,[1] Kuti Baruch,[2] David Lara-Astaiso,[1] Beata Toth,[5] Shalev Itzkovitz,[5] Marco Colonna,[4] Michal Schwartz,[2,7,*] and Ido Amit[1,7,8,*]

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http://dx.doi.org/10.1016/j.cell.2017.05.018

SUMMARY

Alzheimer's disease (AD) is a detrimental neurodegenerative disease with no effective treatments. Due to cellular heterogeneity, defining the roles of immune cell subsets in AD onset and progression has been challenging. Using transcriptional single-cell sorting, we comprehensively map all immune populations in wild-type and AD-transgenic (Tg-AD) mouse brains. We describe a novel microglia type associated with neurodegenerative diseases (DAM) and identify markers, spatial localization, and pathways associated with these cells. Immunohistochemical staining of mice and human brain slices shows DAM with intracellular/phagocytic Aβ particles. Single-cell analysis of DAM in Tg-AD and triggering receptor expressed on myeloid cells 2 (Trem2)$^{-/-}$ Tg-AD reveals that the DAM program is activated in a two-step process. Activation is initiated in a Trem2-independent manner that involves downregulation of microglia checkpoints, followed by activation of a Trem2-dependent program. This unique microglia-type has the potential to restrict neurodegeneration, which may have important implications for future treatment of AD and other neurodegenerative diseases.

et al., 2011; Ziv et al., 2006). In the mouse embryo, microglia migrate from the yolk sac to the CNS at embryonic days 8–9, undergo a stepwise program of development that is synchronized with the brain developmental process, and subsequently acquire a stable phenotype essential for the brain protection and homeostasis (Ginhoux and Prinz, 2015; Matcovitch-Natan et al., 2016). Microglia immune activity is restrained by dedicated immune inhibitory pathways that suppress unwanted inflammatory responses and tissue destruction that are often associated with immune activation (Hanisch and Kettenmann, 2007). These checkpoint mechanisms include direct inhibitory interactions of microglia with neurons through the receptor-ligand pairs CX3CL1-CX3CR1 and CD200-CD200R, soluble molecules present in the CNS milieu (e.g., transforming growth factor β [TGF-β]), and intracellular regulators such as the transcription factor MafB (Butovsky et al., 2015; Kierdorf and Prinz, 2013; Lauro et al., 2015; Matcovitch-Natan et al., 2016; Ransohoff and Cardona, 2010). Nevertheless, these mechanisms may be disadvantageous under extreme conditions when reparative microglial activity is needed.

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by progressive memory decline and cognitive dysfunction, often manifested histologically by the parenchymal deposition of amyloid-beta (Aβ) plaques, the formation of neurofibrillary tangles and neuroinflammation (Hardy and Selkoe, 2002; Holtzman et al., 2011). Numerous studies reported conflicting results regarding the contribution of systemic immunity, recruited monocytes, and tissue-resident microglia to AD onset and disease progression (Baruch et al., 2016; Deardorff

Single-cell transcriptomic analysis of Alzheimer's disease

Hansruedi Mathys[1,2,10], Jose Davila-Velderrain[3,4,10], Zhuyu Peng[1,2], Fan Gao[1,2], Shahin Mohammadi[3,4], Jennie Z. Young[1,2], Madhvi Menon[4,5,6], Liang He[3,4], Fatema Abdurrob[1,2], Xueqiao Jiang[1,2], Anthony J. Martorell[1,2], Richard M. Ransohoff[7], Brian P. Hafler[4,5,6,8], David A. Bennett[9], Manolis Kellis[3,4,11]* & Li-Huei Tsai[1,2,4,11]*

Alzheimer's disease is a pervasive neurodegenerative disorder, the molecular complexity of which remains poorly understood. Here, we analysed 80,660 single-nucleus transcriptomes from the prefrontal cortex of 48 individuals with varying degrees of Alzheimer's disease pathology. Across six major brain cell types, we identified transcriptionally distinct subpopulations, including those associated with pathology and characterized by regulators of myelination, inflammation, and neuron survival. The strongest disease-associated changes appeared early in pathological progression and were highly cell-type specific, whereas genes upregulated at late stages were common across cell types and primarily involved in the global stress response. Notably, we found that female cells were overrepresented in disease-associated subpopulations, and that transcriptional responses were substantially different between sexes in several cell types, including oligodendrocytes. Overall, myelination-related processes were recurrently perturbed in multiple cell types, suggesting that myelination has a key role in Alzheimer's disease pathophysiology. Our single-cell transcriptomic resource provides a blueprint for interrogating the molecular and cellular basis of Alzheimer's disease.

