GRADUATE SCHOOL Life Sciences and Health

MASTER Biologie-Santé Life Sciences and Health

Genetics Genomics Epigenetics Evolution GenE2

Teaching unit "non-coding RNAs and epigenetics"

Single cell omics in oncology

Justine Marsolier

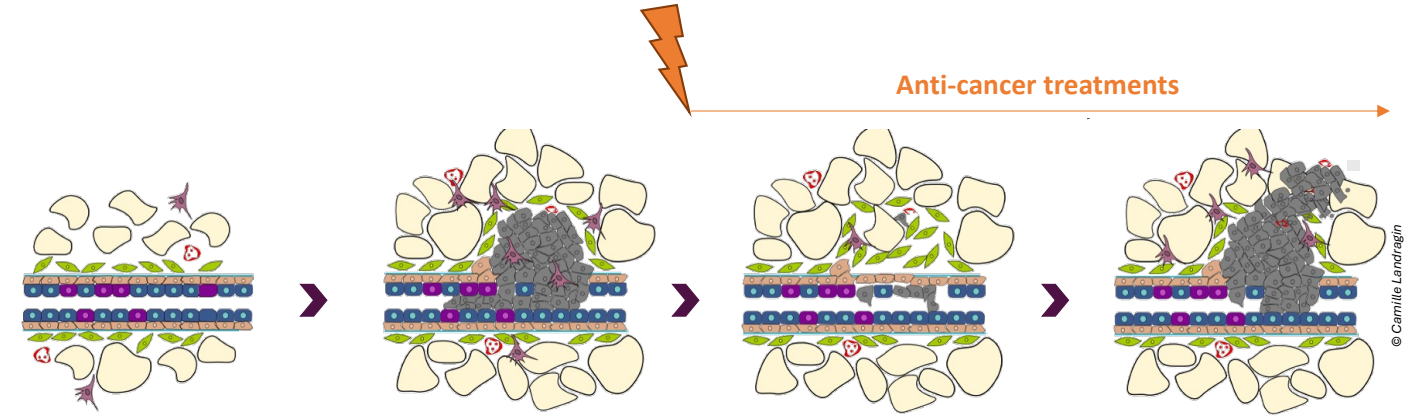
Group Dynamic of Epigenetic Alterations in Cancer

Céline Vallot

Translational Department & UMR3244
Institut Curie, Paris, France

2025

Tumor evolution is a multi-step process

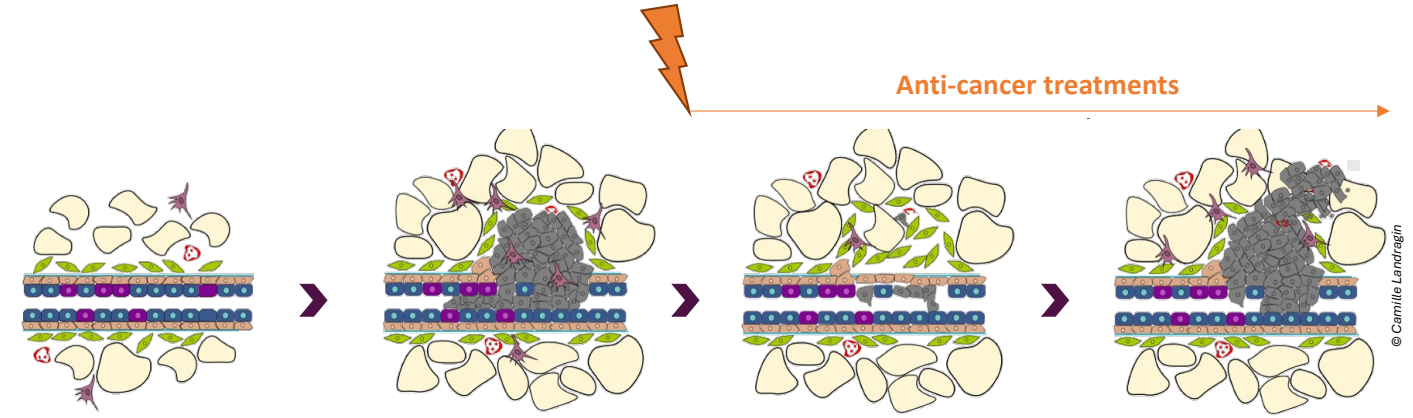


Initiation/Transformation

Tumor Progression

Response & Resistance

Plasticity of the cells during tumor evolution



Initiation/Transformation

Tumor Progression

Response & Resistance

Mutation, Insertion, Deletion

Transcriptome

DNA methylation

Histones modifications

Genetic alterations

Non-genetic alterations

LETTER

doi:10.1038/nature22794

Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance

Sydney M. Shaffer[1,2], Margaret C. Dunagin[1], Stefan R. Torborg[1,3], Eduardo A. Torre[1,2], Benjamin Emert[2,4], Clemens Krepler[5], Marilda Beqiri[5], Katrin Sproesser[5], Patricia A. Brafford[5], Min Xiao[5], Elliott Eggan[2], Ioannis N. Anastopoulos[2], Cesar A. Vargas-Garcia[6], Abhyudai Singh[6,7], Katherine L. Nathanson[2], Meenhard Herlyn[5] & Arjun Raj[1,8]

Model: Melanoma (V600E mutation - BRAF protein)

Therapy: Vemurafenib = BRAF inhibitor (inhibits the mutated BRAF protein)

-> a small subset of cancer cells develop drug resistance

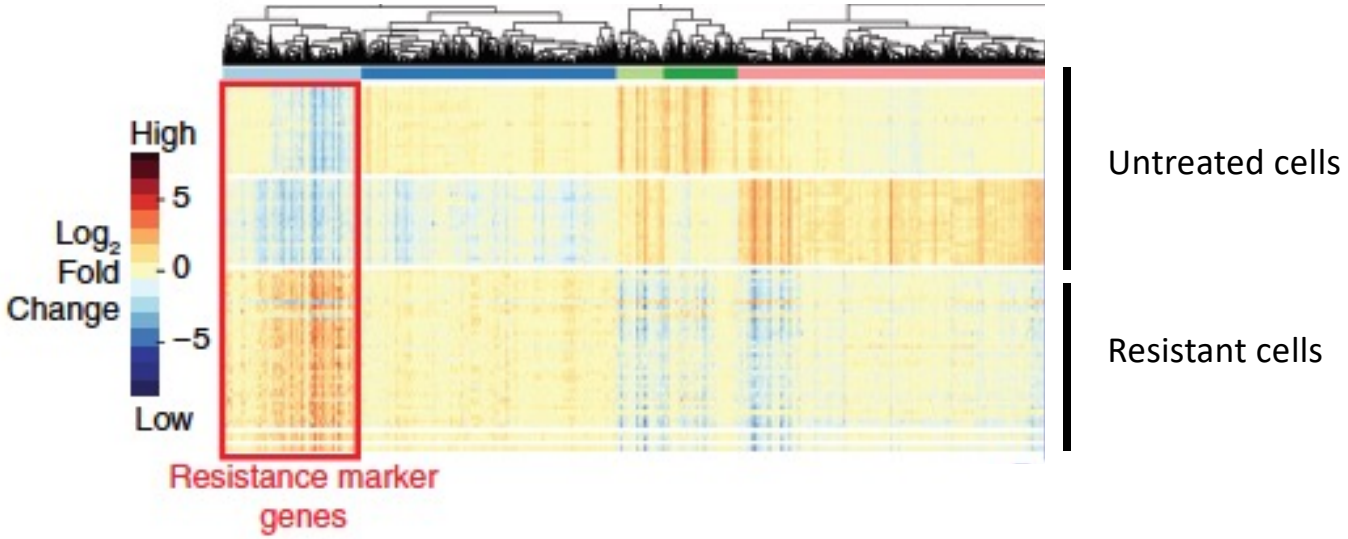
Objectif: To understand resistance at the single-cell level (cultured patient-derived melanoma cells)

Hypothesis: Single-cell gene expression differences marked pre-resistant cells in the population before treatment.

Expression of marker genes increased in resistant cells

Transcriptomic profiles (bulk-RNA-seq)

Heatmap representation



WNT5A, AXL, EGFR, PDGFRB, and JUN.

Shaffer et al., 2017

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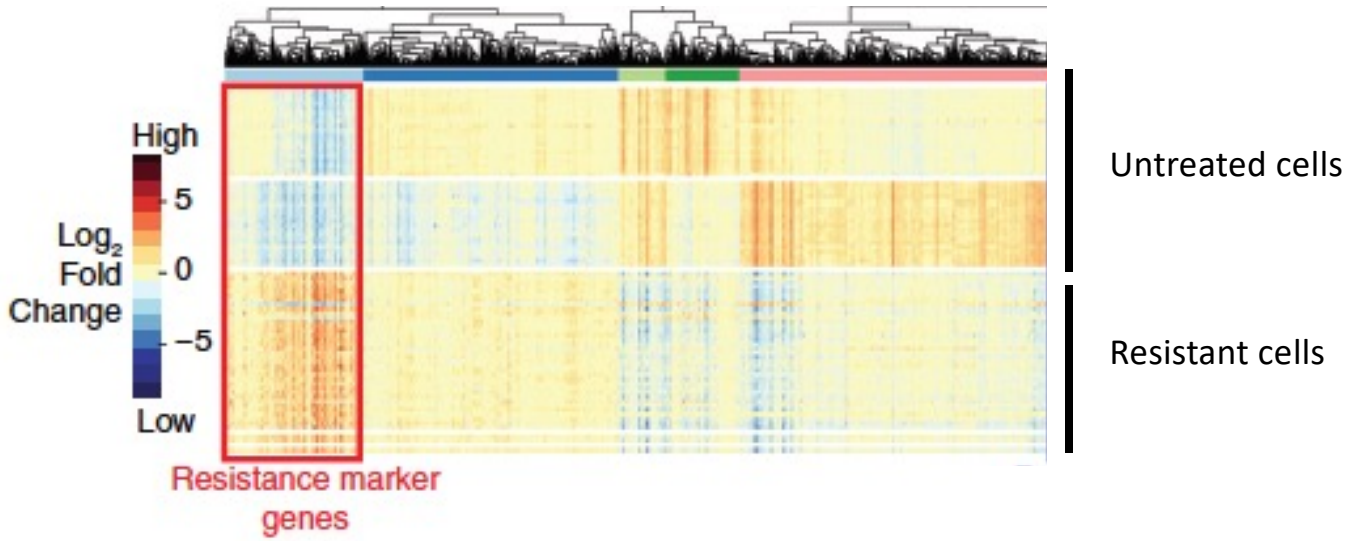


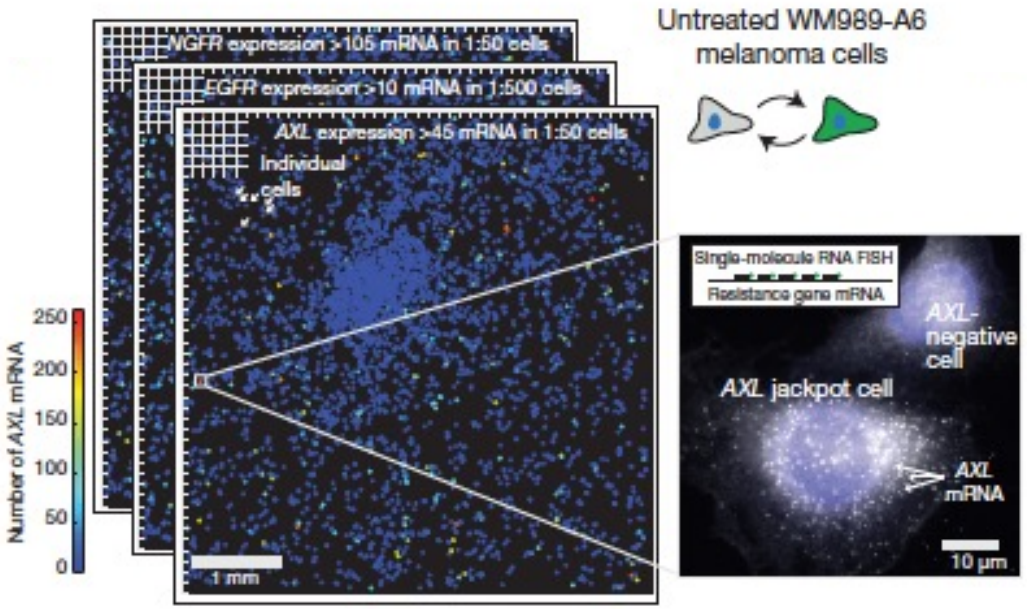
WNT5A, AXL, EGFR, PDGFRB, and JUN.

Can the low average expression of these markers in untreated cells mask rare individual cells with high expression for these resistant markers ?

