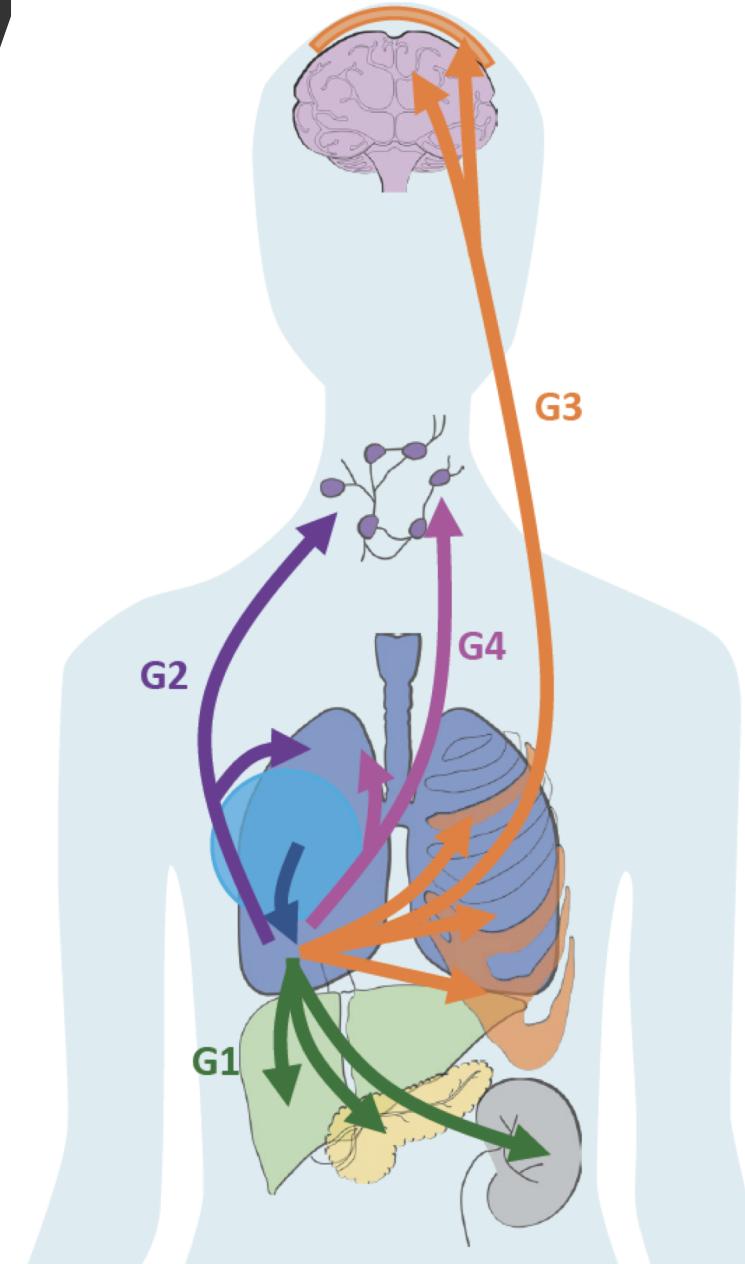
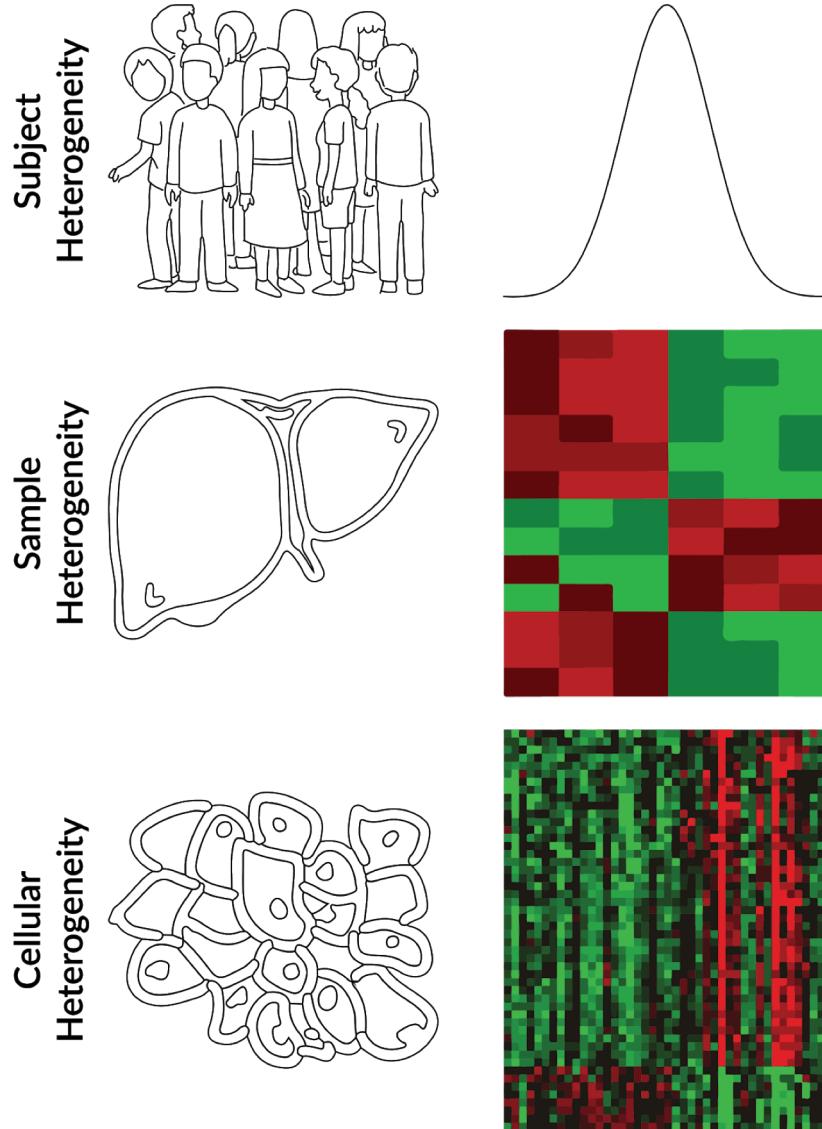
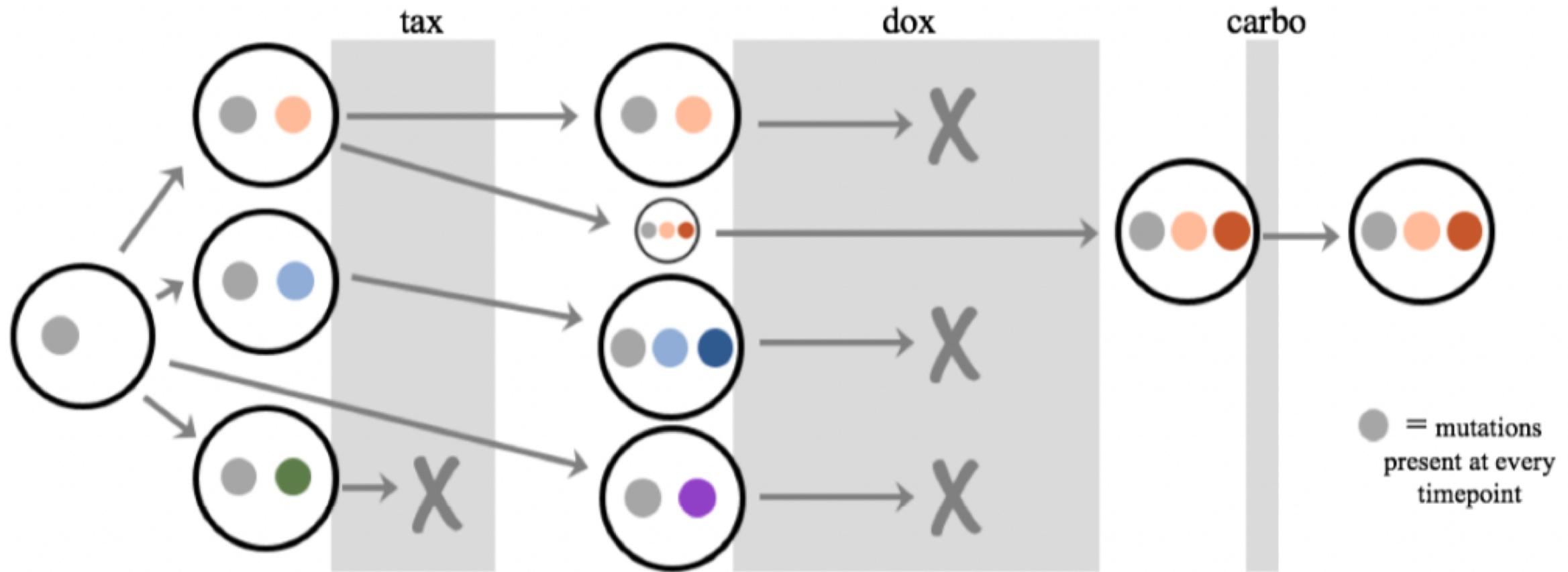