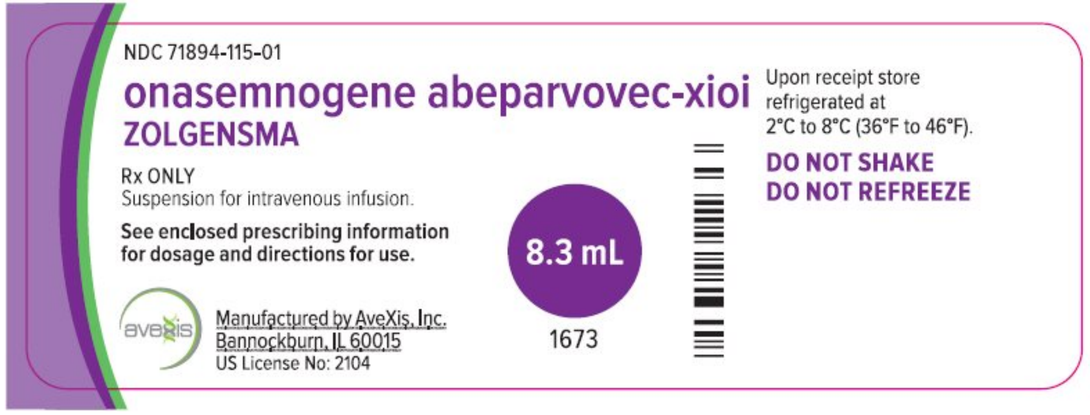
A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation

Alexandra Grubman[1,2,3,9], Gabriel Chew[4,9], John F. Ouyang[4,9], Guizhi Sun[1,2,3], Xin Yi Choo[1,2,3,5], Catriona McLean[6], Rebecca K. Simmons[7,8], Sam Buckberry[7,8], Dulce B. Vargas-Landin[7,8], Daniel Poppe[7,8], Jahnvi Pflueger[7,8], Ryan Lister[7,8], Owen J. L. Rackham[4*], Enrico Petretto[4*] and Jose M. Polo[1,2,3*]

There is currently little information available about how individual cell types contribute to Alzheimer's disease. Here we applied single-nucleus RNA sequencing to entorhinal cortex samples from control and Alzheimer's disease brains ($n = 6$ per group), yielding a total of 13,214 high-quality nuclei. We detail cell-type-specific gene expression patterns, unveiling how transcriptional changes in specific cell subpopulations are associated with Alzheimer's disease. We report that the Alzheimer's disease risk gene *APOE* is specifically repressed in Alzheimer's disease oligodendrocyte progenitor cells and astrocyte subpopulations and upregulated in an Alzheimer's disease-specific microglial subopulation. Integrating transcription factor regulatory modules with Alzheimer's disease risk loci revealed drivers of cell-type-specific state transitions towards Alzheimer's disease. For example, transcription factor EB, a master regulator of lysosomal function, regulates multiple disease genes in a specific Alzheimer's disease astrocyte subpopulation. These results provide insights into the coordinated control of Alzheimer's disease risk genes and their cell-type-specific contribution to disease susceptibility. These results are available at http://adsn.ddnetbio.com.

AAV-based gene therapy for the nervous system is becoming a reality

Biallelic RPE65 mutation-associated retinal dystrophy

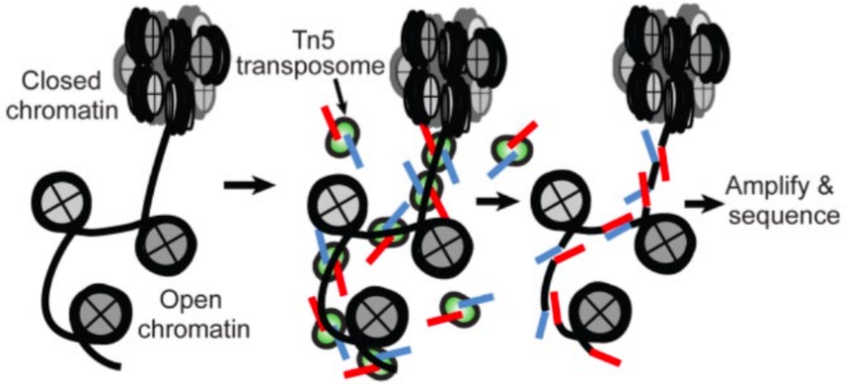


SMN1 mutation-associated Spinal Muscular Atrophy

A genetic cell classification leads directly to genetic tools to target cell types