Shaffer et al., 2017

A population of rare cells expressed resistance genes at high levels before drug exposure

High-throughput single-molecule RNA FISH



Shaffer et al., 2017

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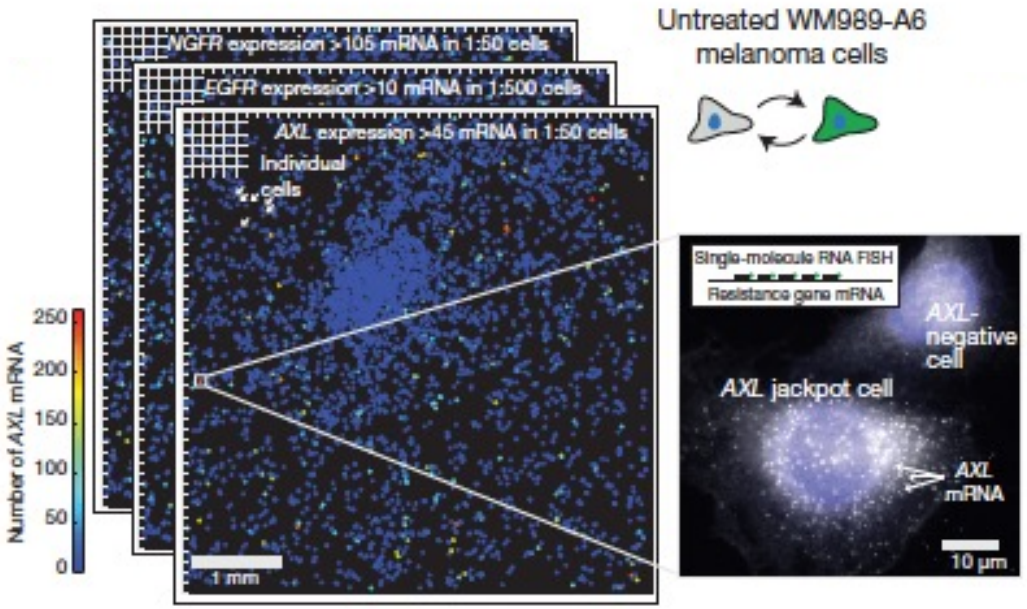
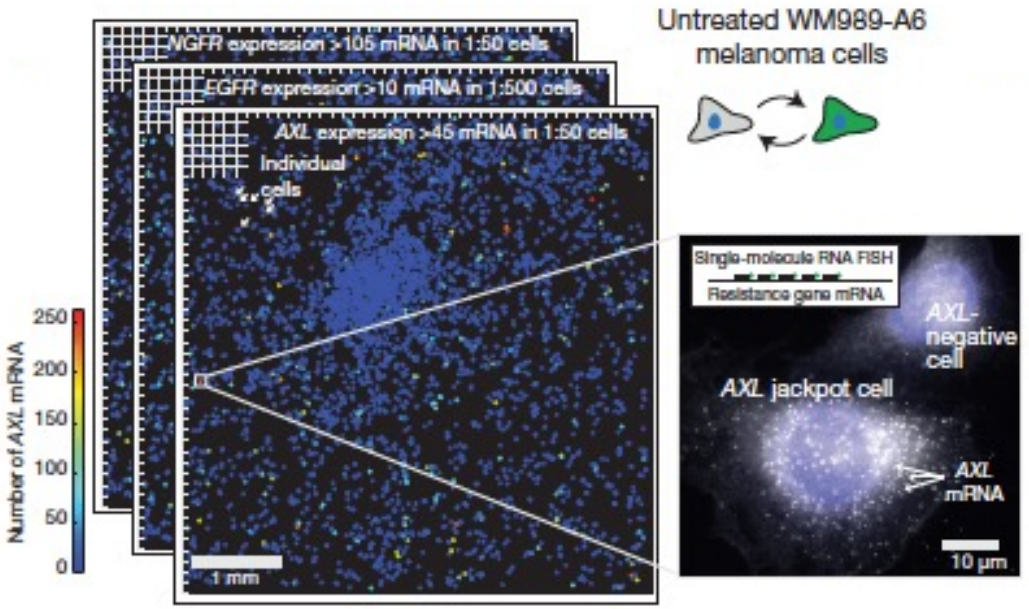


After 4 weeks of treatment with vemurafenib
= resistant colonies expressed these markers at more uniformly high levels

Shaffer et al., 2017

A population of rare cells expressed resistance genes at high levels before drug exposure

High-throughput single-molecule RNA FISH

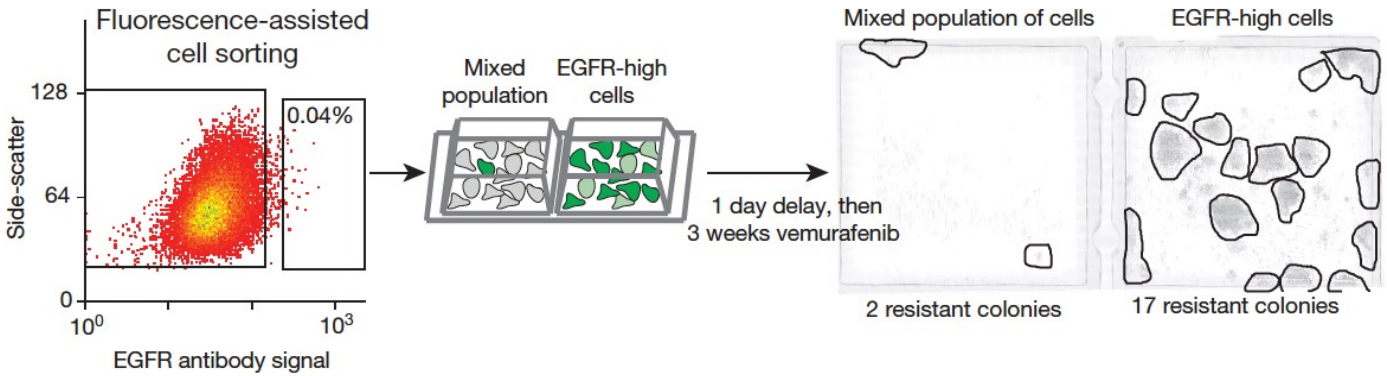


Did sporadic marker gene expression marked untreated cells that ultimately become resistant after drug exposure ?

Shaffer et al., 2017

EGFR-high cells produced more resistant colonies than EGFR-mixed cells

Fluorescence-activated cell sorting of EGFR expressing cells – 3 weeks of treatment



Shaffer et al., 2017

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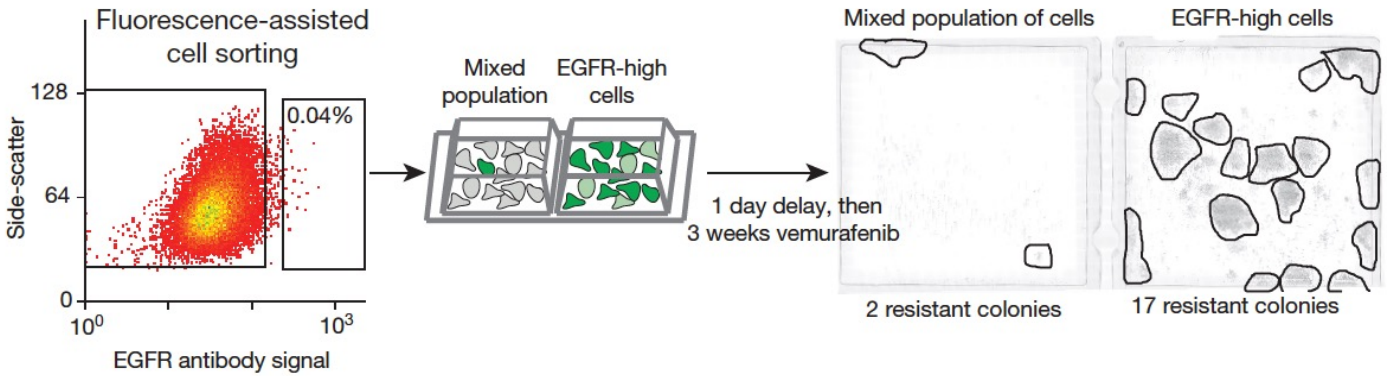


Human melanoma cells can display transcriptional variability at the single cell level

(i.e. infrequent, semi-coordinated transcription of a limited number of resistance markers at high levels in a very small percentage of cells in the initial population = jackpot cells)

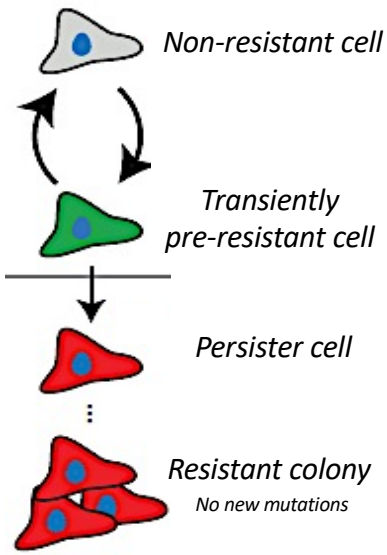
=

Prediction of which cells will ultimately resist drug treatment.

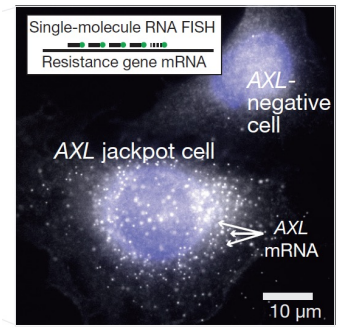
Shaffer et al., 2017

Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance

Melanoma and BRAFi targeted therapy



Pre-resistant cells *= cells that give rise to resistant colonies upon addition of drug.*



Rare cell diversity = **HETEROGENEITY** of the initial population

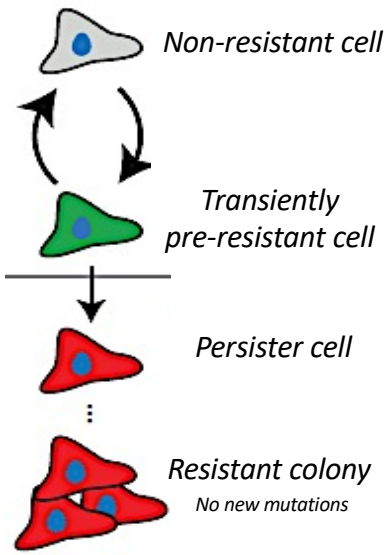
+

Acquisition of new transcriptomic features = **PLASTICITY**

Shaffer et al., 2017

Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance

Melanoma and BRAFi targeted therapy



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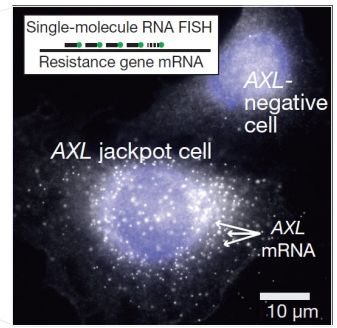
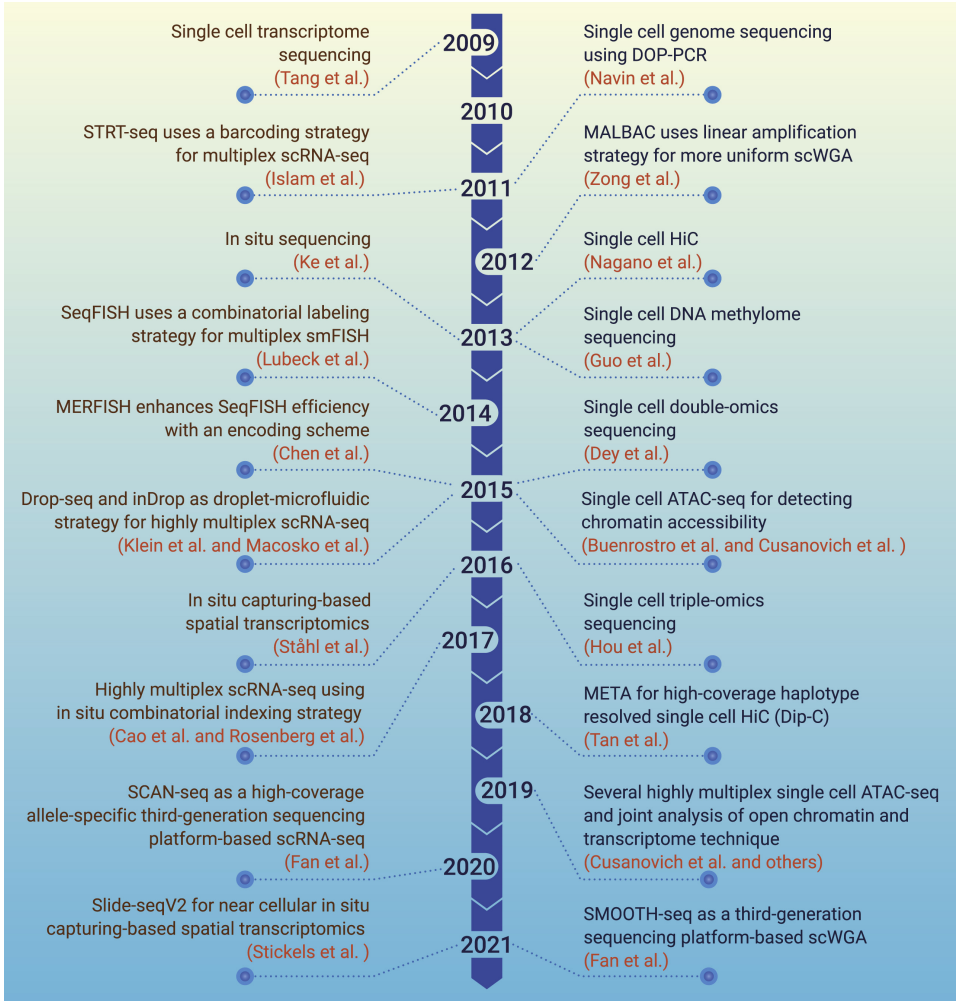


RNA-FISH ~ 1000 cells

How can we studied non-genetic alterations on several thousands of cells at the same time, at the single cell level to study heterogeneity and detect rare cell subpopulations ?