Heterogeneity

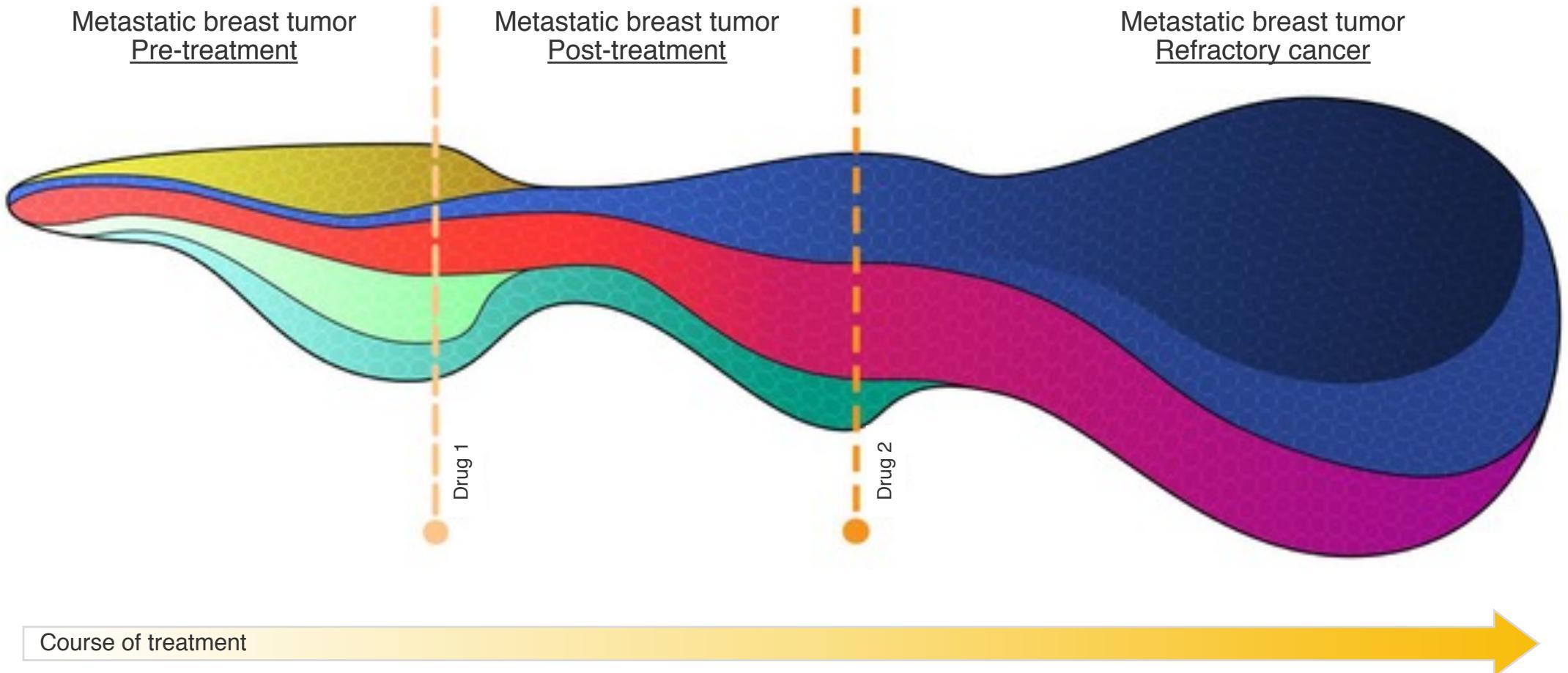


Tumor Heterogeneity



Question: What is the functional impact of tumor evolution?