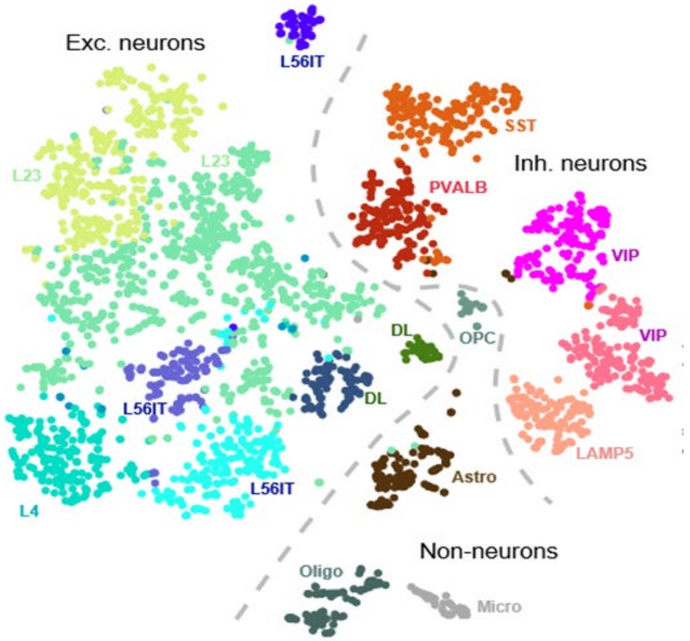
Gene regulatory regions are found in open regions of the genome that are potential “enhancers” of gene expression

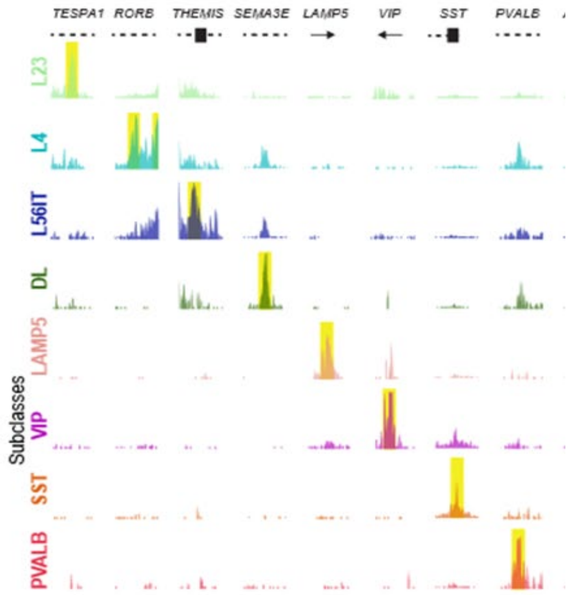
Single cell “ATAC-Seq”