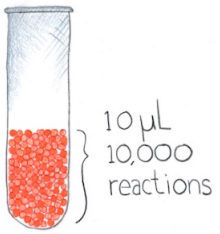
Shaffer et al., 2017

Single-cell transcriptomic technologies : A rapidly-evolving field



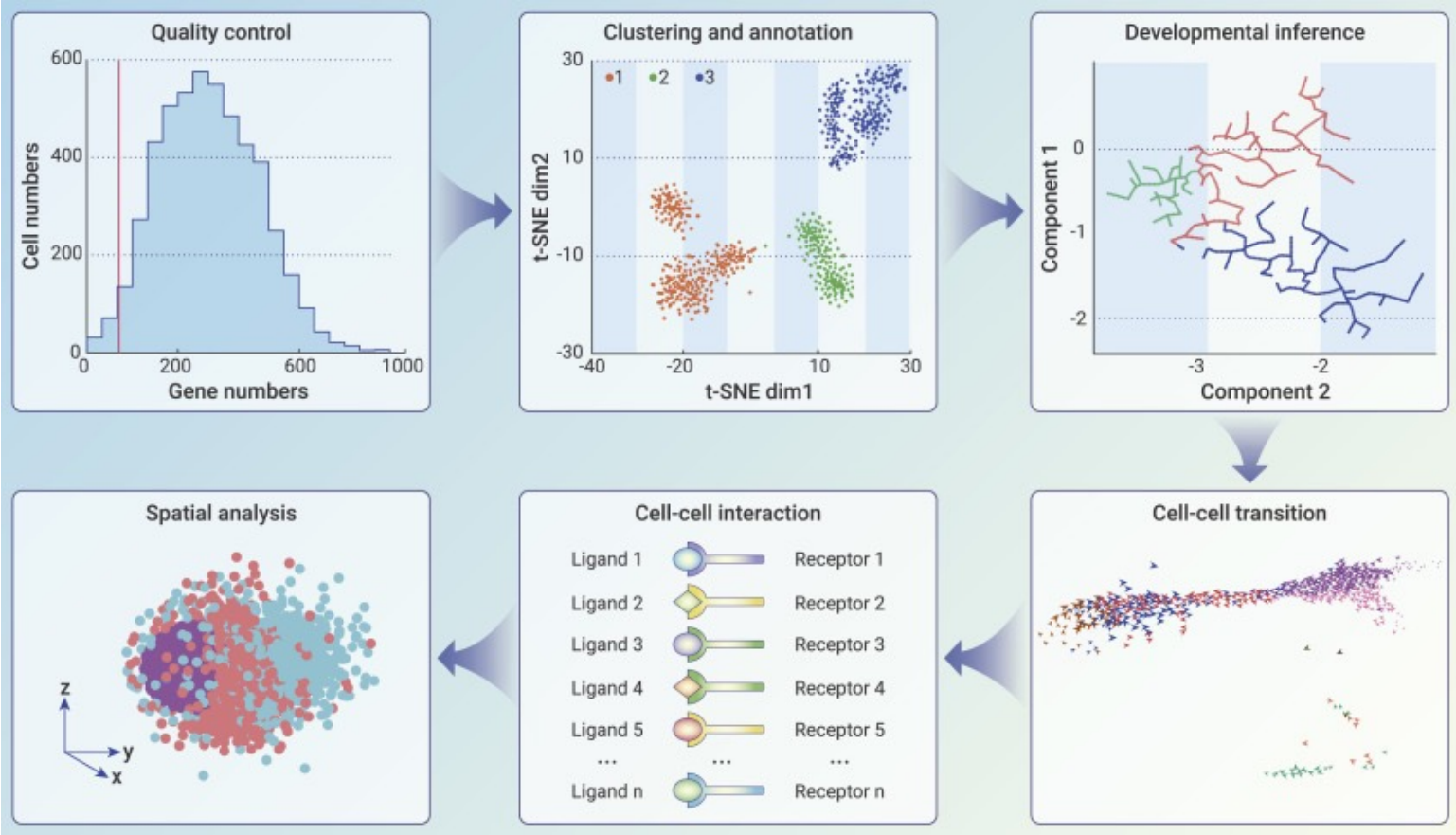
Weng et al.,2022

How to make reactions with one cell?

FACS	Droplet micro-fluidics
	
96 or 384 cells	1000 to 50,000 cells and more
Reaction volume ~1-2µl	~150pl
High gene coverage	High cell coverage

10000 X

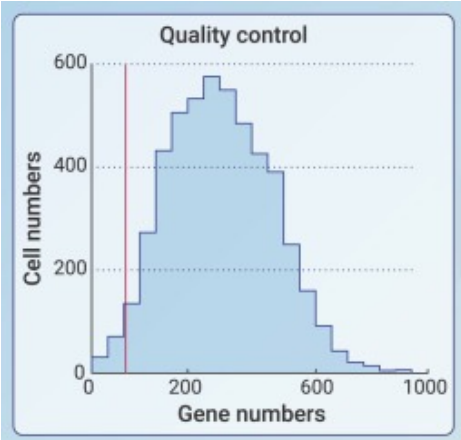
Bioinformatics analysis of single-cell sequencing data



Algorithm and software development in bioinformatics are critical to translate scRNA-seq data into biological and medical data and further applications.

Weng et al.,2022

1- Quality Control



Limited RNA content of single cells
+ the stochastic nature of current scRNA-seq techniques

↓

mRNAs within one cell cannot be fully captured and sequenced

<u>Indices for quality control during scRNA-seq data analysis:</u>

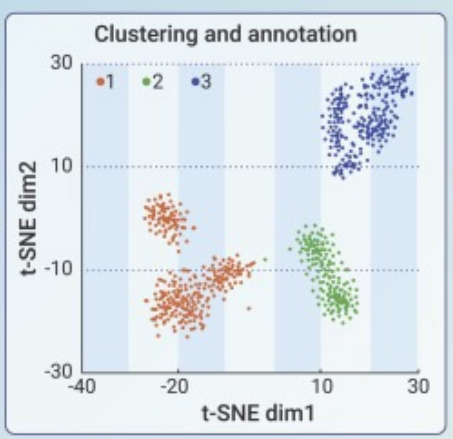
- Low or high gene numbers
- Percentage of mitochondrial genes

The aim of quality control
= to control the rates of empty cells, doublets or multiplets, and cells with bad states.

-> such quality control metrics vary across different biological specimens, and thus no consistent cutoffs exist.

Weng et al.,2022

2 – Visualization, clustering and cell-type annotation



<u>Critical task of scRNA-seq data analysis:</u>
Visualization of cell clusters and their mutual relationships.

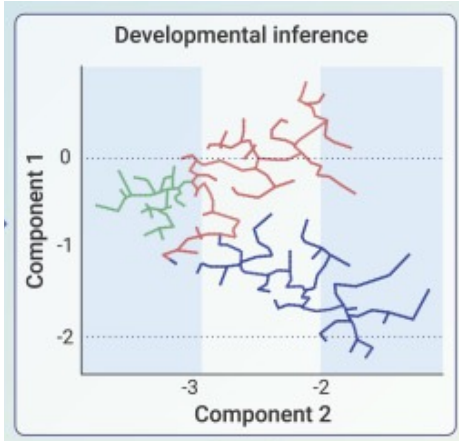
PCA - principal-component analysis
T-SNE - t-distributed stochastic neighbor embedding
UMAP - Uniform Manifold Approximation and Projection

After clustering, the cell type of each cluster can be predicted:

- with artificial intelligence-based methods (SingleR),
- with cell marker-based methods (CellAssign).

Weng et al.,2022

3 - Trajectory analysis



Developmental inference = cells along a specific developmental trajectory were sampled by scRNA-seq and thus could be reconstructed via examining the similarity of cellular expression profiles.

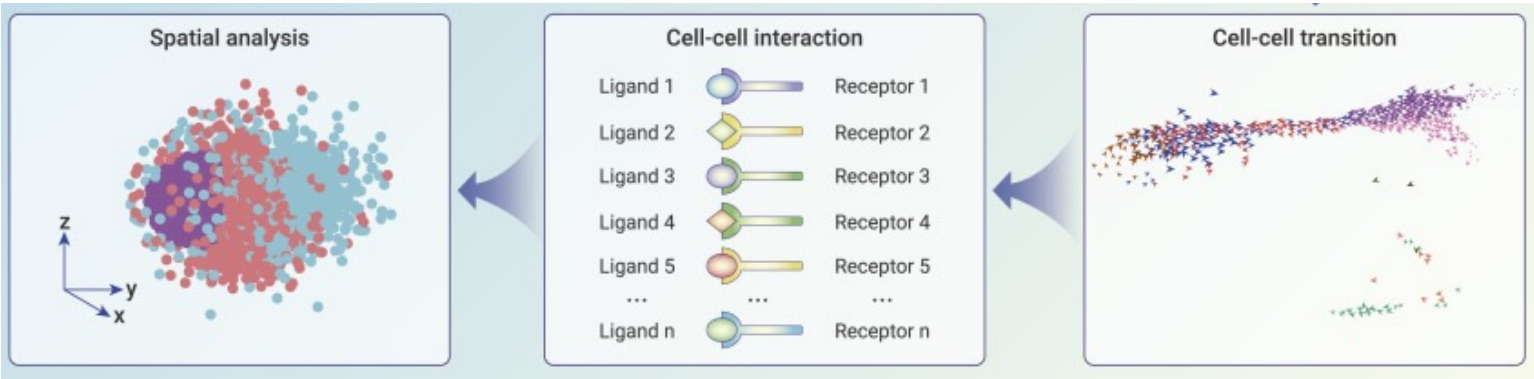
Monocle infers cellular developmental trajectories based on gene expression similarity.

RNA velocity derives the probability and direction of cell-to-cell transitions.

Results are predictive and validations are needed to confirm the predictions.

Weng et al.,2022

4 - Single-cell regulatory network & Cell-cell communication



To construct the single-cell regulatory network:

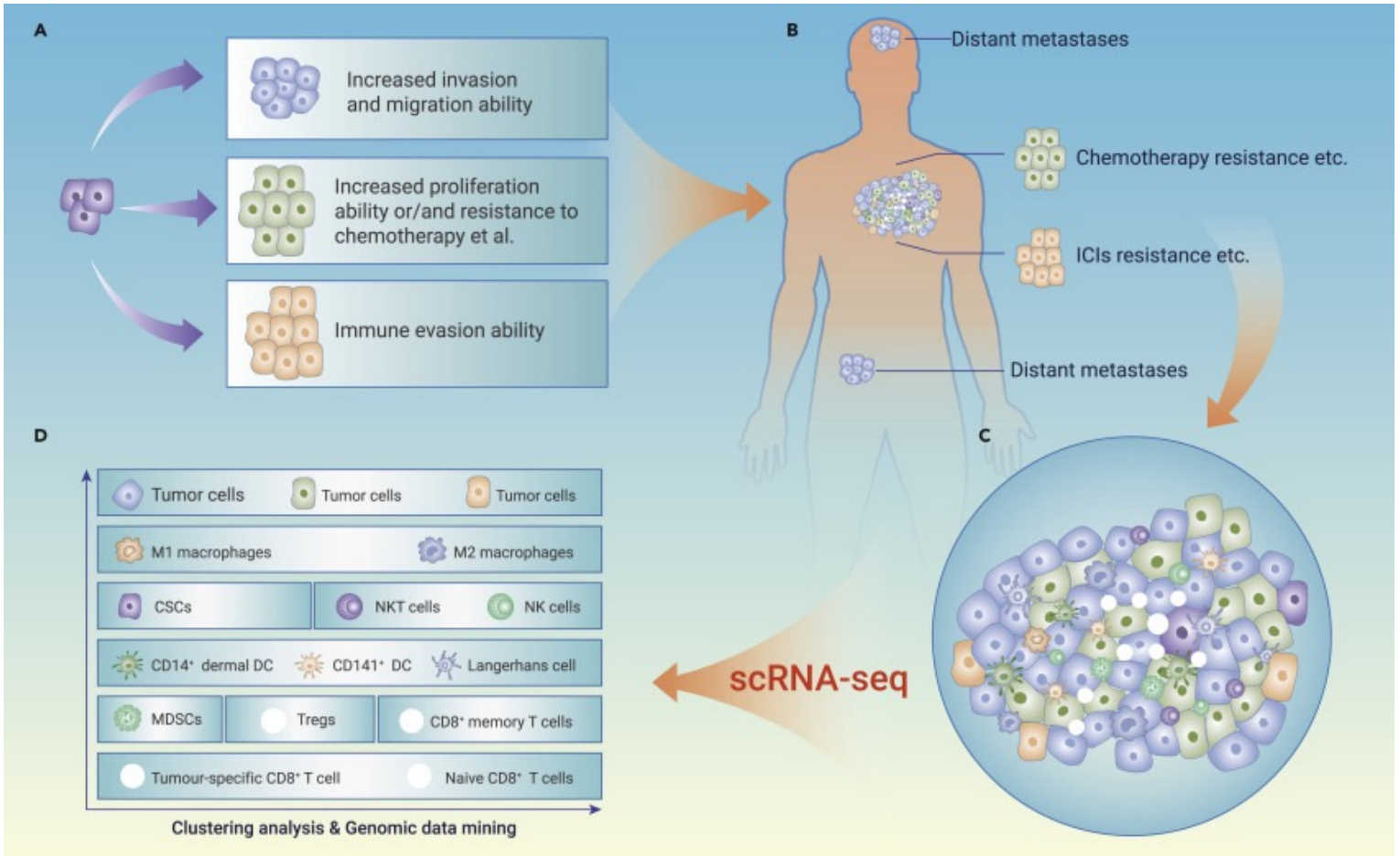
- SCENIC conducts regulon analysis based on scRNA-seq data and can provide quantitative analysis of the roles of diverse transcription factors in shaping the observed scRNA-seq data.
- NicheNet compiles a ligand-target database and tries to prioritize those extrinsic factors critical to the observed gene expression profiles.