Tumor Heterogeneity

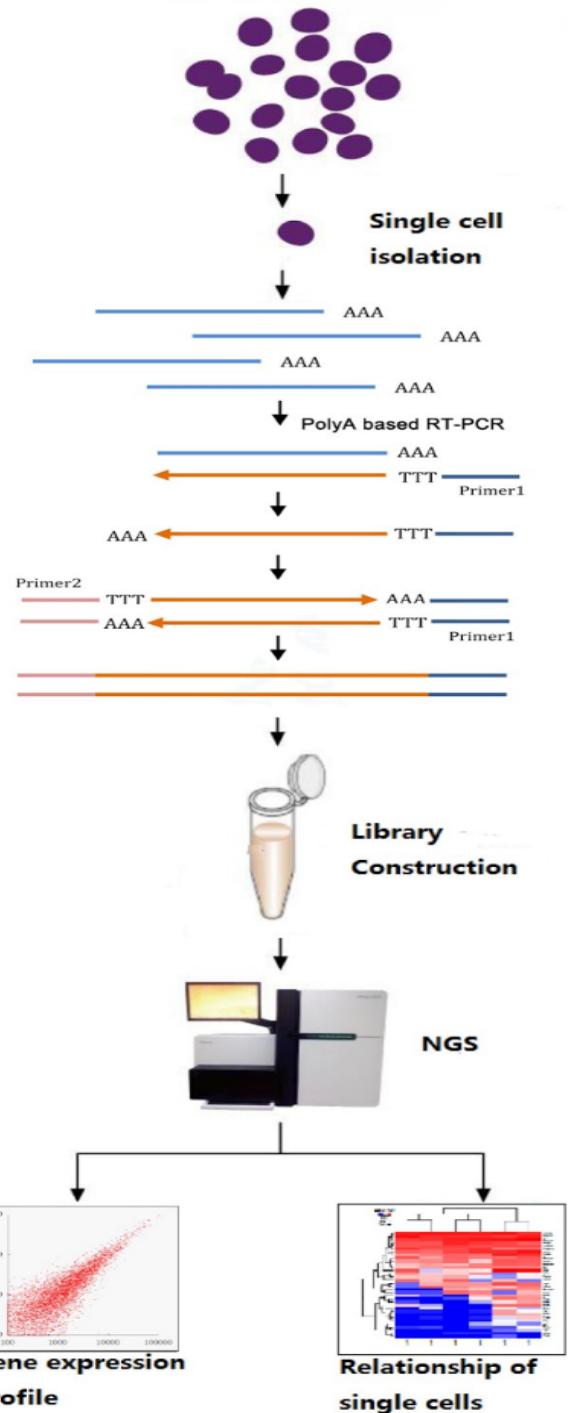


Single Cell Profiling

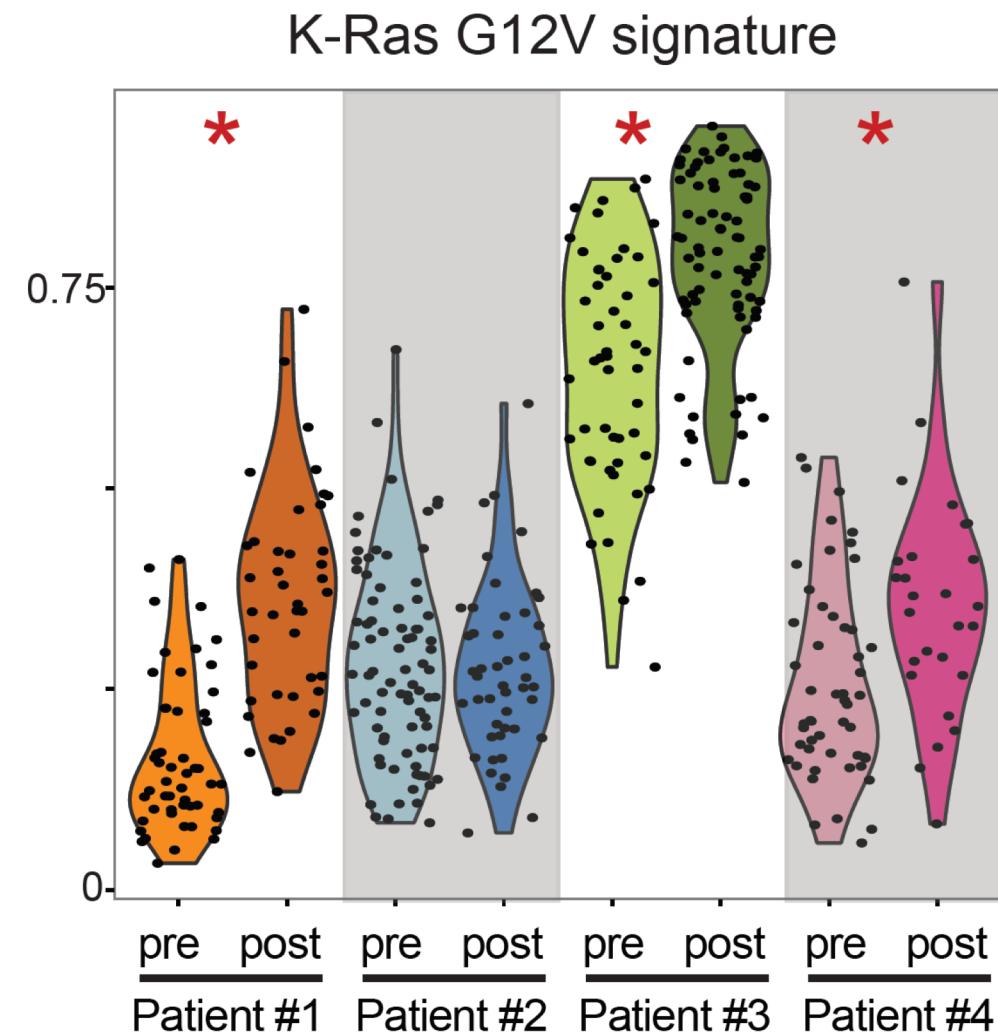
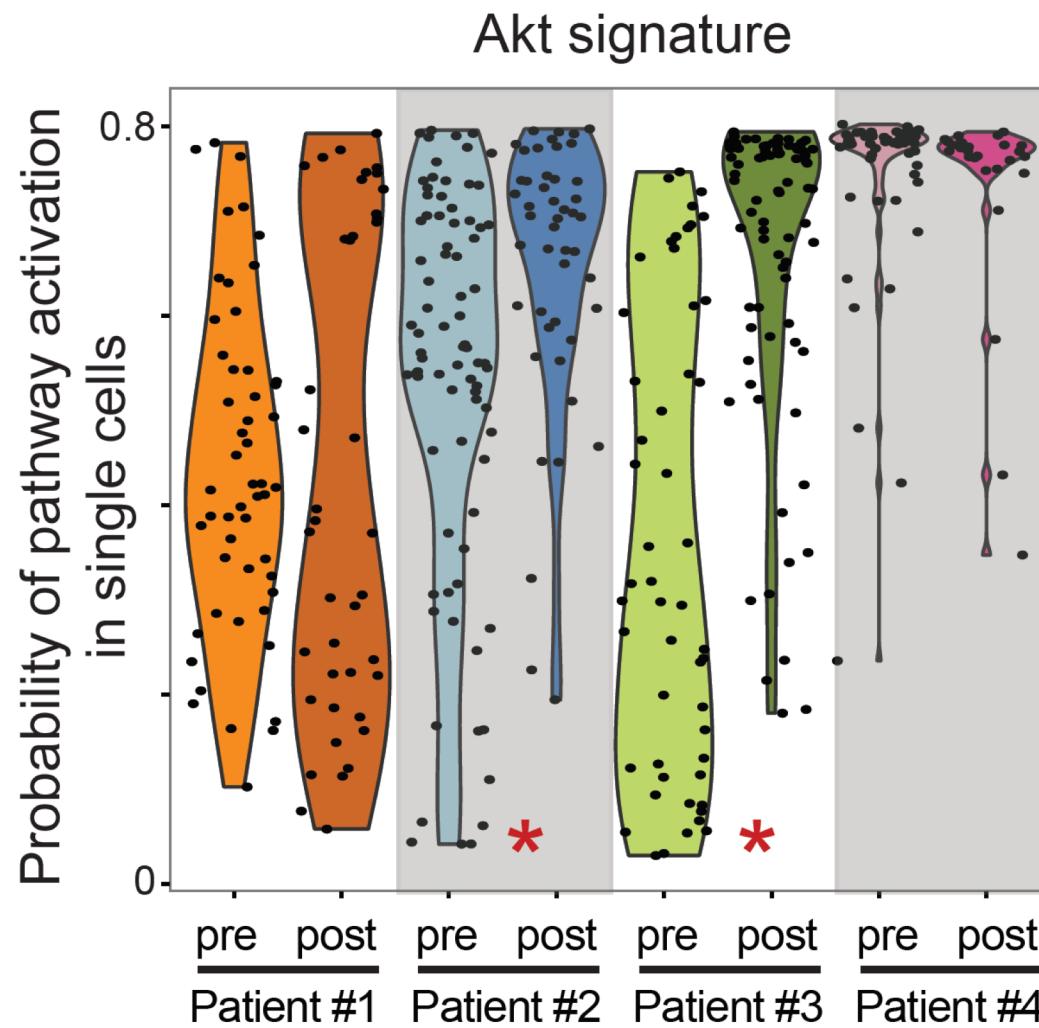
Applications for Single Cell Sequencing:

- **Cancer:** intra-tumoral heterogeneity
- **Development:** characterize every cell in blastocyst
- **Other applications:** novel/rare cell type discovery
- **Infectious Diseases:** combine with metagenomics to explore host response (e.g. TB)
- And more!

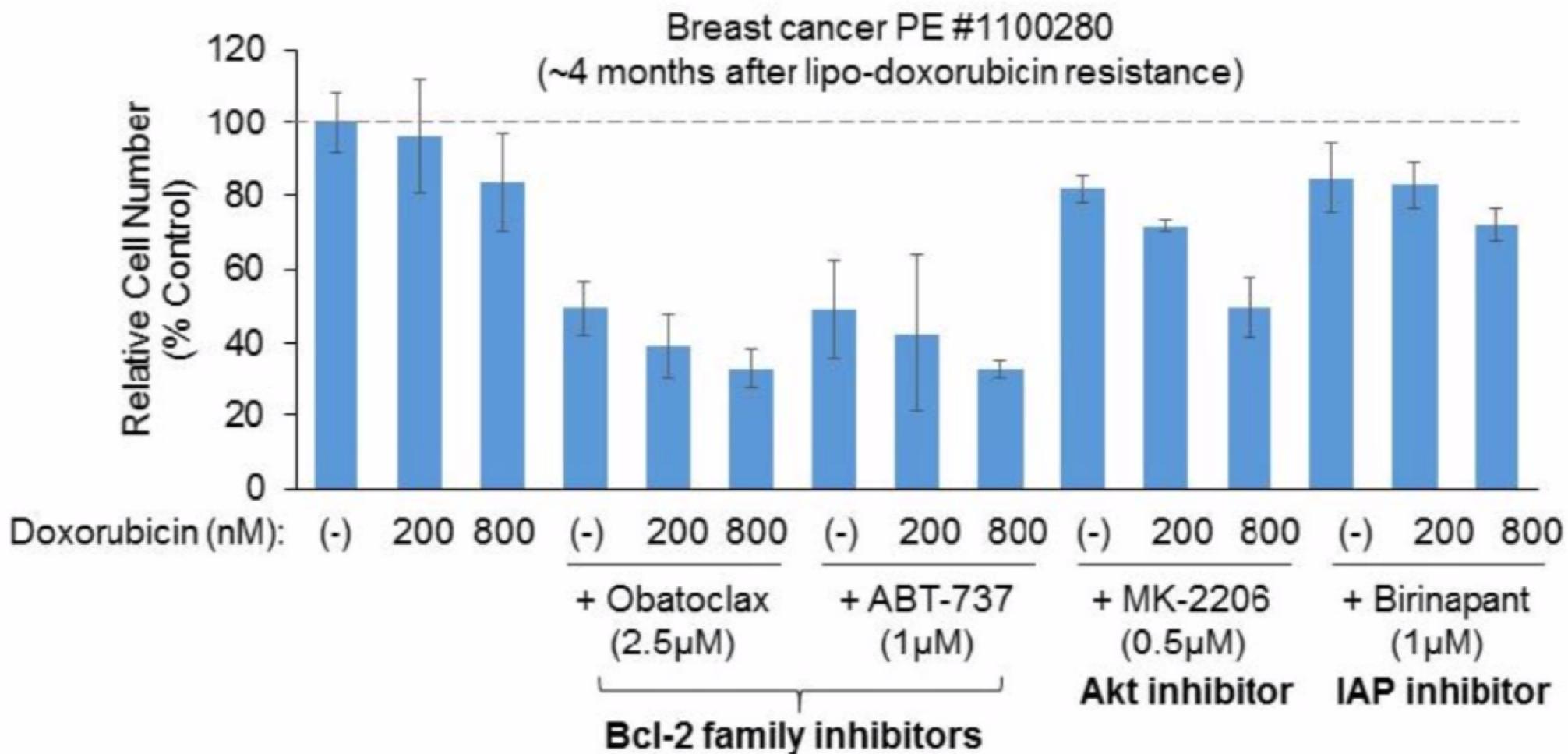
The Future of RNA-sequencing



Pathway Activity in Breast Cancer



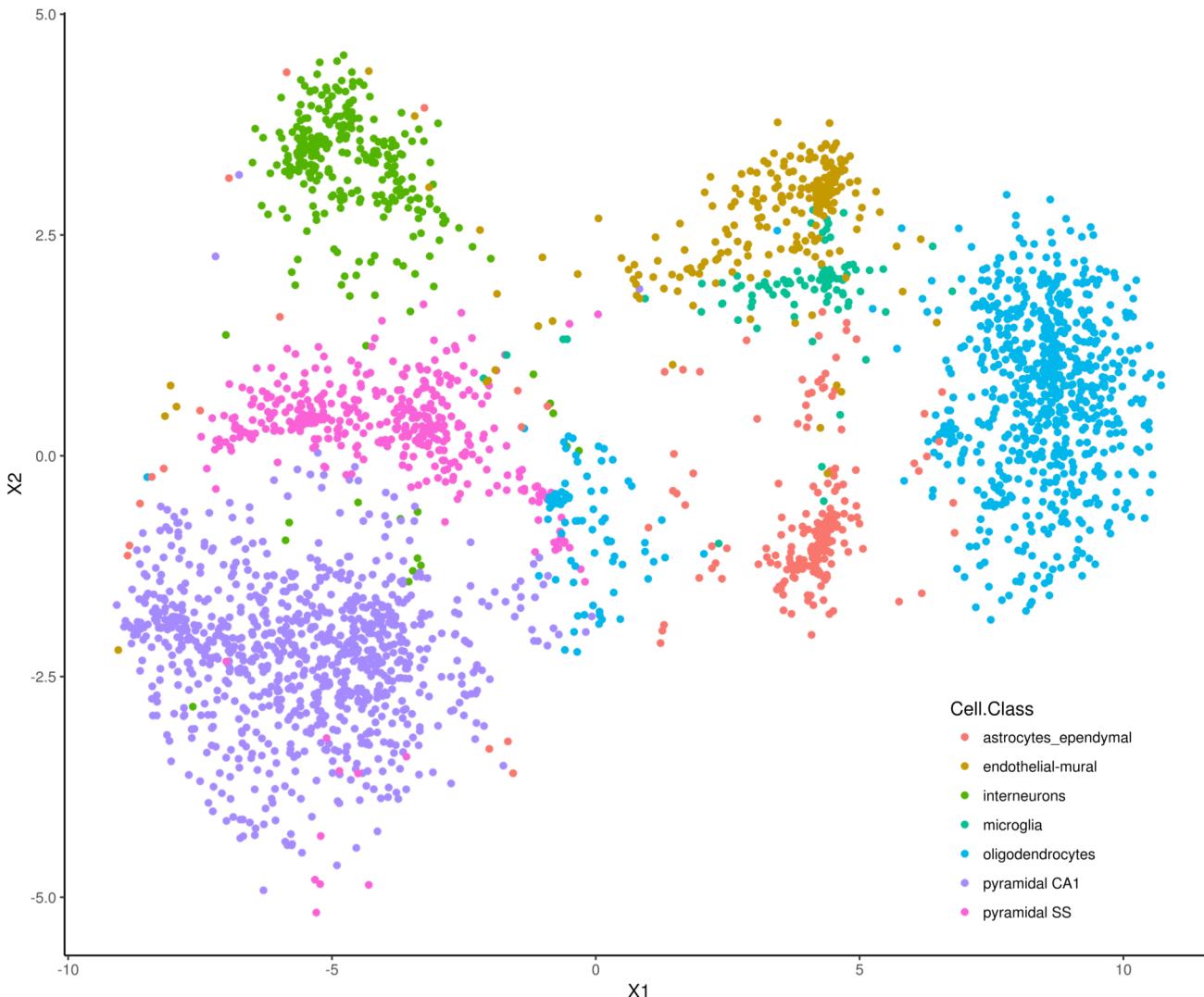
Recovering Efficacy of Chemotherapy