Buenrostro et al., (2013) Nat. Methods, (2015) Nature

Different cell types have different potential enhancers





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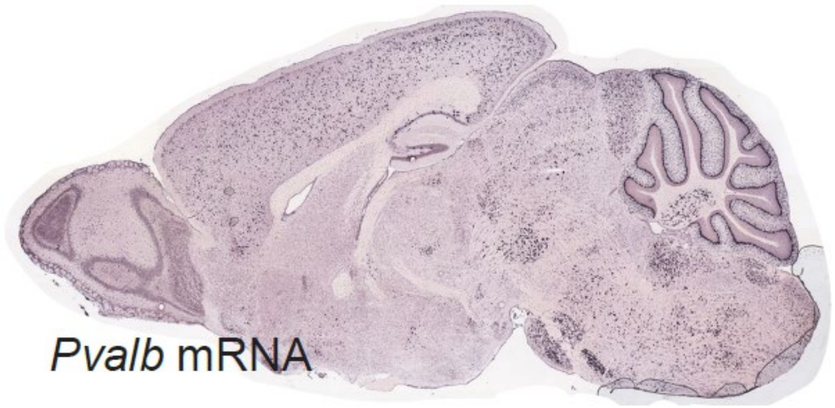
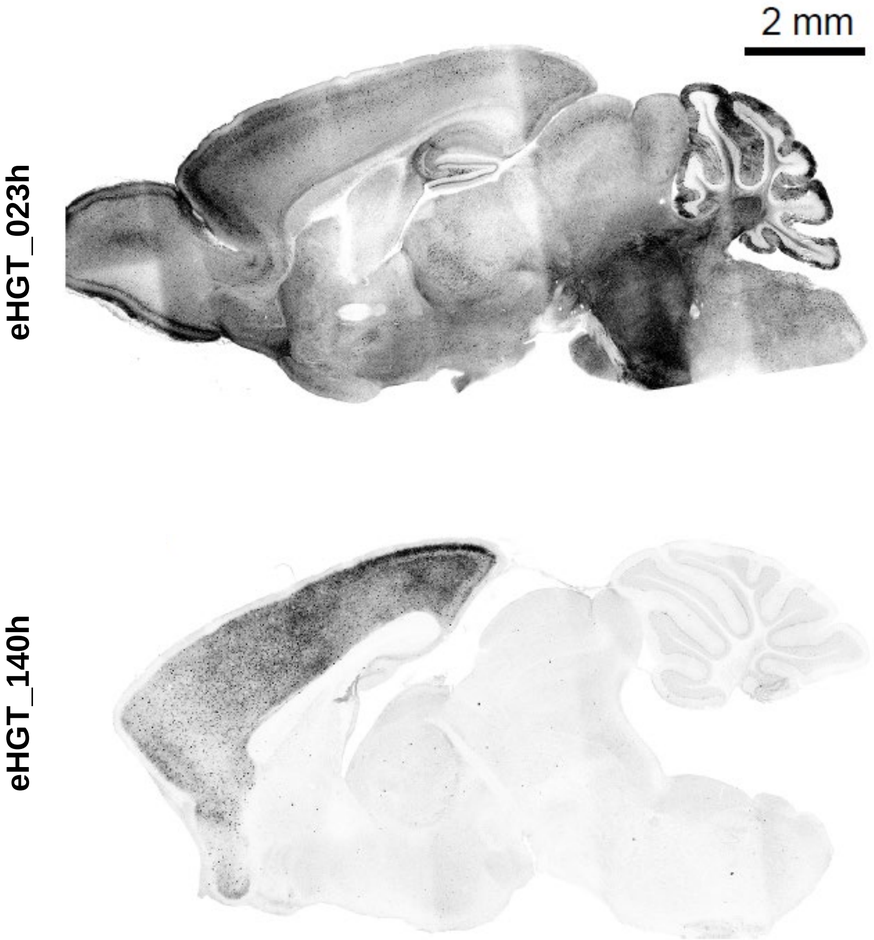
Future of precision medicine: Gene therapy targeted to specific cell types affected in disease



Short enhancers drive gene expression in different cell types following viral infection



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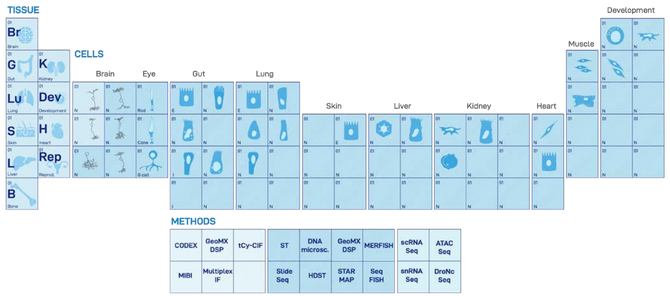
The brain atlas is the foundation for a new era in understanding and treating brain disease

Do neurological, neuropsychiatric, or neurodegenerative diseases involve pathology of specific cell types?