To interrogate the potential cell-to-cell crosstalks and even spatial relationships:

- CellphoneDB and CSOmap are two typical methods of the ligand-receptor interaction-dependent cell-cell communication algorithms.
- NovoSpaRc derives cellular spatial relationships only based on scRNA-seq data without ligand-receptor interactions (Cells with similar gene expression profiles should have a high probability to share spatial niches).

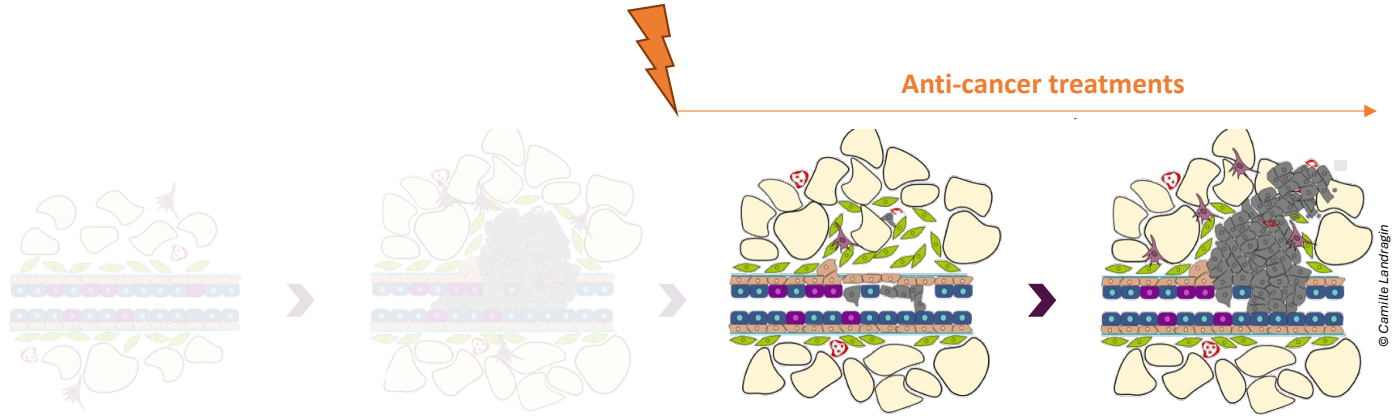
Weng et al.,2022

The applications of sc technologies in oncology



Weng et al.,2022

Plasticity of the cells during tumor evolution

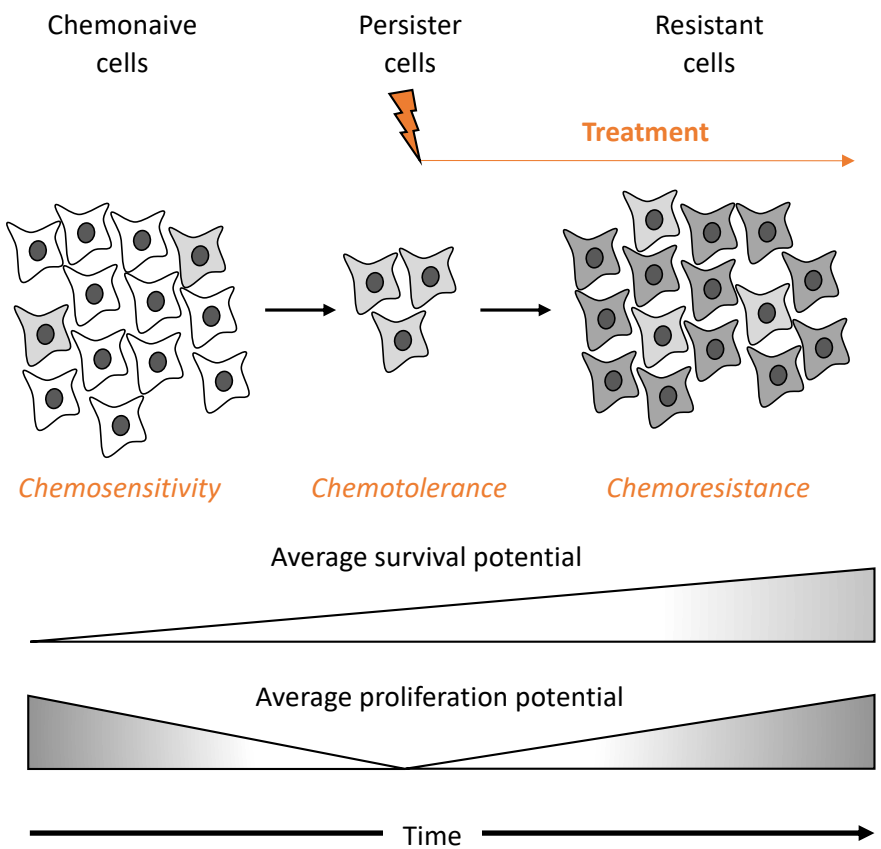


Initiation/Transformation

Progression Tumorale

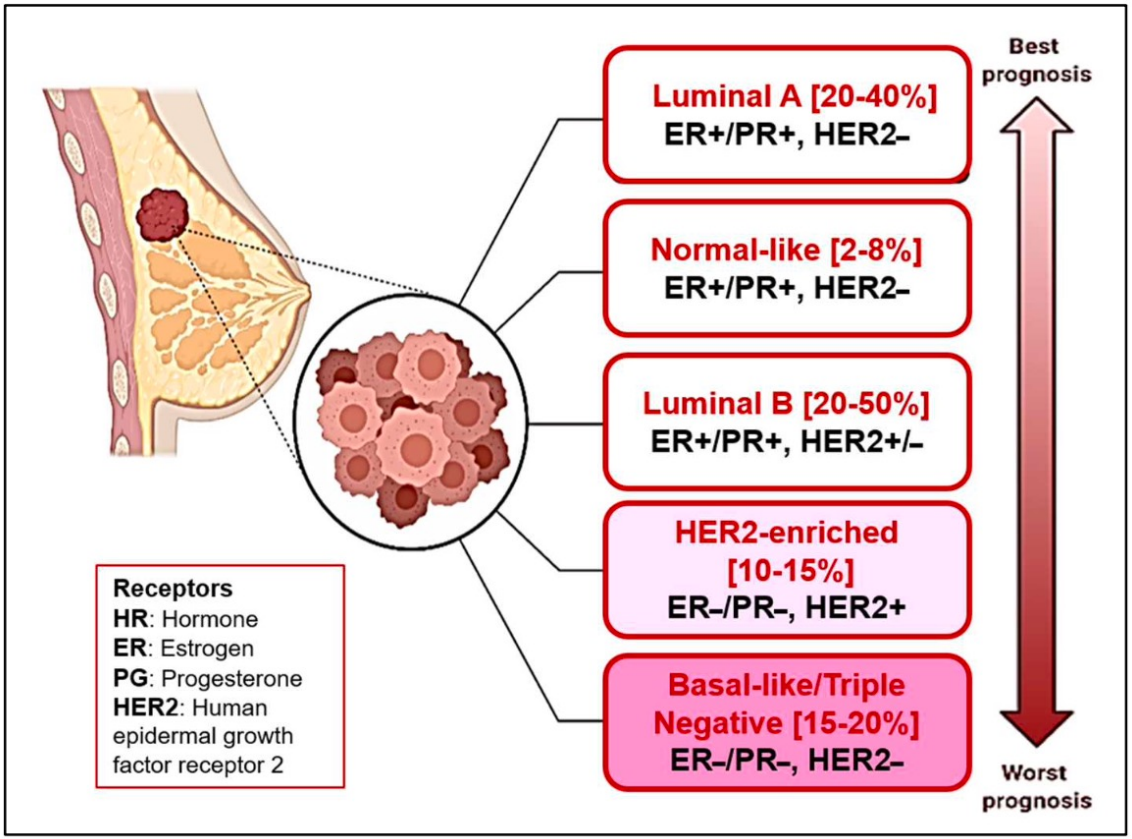
Response & Resistance

Drug persister cells constitute a reservoir of cells from which drug resistant cells will emerge



Adapted from Chisholm et al., 2015

Clinical setting: Triple Negative Breast Cancers



Drug resistance develops following use of chemotherapies

Alalhareth et al., 2025

Chemoresistance Evolution in Triple-Negative Breast Cancer Delineated by Single-Cell Sequencing

Charissa Kim,[1,2,6] Ruli Gao,[1,6] Emi Sei,[1] Rachel Brandt,[1] Johan Hartman,[3] Thomas Hatschek,[3] Nicola Crosetto,[4] Theodoros Foukakis,[3,*] and Nicholas E. Navin[1,2,5,7,*]

[1]Department of Genetics, UT MD Anderson Cancer Center, Houston, TX 77030, USA
[2]Graduate School of Biological Sciences, UT MD Anderson Cancer Center, Houston, TX 77030, USA
[3]Department of Oncology-Pathology, Karolinska Institute, SE-17176 Stockholm, Sweden
[4]Department of Medical Biochemistry and Biophysics, Karolinska Institute, SE-17177 Stockholm, Sweden
[5]Department of Bioinformatics and Computational Biology, UT MD Anderson Cancer Center, Houston, TX 77030, USA
[6]These authors contributed equally
[7]Lead Contact
*Correspondence: theodoros.foukakis@ki.se (T.F.), nnavin@mdanderson.org (N.E.N.)
https://doi.org/10.1016/j.cell.2018.03.041

Model: TNBC

Therapy: Neo-adjuvant chemotherapy

An unresolved question:

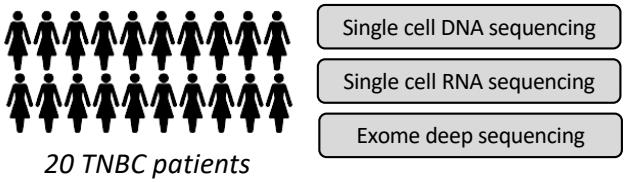
Chemoresistance -> selection and expansion of rare pre-existing subclones (adaptive resistance)
-> induction of new mutations that confer a chemoresistant phenotype (acquired resistance)

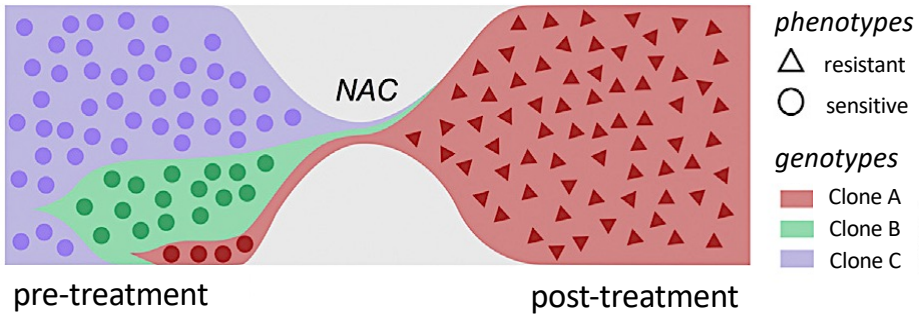
Author's hypothesis:

Genomic aberrations associated with chemoresistance are pre-existing in the tumor mass and adaptively selected in response to chemotherapy.