Takehome message: Adding an anti-apoptotic inhibitor (e.g. BCL2) in this case will re-sensitize refractory cancer to standard chemotherapy!

Critical Gaps in scRNA-seq data

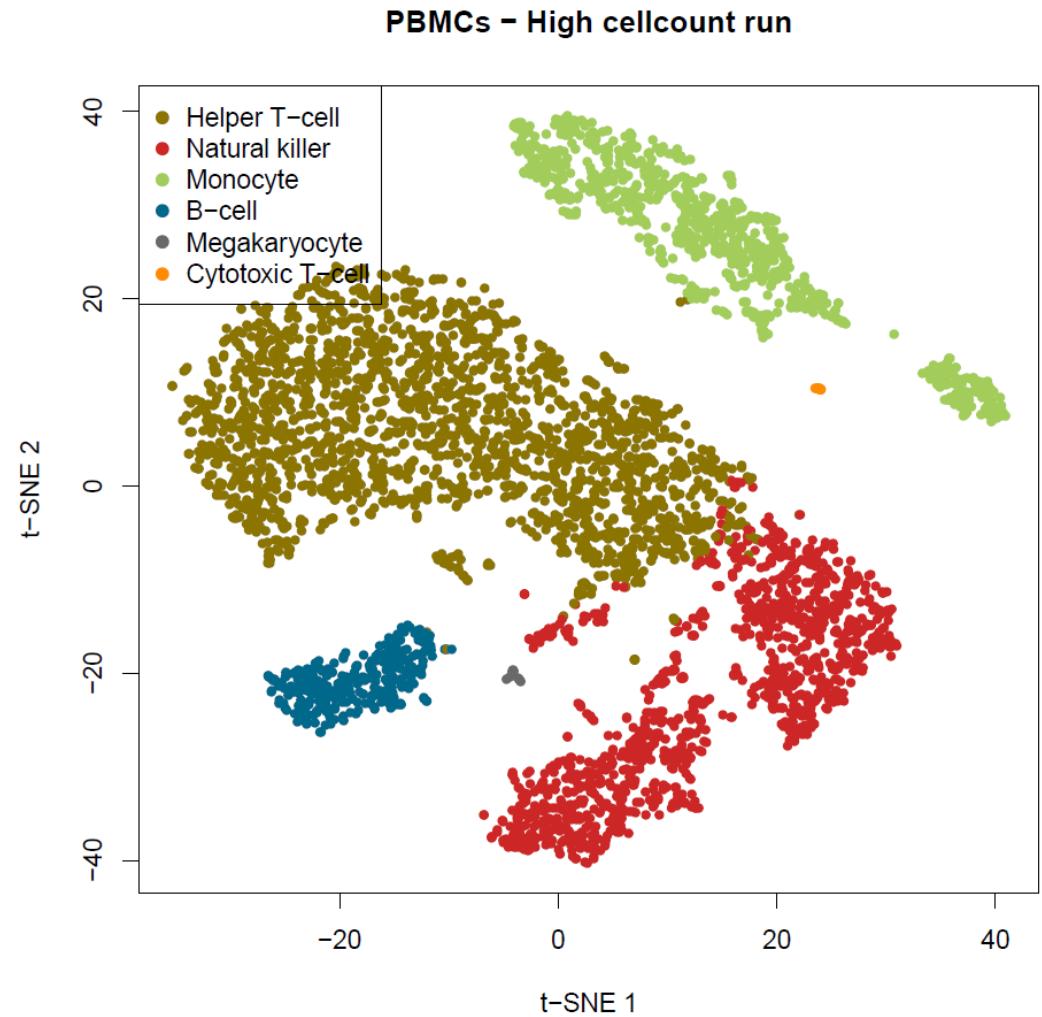
- Complex data
 - New analysis challenges
- Interactive, Simple Analysis?
 - Inexperienced Users
 - Optimizing Parameters
 - Filtering Failed Samples
 - Filtering Low expression genes
- Some packages already exist
 - QC
 - Clustering
 - Full Analysis Portal?