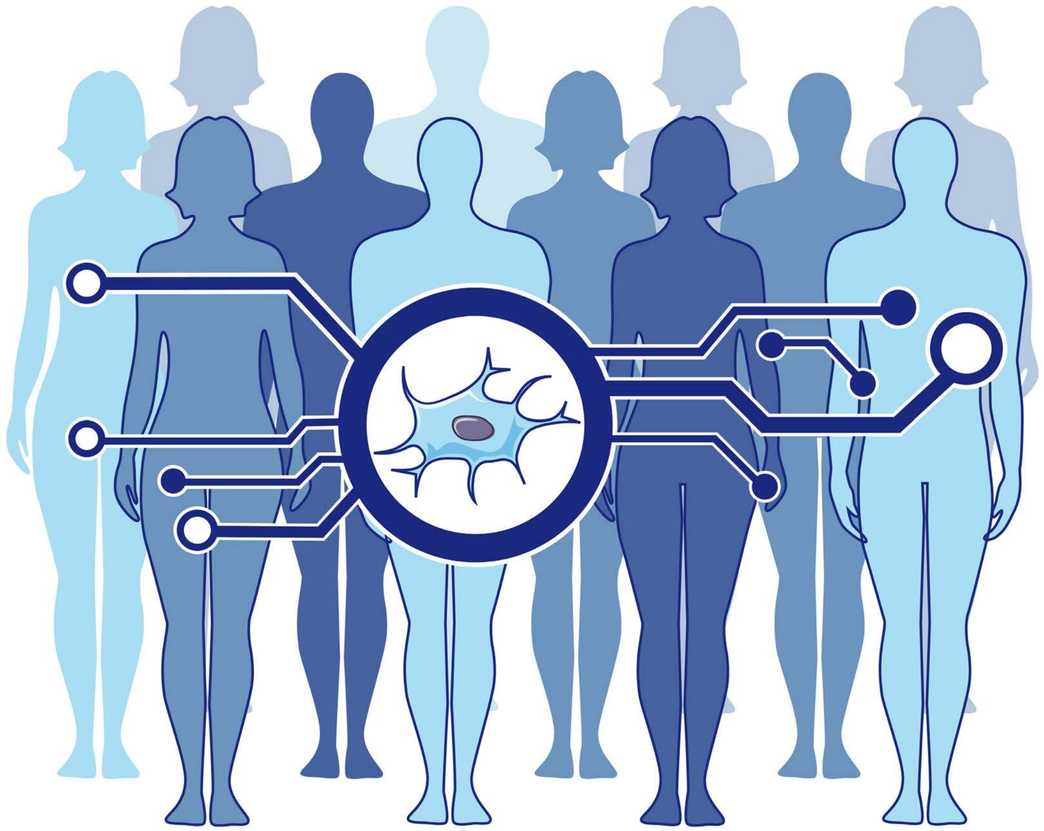
The molecular tools are available now to probe this question by building on the baseline “periodic table”:

- **Are some cell types selectively vulnerable or resistant?**
- **What molecular pathways are perturbed in which cell types?**
- **Where is the best cellular and molecular target for intervention, potentially using gene therapy applications?**

NIMH National Institute of Mental Health

BICCN

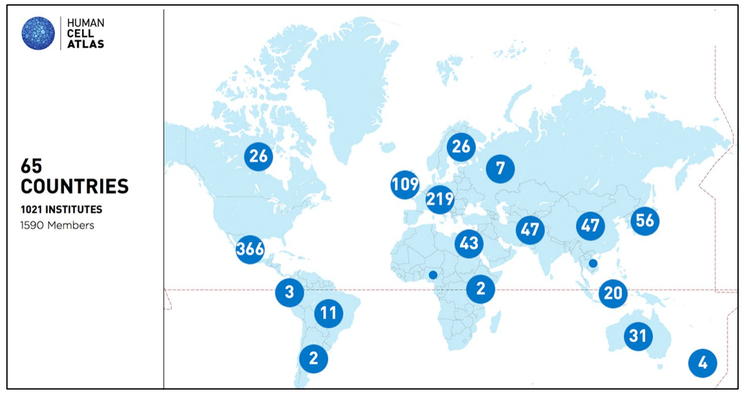
NIH BRAIN Initiative Cell Census Network will complete the entire mouse brain and a draft of human brain in 2020



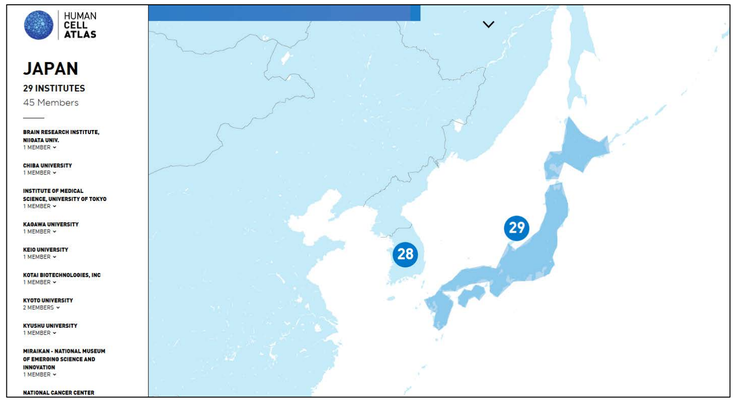
Humans are Diverse.
So Are their Cells.
No One Lab or Country
Should Build the Atlas
Alone.