Chemoresistance evolution in TNBC is driven by genetic & transcriptomic mechanisms through combined selective and adaptive modes of evolution

Breast cancer and NAC therapy





Adaptive genome evolution
+
Transcriptional reprogramming
= SELECTION + PLASTICITY

Kim et al., 2018

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

Resistance to neoadjuvant chemotherapy in triple-negative breast cancer mediated by a reversible drug-tolerant state

Gloria V. Echeverria[1], Zhongqi Ge[1,2], Sahil Seth[3,4,5], Xiaomei Zhang[1], Sabrina Jeter-Jones[1], Xinhui Zhou[1], Shirong Cai[1], Yizheng Tu[1], Aaron McCoy[1], Michael Peoples[4,5], Yuting Sun[4,5], Huan Qiu[6], Qing Chang[4,5], Christopher Bristow[4,5], Alessandro Carugo[4,5], Jiansu Shao[1], Xiaoyan Ma[4,5], Angela Harris[4,5], Prabhjot Mundi[7], Rosanna Lau[8], Vandhana Ramamoorthy[4,5], Yun Wu[8], Mariano J. Alvarez[7,9], Andrea Califano[7], Stacy L. Moulder[10], William F. Symmans[8], Joseph R. Marszalek[4,5], Timothy P. Heffernan[4,5], Jeffrey T. Chang[2,6], Helen Piwnica-Worms[1]*

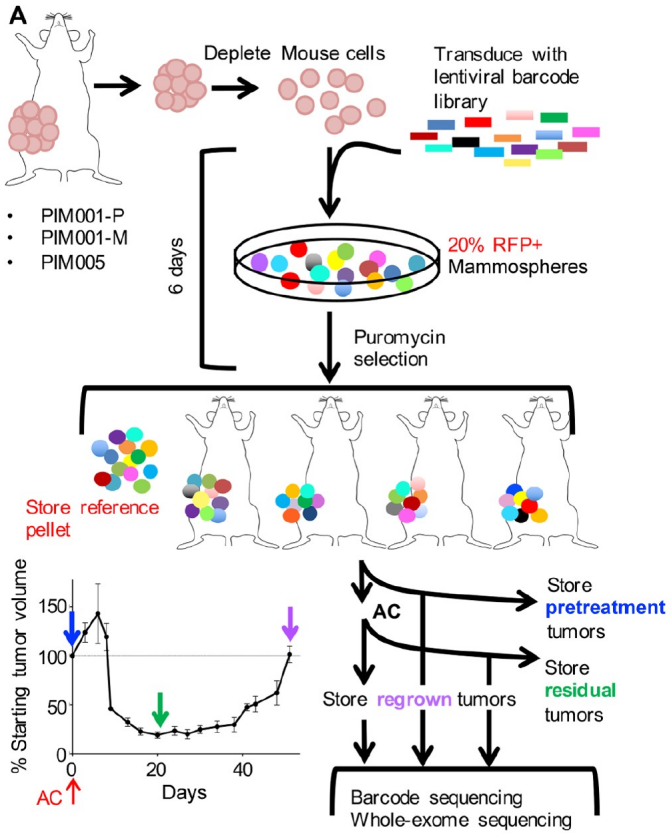
Model: TNBC

Therapy: Neo-adjuvant chemotherapy

Objectif:
To understand the earliest steps of chemoresistance

Resistance to chemotherapy in breast cancer is mediated by a reversible drug-tolerant state

Breast cancer and neo-adjuvant chemotherapy

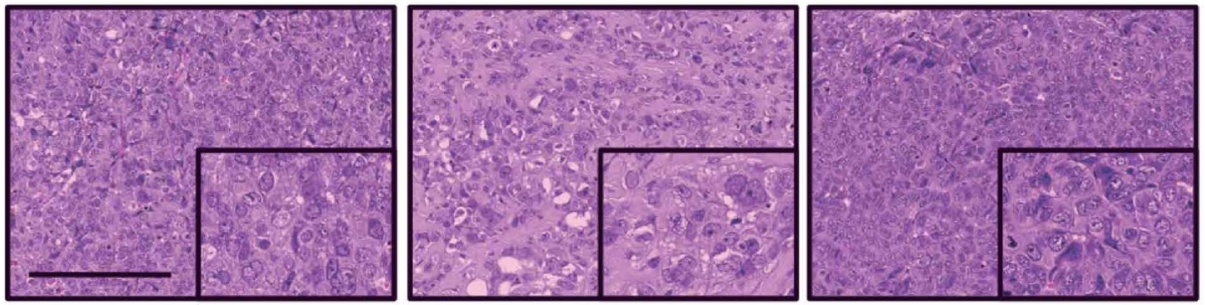


Echeverria et al., 2019

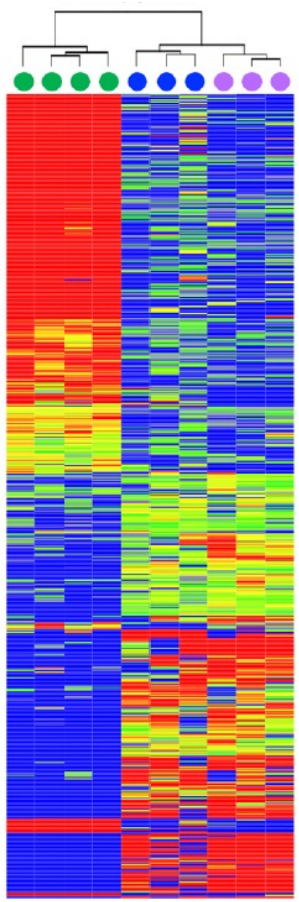
The reversible drug-tolerant state involved transcriptional reprogramming

Histological features

Chemosensitive tumor | Residual tumor | Residual tumor - regrow



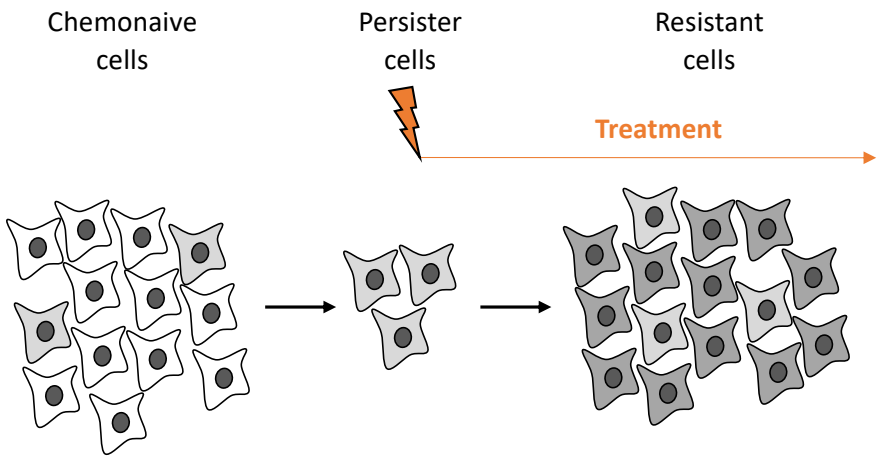
Transcriptomic profiles (bulk-RNA-seq)



Reversibility of the drug-tolerant state
+
Transcriptional reprogramming
= PLASTICITY

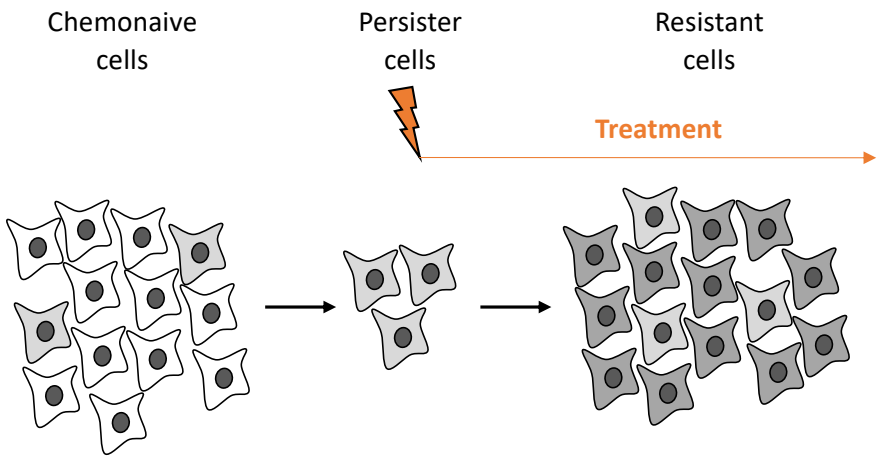
Echeverria et al., 2019

Genetic and transcriptomic mechanisms drive cancer evolution towards drug tolerance/resistance



Molecular basis of drug tolerance ?

Genetic and transcriptomic mechanisms drive cancer evolution towards drug tolerance/resistance



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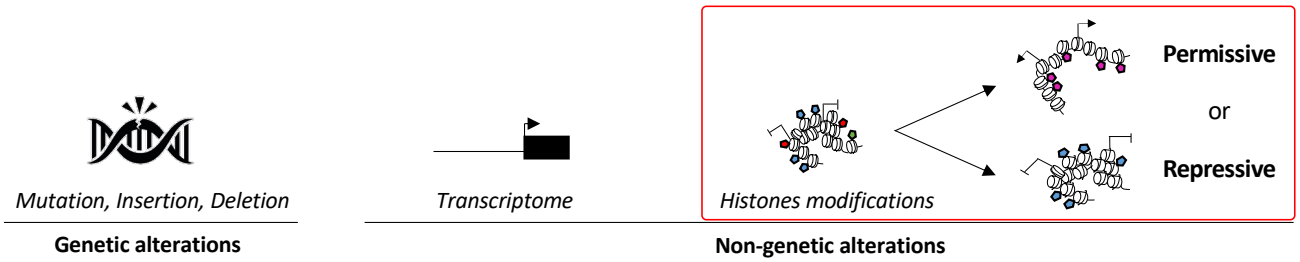
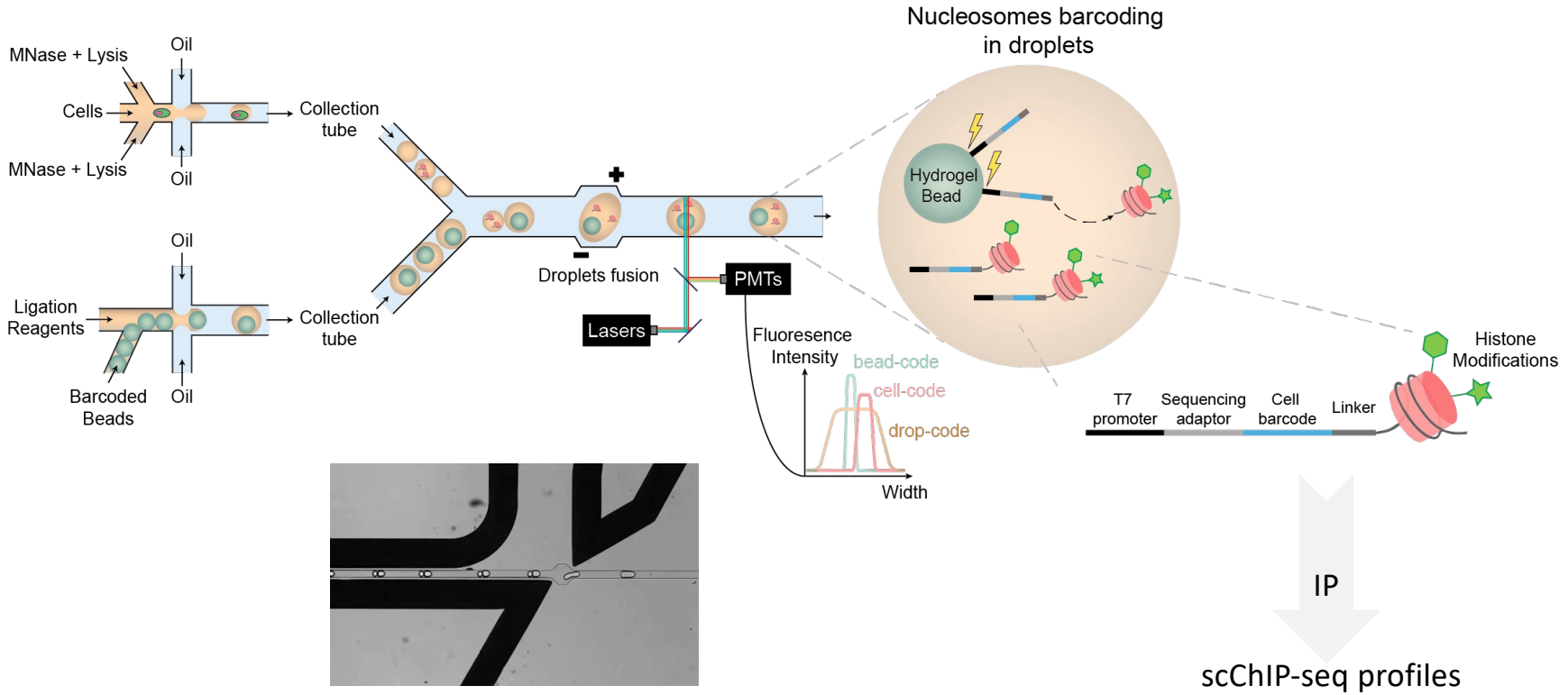