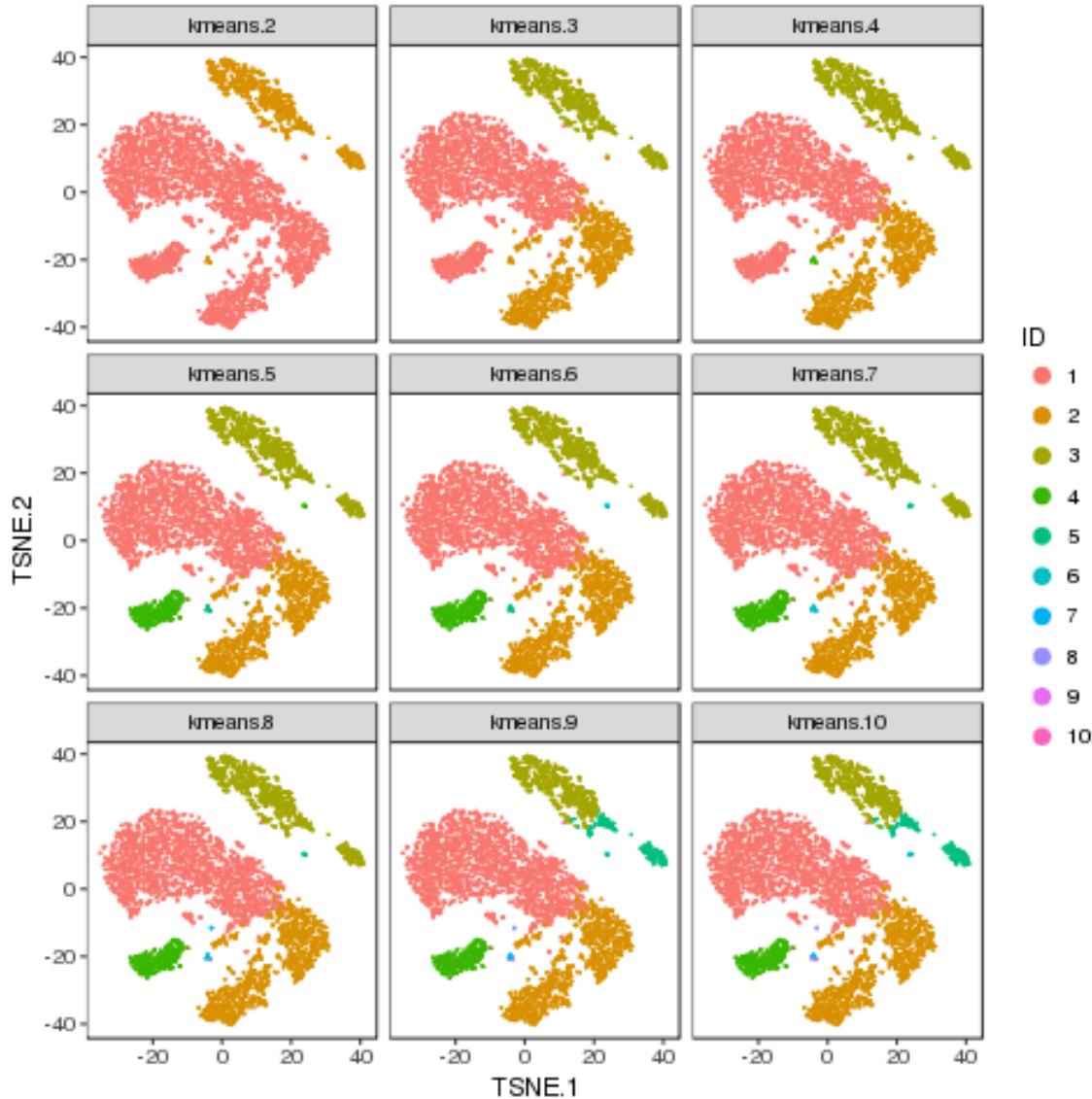
Clustering consistency in PBMCs

First dataset produced by the Single Cell Sequencing Core:

- 10X Genomics
- 2 PBMC datasets, each given one NextSeq run:
 - “HIGH”: ~5,000 cells (27,000 reads/cell)
 - “LOW”: ~800 cells (120,000 reads/cell)
- First pass analysis: Cell Ranger



Complicating factor: cell typing/clustering

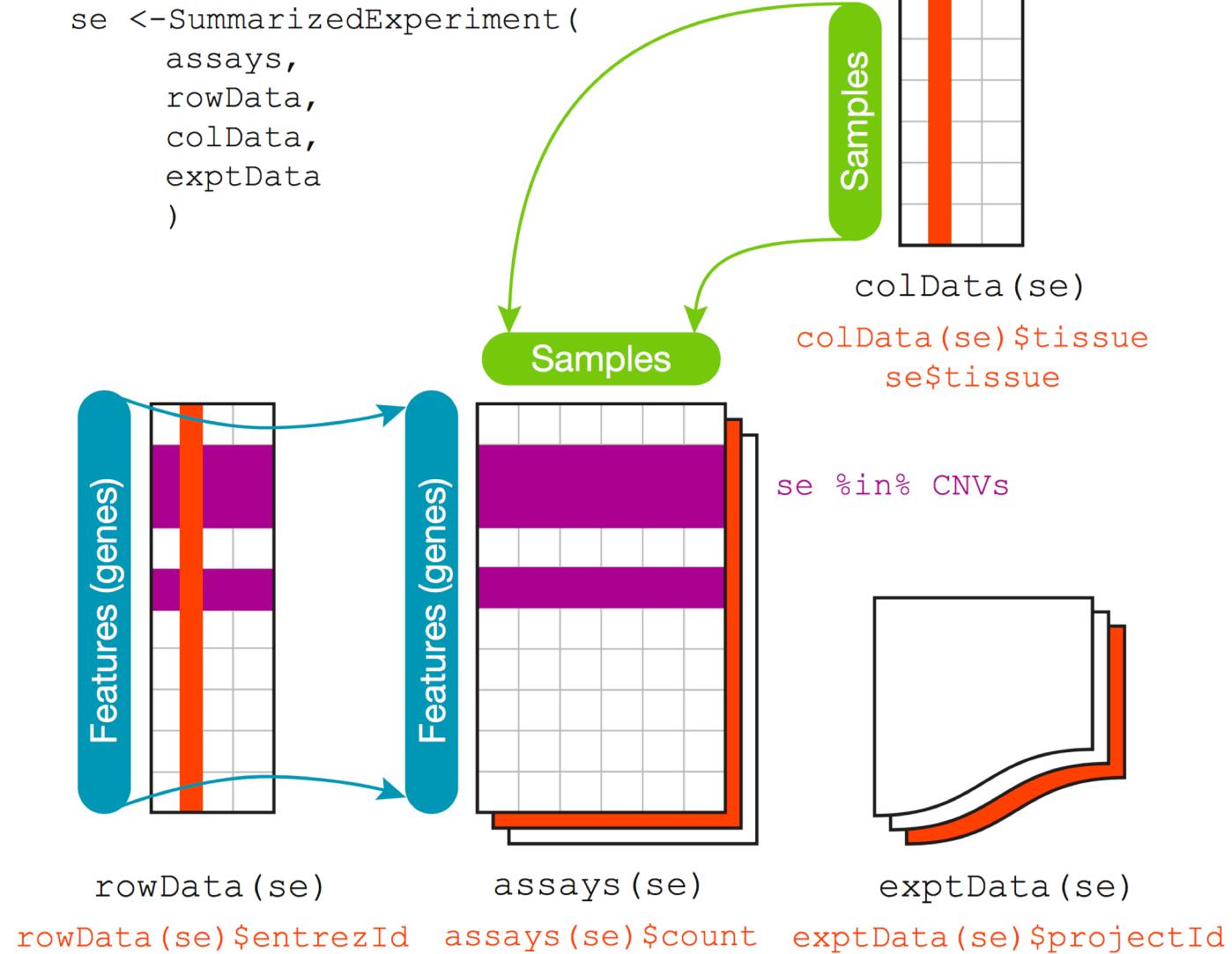


- “Cell type” isn’t always clear-cut
- Overlapping transcriptional clusters
- When is a cluster a new subtype vs arbitrary splitting
- Can rely on expert knowledge and manual designation
- Necessary in order to perform diffExp or differential abundance