HCA membership: global and rapidly growing

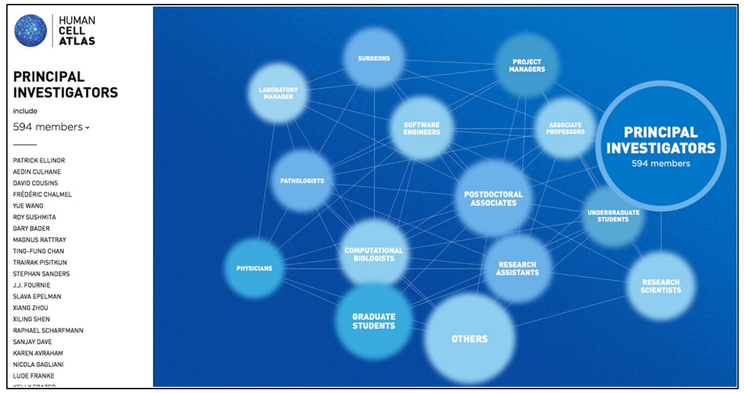
1756 Members, 70 Countries, 1030 Institutes



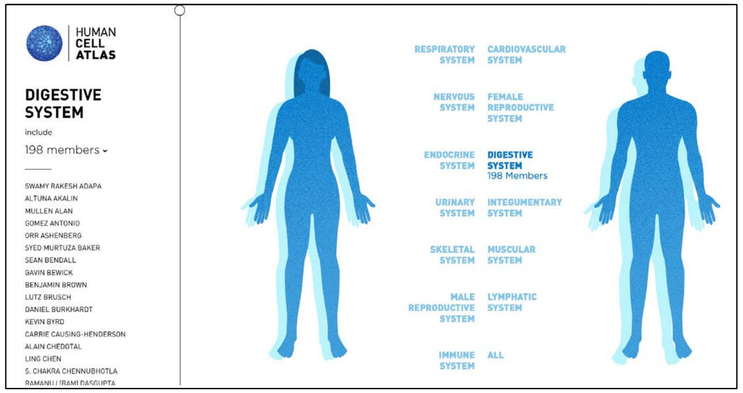
Japan: 45 Members, 29 Institutes



1756 members, 641 PI



14 organs/systems: e.g. digestive system; 198 members



https://www.humancellatlas.org/join-hca/

Learning more about the HCA

https://www.humancellatlas.org/

The Human Cell Atlas

Abstract The recent advent of methods for high-throughput single-cell molecular profiling has catalyzed a growing sense in the scientific community that the time is ripe to complete the 150-year-old effort to identify all cell types in the human body. The Human Cell Atlas Project is an international collaborative effort that aims to define all human cell types in terms of distinctive molecular profiles (such as gene expression profiles) and to connect this information with classical cellular descriptions (such as location and morphology). An open comprehensive reference map of the molecular state of cells in healthy human tissues would propel the systematic study of physiological states, developmental trajectories, regulatory circuitry and interactions of cells, and also provide a framework for understanding cellular dysregulation in human disease. Here we describe the idea, its potential utility, early proofs-of-concept, and some design considerations for the Human Cell Atlas, including a commitment to open data, code, and community.