High-throughput single-cell chromatin profiling of complex biological samples

>15,000 cells



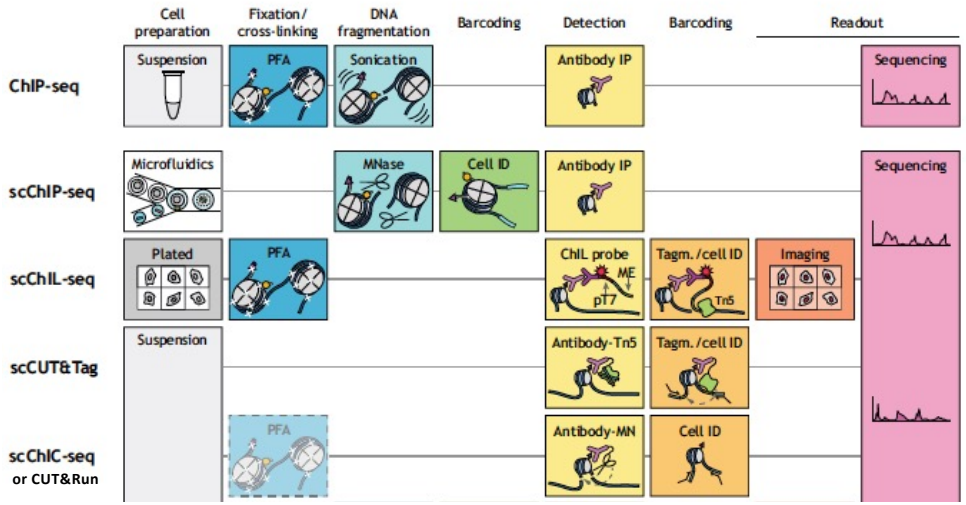
IP

scChIP-seq profiles for ~ 3,000 cells

With K. Grosselin,
A. Griffiths (ESPCI)
A. Gérard (HiFiBio)

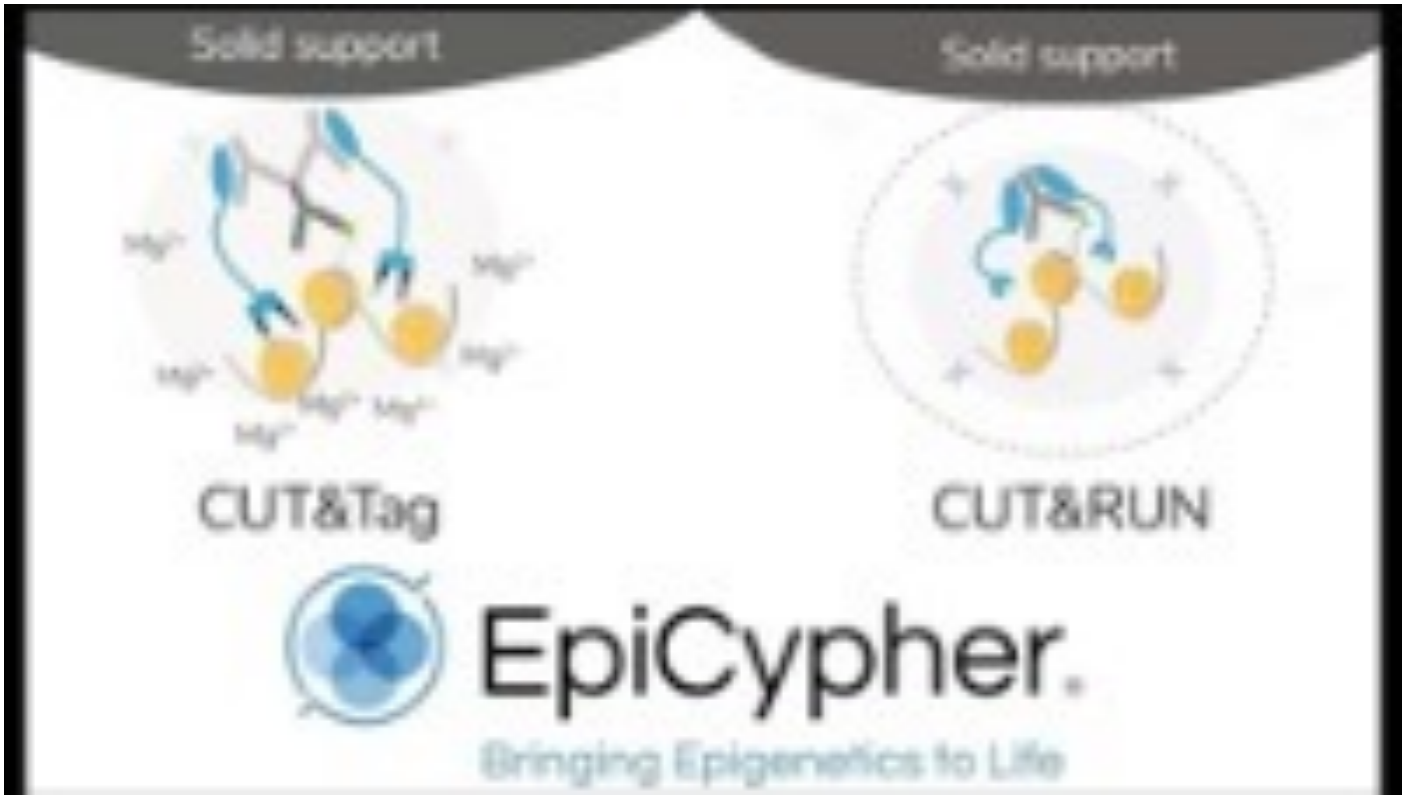
Grosselin et al., Nat Genet 2019

Mapping chromatin modifications at the single-cell level



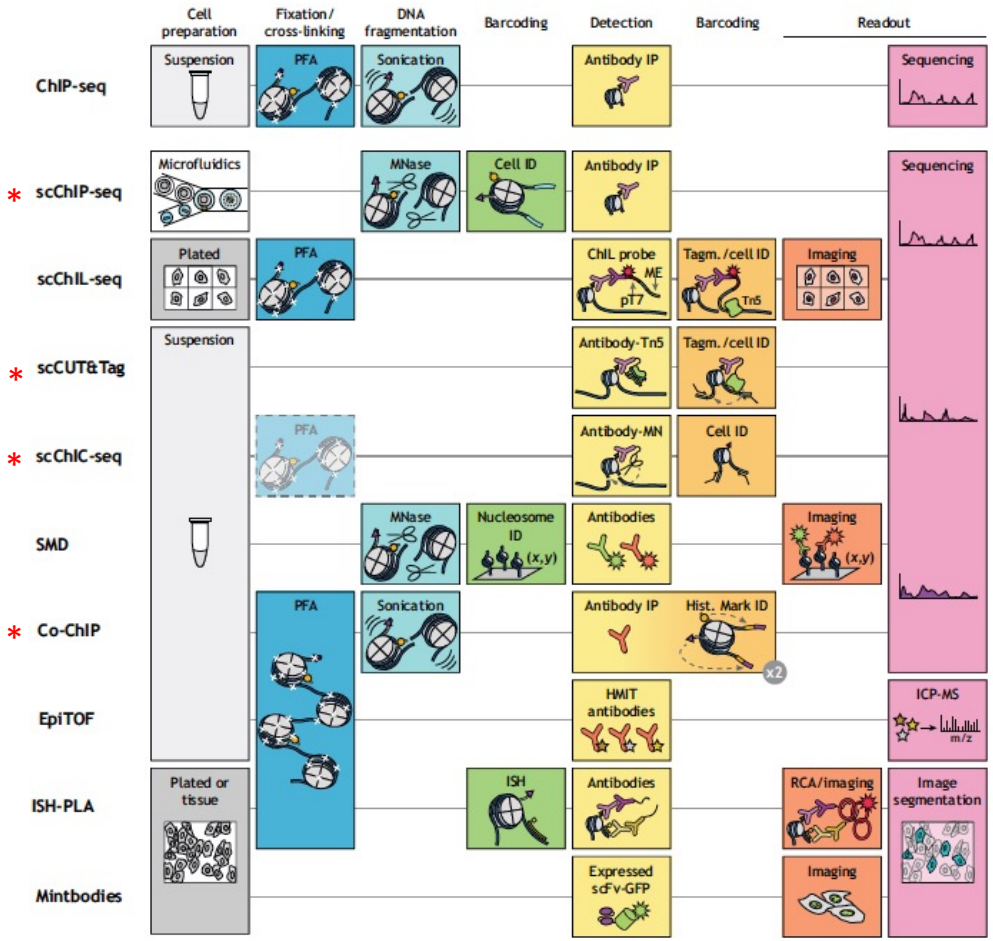
Ludwig and Bintu, 2019

CUT&Tag and CUT&Run technologies



https://youtu.be/_LxnNnlnEs8

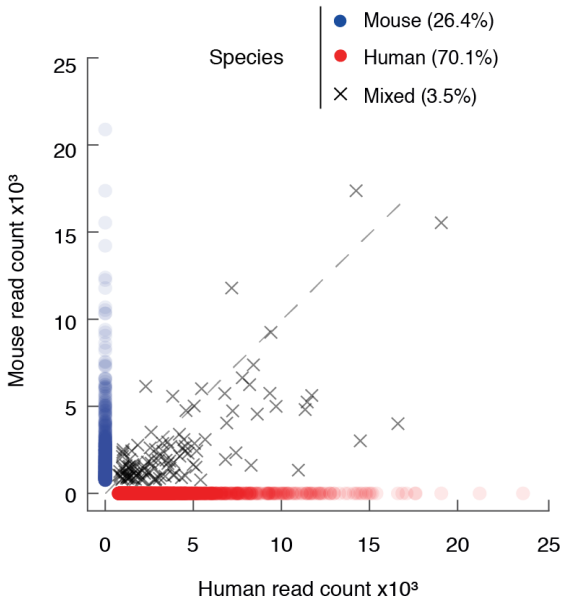
Mapping chromatin modifications at the single-cell level