Single Cell Toolkit

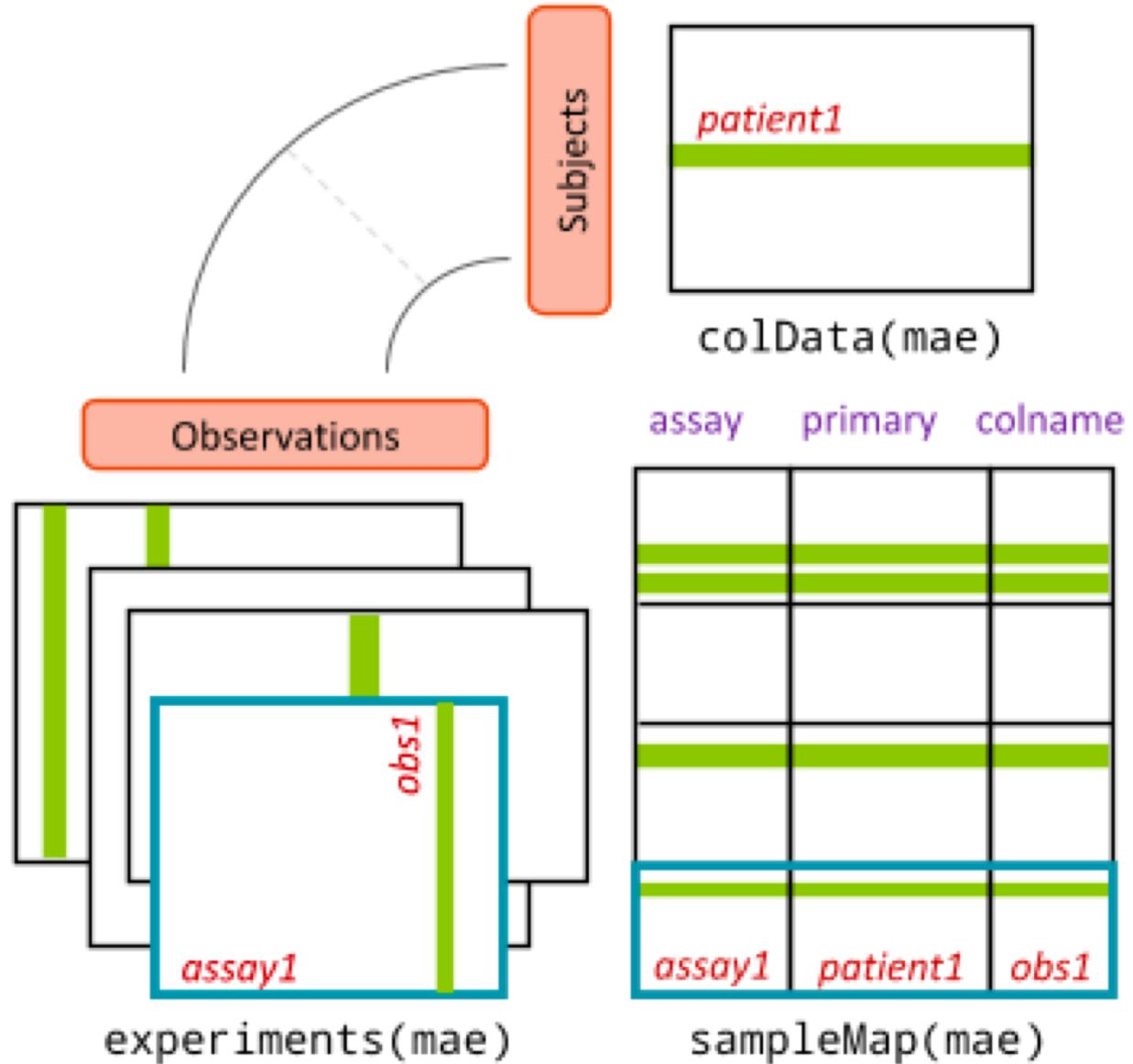
- Standard R package *with* a Shiny toolkit
- R functions on top of a SingleCellExperiment (SCE) object
 - Can download analysis performed in the toolkit and continue on the command line
- SCE object can be brought in/out of Shiny at any stage
 - Great for common tasks:
 - Interactive clustering/visualization
 - Differential gene/pathway analysis
- (Also works for bulk RNA-seq analysis)

SummarizedExperiment



MultiAssayExperiment

- Combine multiple data types in similar objects with functionality to query across features and cells.