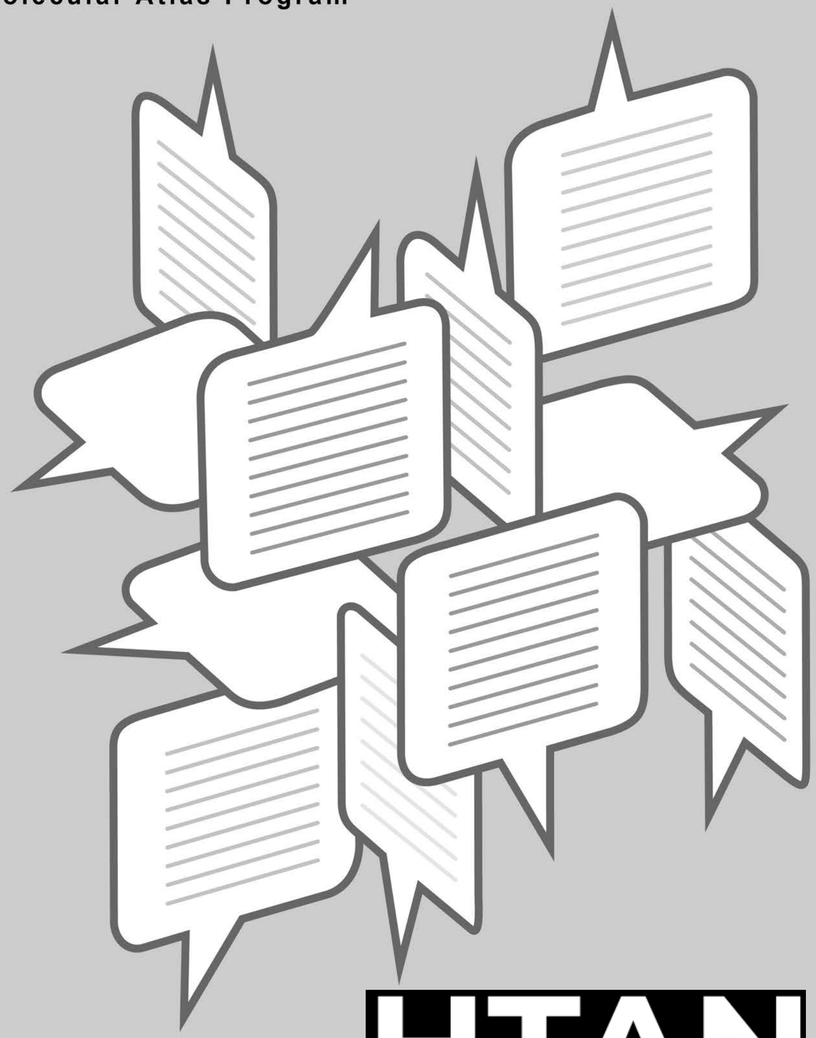
DOI: https://doi.org/10.7554/eLife.27041.001

AVIV REGEV*, SARAH A TEICHMANN*, ERIC S LANDER*, IDO AMIT, CHRISTOPHE BENOIST, EWAN BIRNEY, BERND BODENMILLER, PETER CAMPBELL, PIERO CARNINCI, MENNA CLATWORTHY, HANS CLEVERS, BART DEPLANCKE, IAN DUNHAM, JAMES EBERWINE, ROLAND EILS, WOLFGANG ENARD, ANDREW FARMER, LARS FUGGER, BERTHOLD GÖTTGENS, NIR HACOHEN, MUZLIFAH HANIFFA, MARTIN HEMBERG, SEUNG KIM, PAUL KLENERMAN, ARNOLD KRIEGSTEIN, ED LEIN, STEN LINNARSSON, EMMA LUNDBERG, JOAKIM LUNDEBERG, PARTHA MAJUMDER, JOHN C MARIONI, MIRIAM MERAD, MUSA MHLANGA, MARTIJN NAWIJN, MIHAI NETEA, GARRY NOLAN, DANA PE'ER, ANTHONY PHILLIPAKIS, CHRIS P PONTING, STEPHEN QUAKE, WOLF REIK, ORIT ROZENBLATT-ROSEN, JOSHUA SANES, RAHUL SATIJA, TON N SCHUMACHER, ALEX SHALEK, EHUD SHAPIRO, PADMANEE SHARMA, JAY W SHIN, OLIVER STEGLE, MICHAEL STRATTON, MICHAEL J T STUBBINGTON, FABIAN J THEIS, MATTHIAS UHLEN, ALEXANDER VAN OUDENAARDEN, ALLON WAGNER, FIONA WATT, JONATHAN WEISSMAN, BARBARA WOLD, RAMNIK XAVIER, NIR YOSEF AND HUMAN CELL ATLAS MEETING PARTICIPANTS

https://www.humancellatlas.org/publications/ **and:** URL: https://arxiv.org/abs/1810.05192v1

HuBMAP
Human BioMolecular Atlas Program

LifeTime



HTAN
HUMAN TUMOR ATLAS NETWORK

Funders

Partnership across programs and funders

- Related and complementary initiatives
- Diverse funded data collected projects across the globe
- Support for central efforts: DCP, meetings, ethics, equity
- Discounts to help enable research:
  - Biolegend: Discount on reagents
  - Nanostring: Discount of reagents, instruments
  - Takara: Discount on reagents
  - 10x Genomics: New discount plan