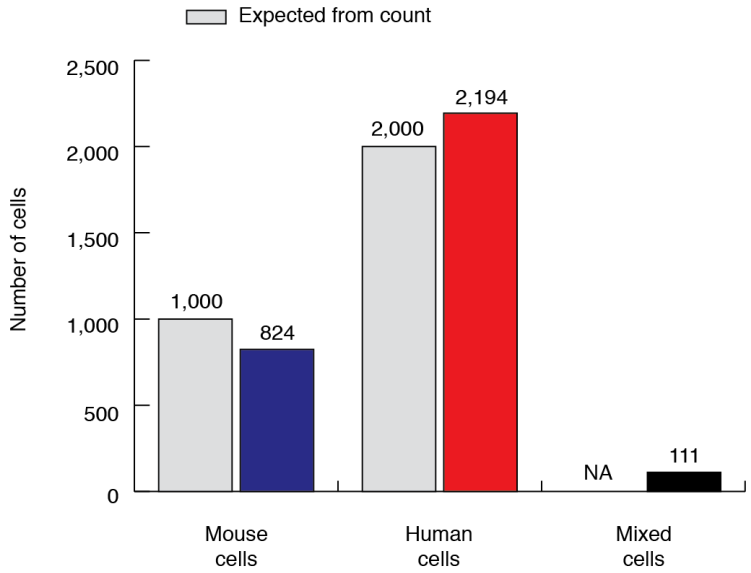


Ludwig and Bintu, 2019

Human and mouse cells mixture confirmed single-cell resolution

Microfluidic QC



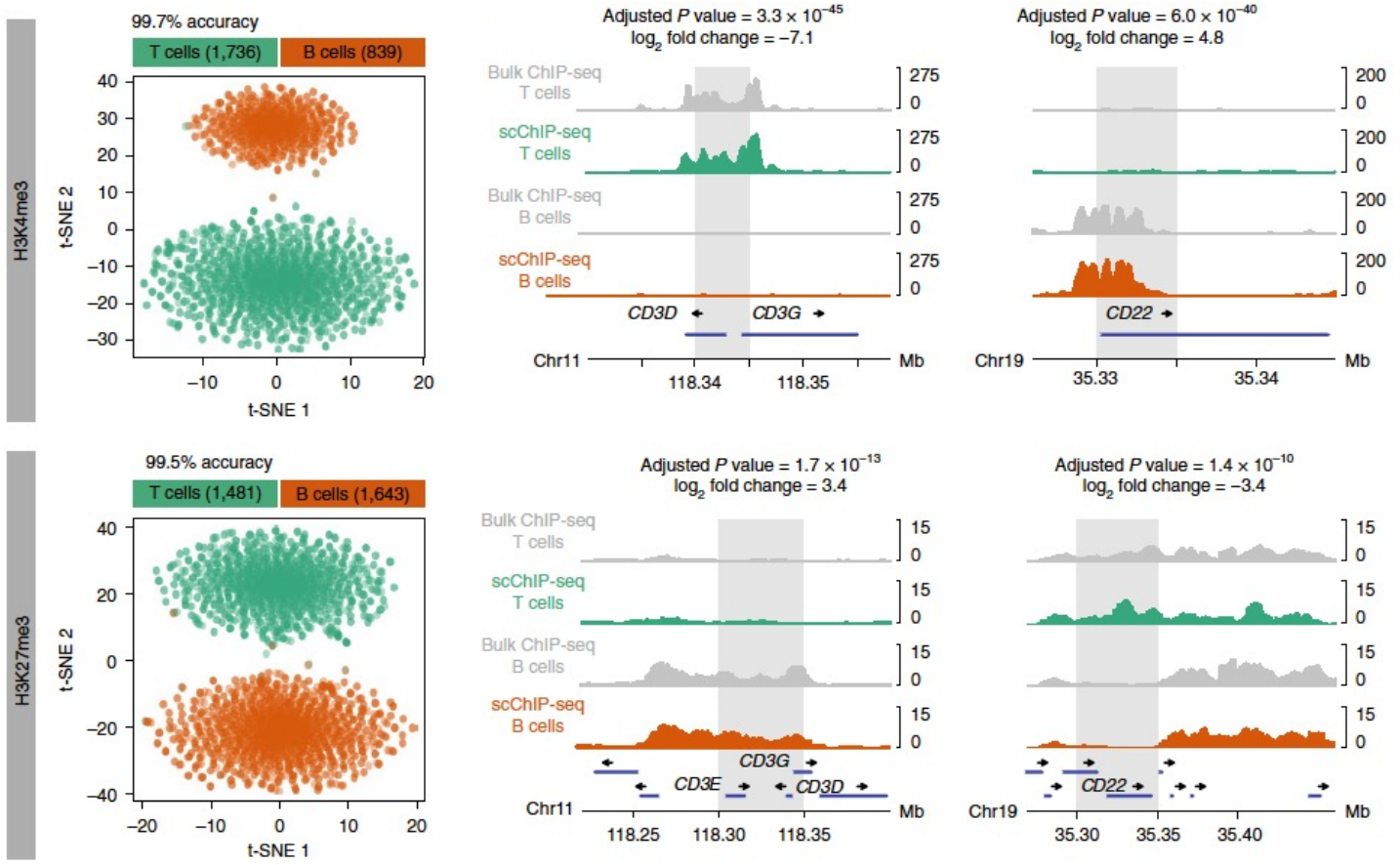


Mixture : 1/3 mouse cells and 2/3 of human cells

Grosselin et al., Nat Genet 2019

Reconstructing cell-type-specific chromatin states from single-cell ChIP-seq profiles

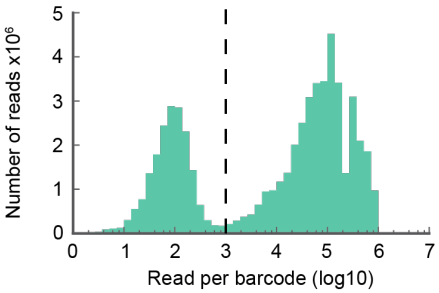
Chromatin profiles



Grosselin et al., Nat Genet 2019

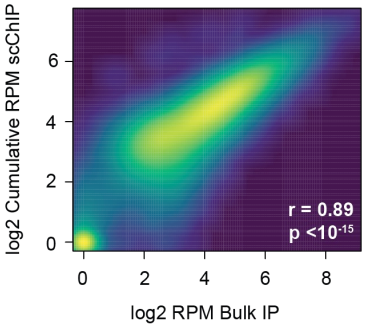
Single-cell chromatin profiling by drop-seq achieves high coverage and similarities to bulk

Data QC analysis

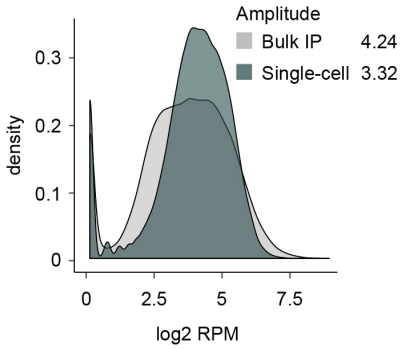


An average coverage of up to 10,000 loci/cell

Previously <1,000 loci
(Rotem *et al.* , 2015)



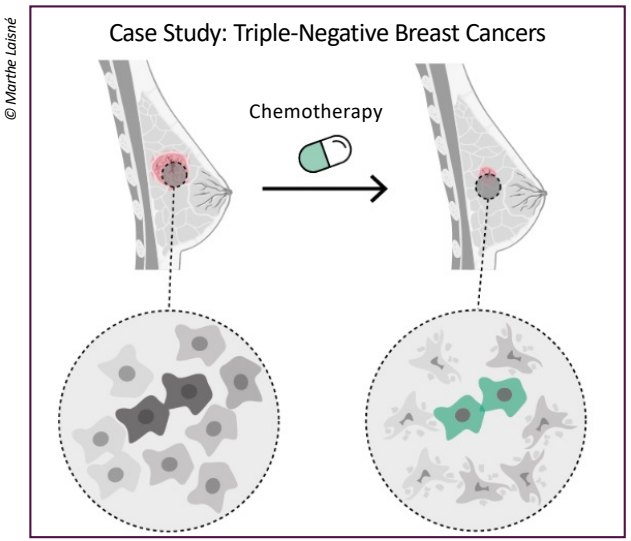
Recapitulates bulk histone enrichment profiles for active and repressive histone marks
H3K4me3, H3K27me3



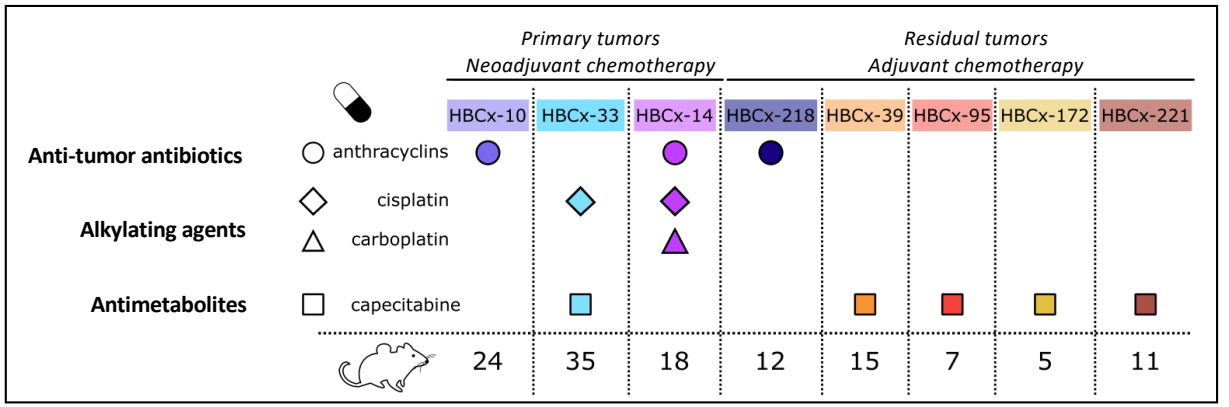
Similar amplitude of signal
Log2fold-change 1.89

Grosselin et al., Nat Genet 2019

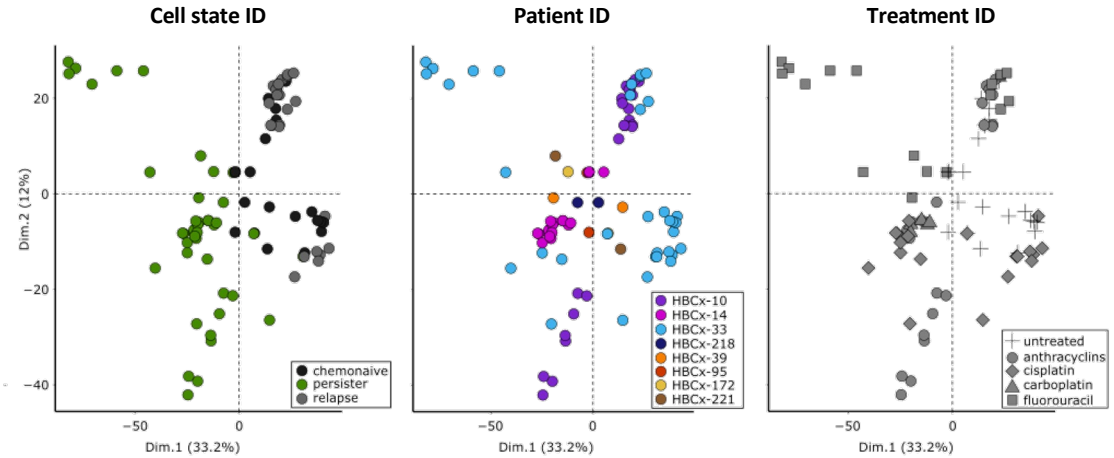
Persister cells share a common expression program



© Marthe Laisné

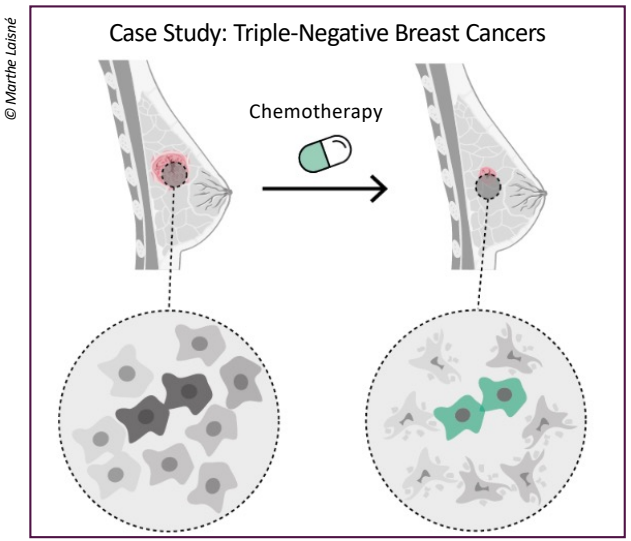


Gene expression



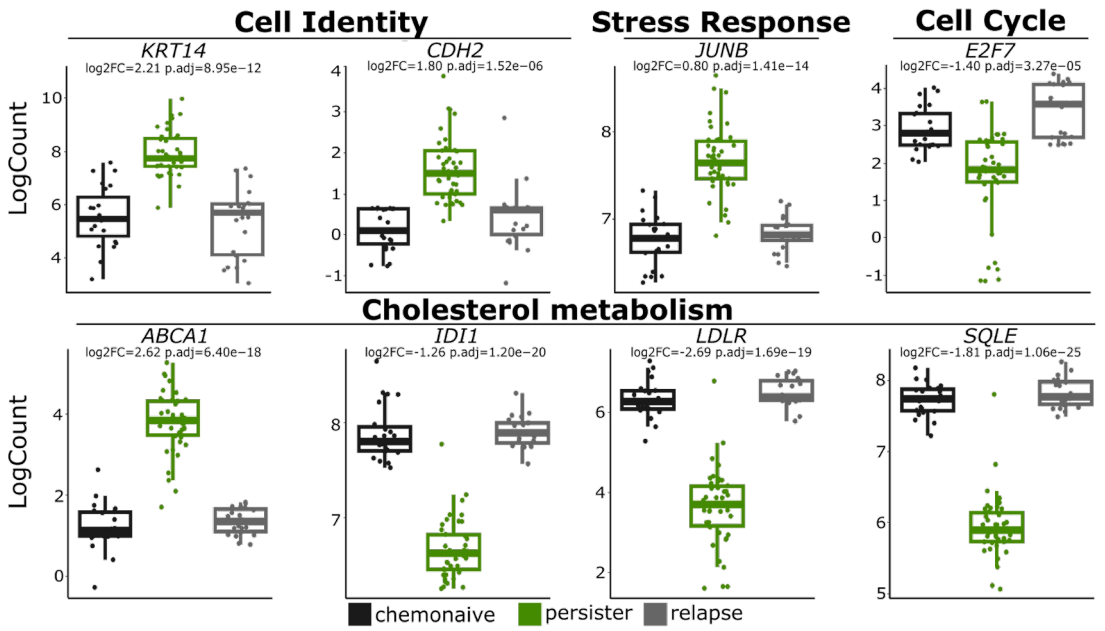
Marsolier & Prompsy et al., 2022
Baudre & Jouault et al., 2025

Persister cells share a common expression program

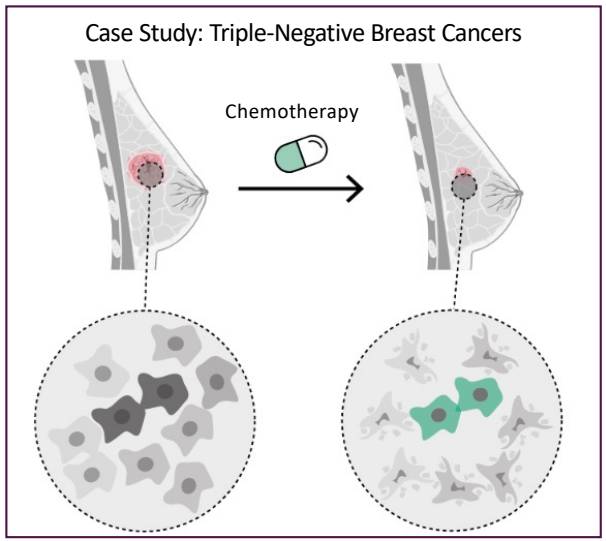


© Marthe Laisné

Gene expression

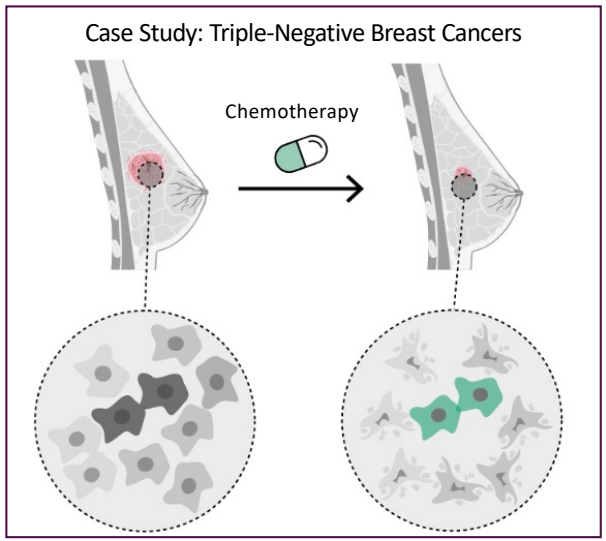


Marsolier & Prompsy et al., 2022
Baudre & Jouault et al., in preparation



Mechanisms regulating the activation of persister genes?