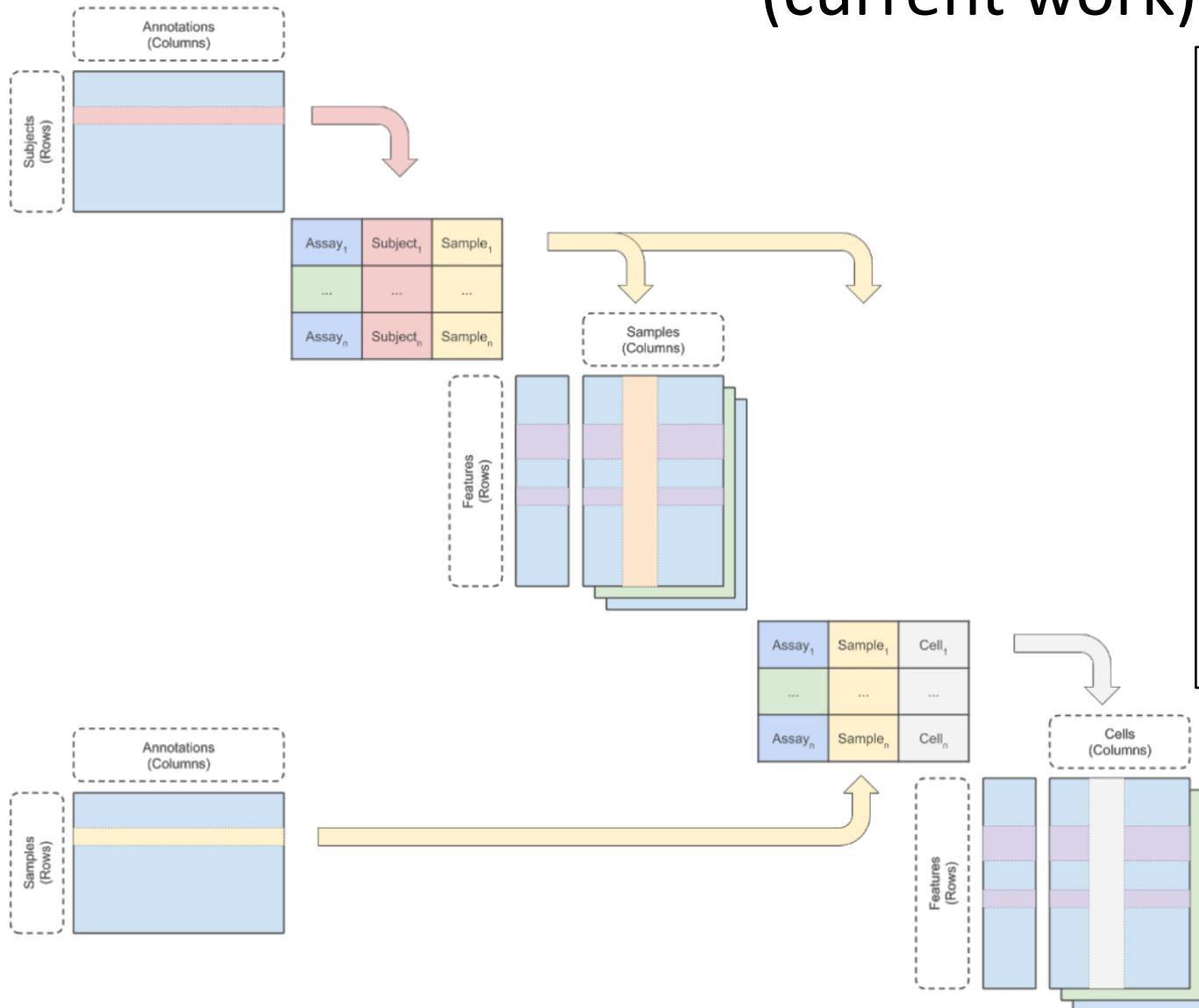


SingleCellExperiment

- <https://bioconductor.org/packages/devel/bioc/html/SingleCellExperiment.html>
- reducedDims
 - PCA, TSNE, any other sample size matrix of dimensionality reduction data
- isSpike – indicate which probes are spike ins
- sizeFactors – add scaling factors
- Named assays: counts, normcounts, logcounts, cpm, tpm
 - Just a convention
- DelayedArray – run common array functions without loading it into memory

CoAssayExperiment

(current work)



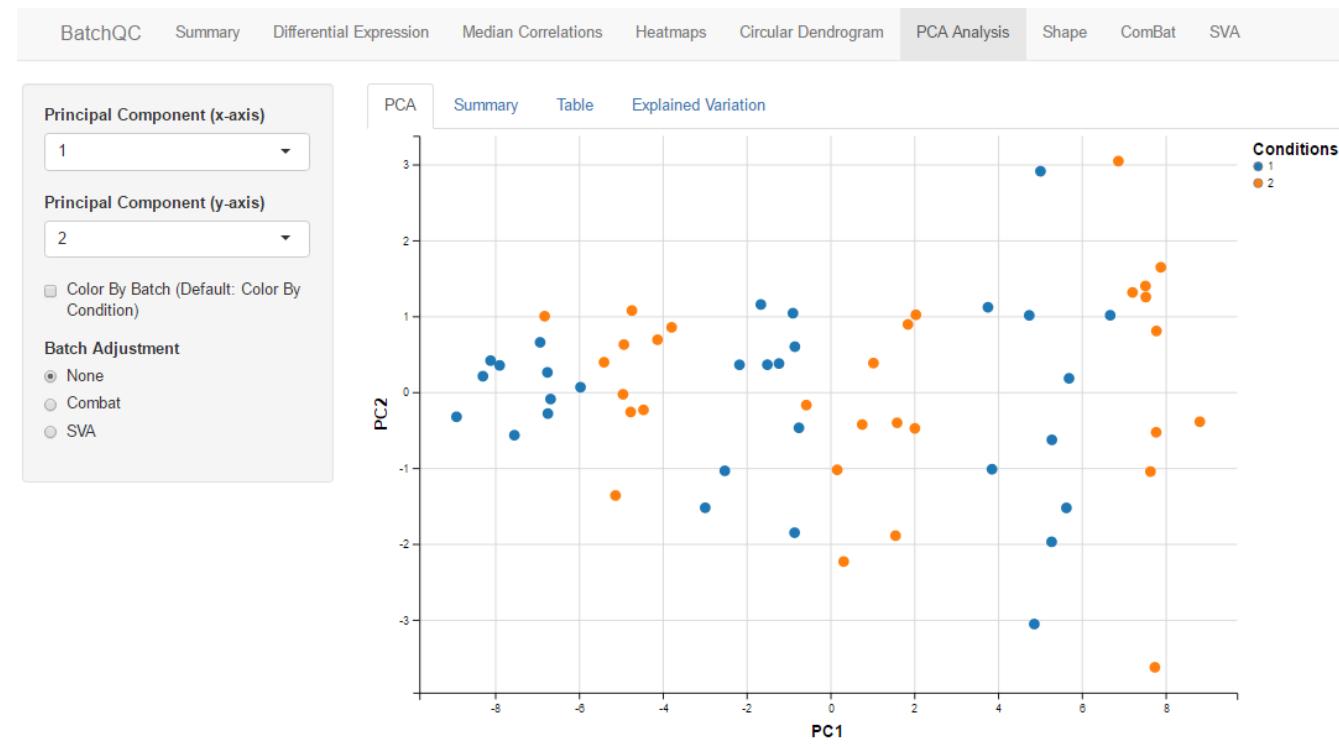
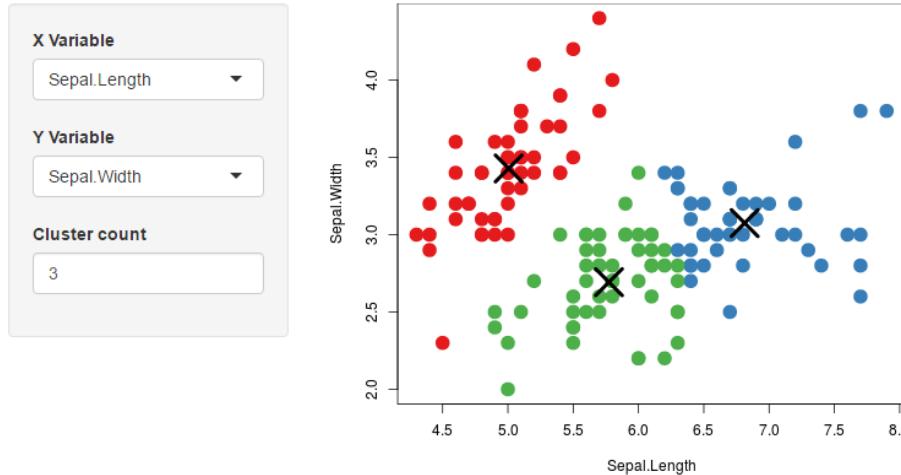
Possible Data:

- Single measure for each subject
 - Treatment, condition, sex, survival
- Bulk Genomic assays
 - Bulk RNA-seq, DNA-seq, epigenetics, proteomics
- Single cell assays (different cells)
 - scRNA-seq, scDNA-seq, scATAC-seq
- Single cell assay (same cells)
 - scRNA-seq, CITE-seq

Shiny

- Simple Web Apps Written in R
- Interactive and Reactive
- Customizable, Modular

Iris k-means clustering



SingleCellITK

Data Upload

QC and Filtering

Visualization and
Clustering

ComBat Batch
Correction

Differential
Expression

MAST

Pathway Activity
Analysis

Experimental
Design

Data Export