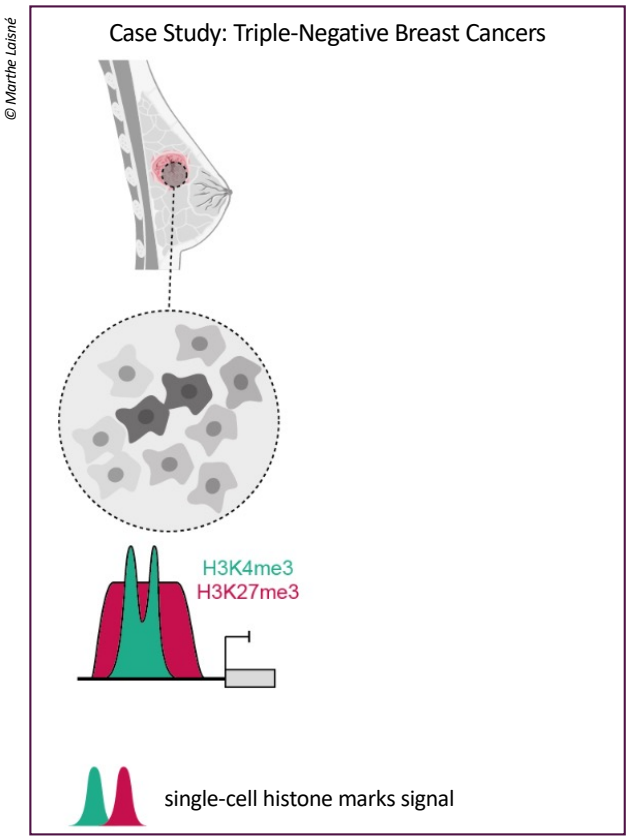
- Chromatin landscapes
- Transcription factors



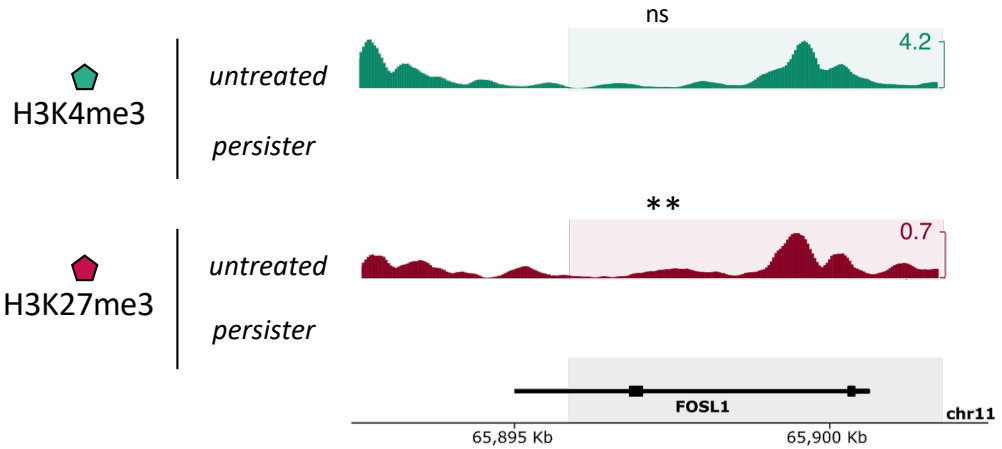
Mechanisms regulating the activation of persister genes?

Chromatin landscapes

A fraction of the persister genes is in a bivalent chromatin configuration before treatment

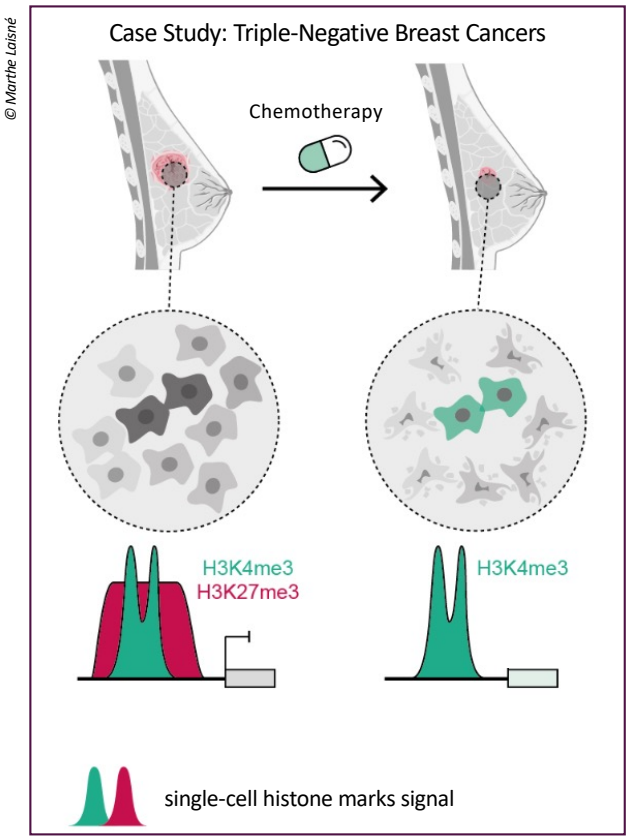


Histone marks profiles

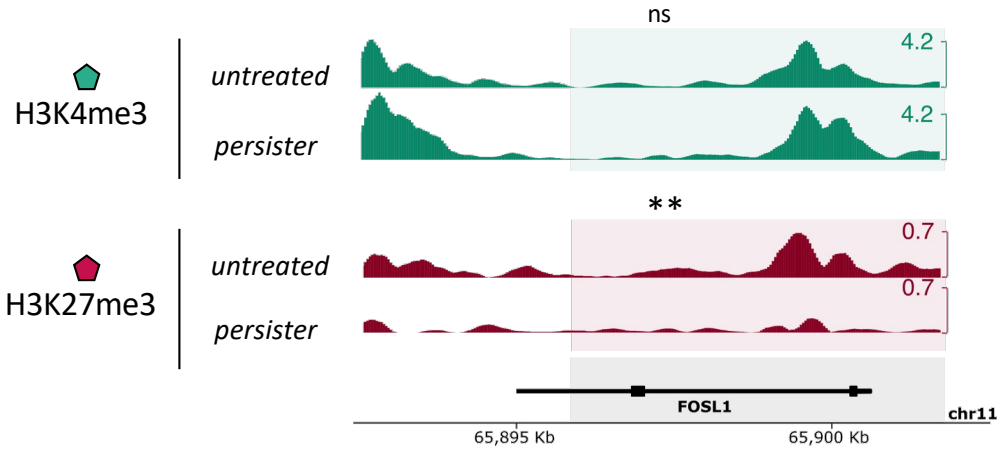


Marsolier & Prompsy et al., 2022

Recurrent redistribution of H3K27 methylation under chemotherapy treatment

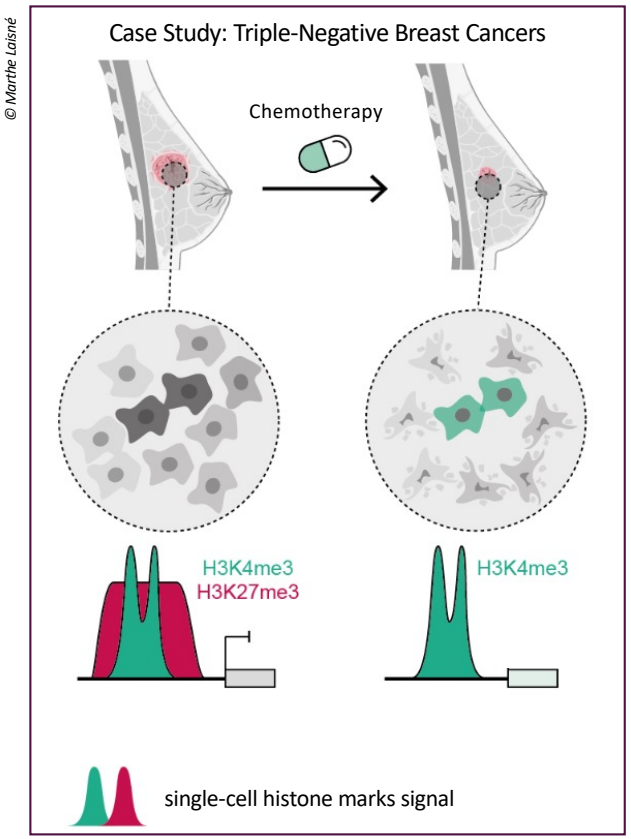


Histone marks profiles

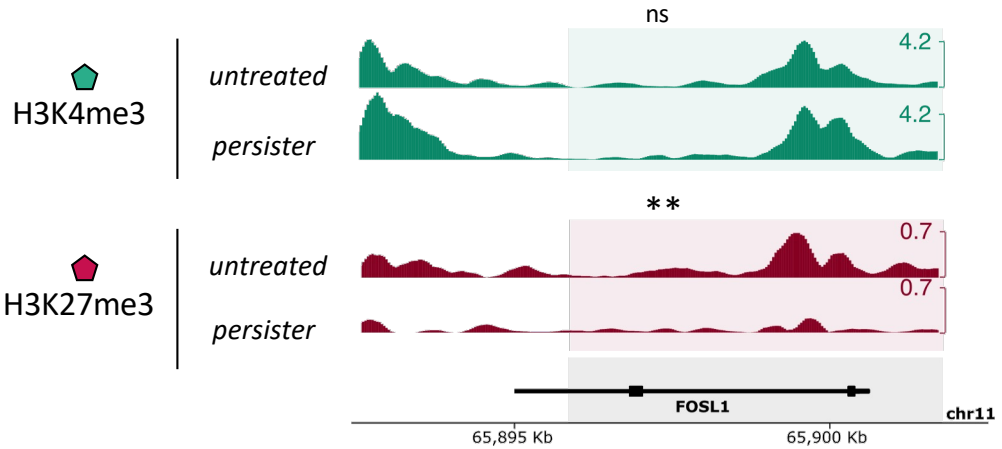


Organized lost of H3K27me3

Marsolier & Prompsy et al., 2022



Histone marks profiles

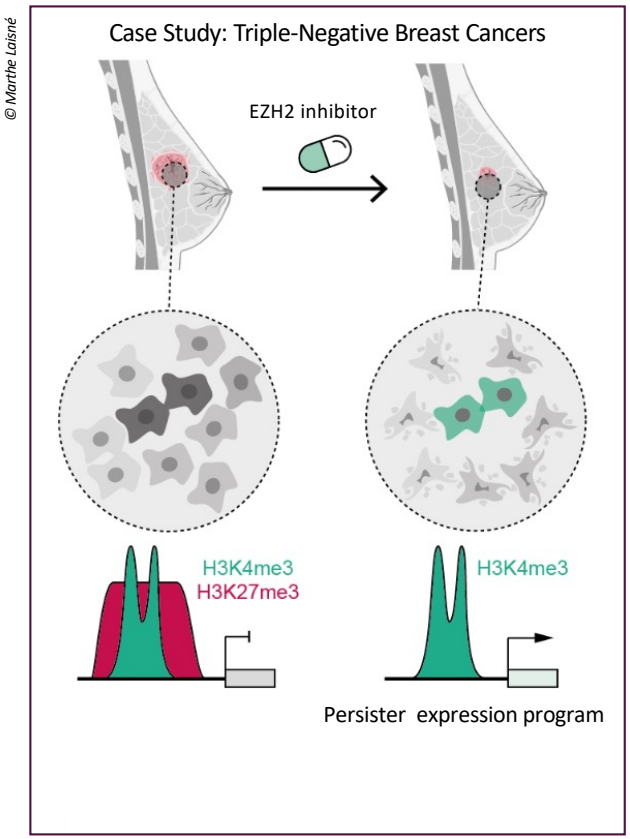


Organized lost of H3K27me3

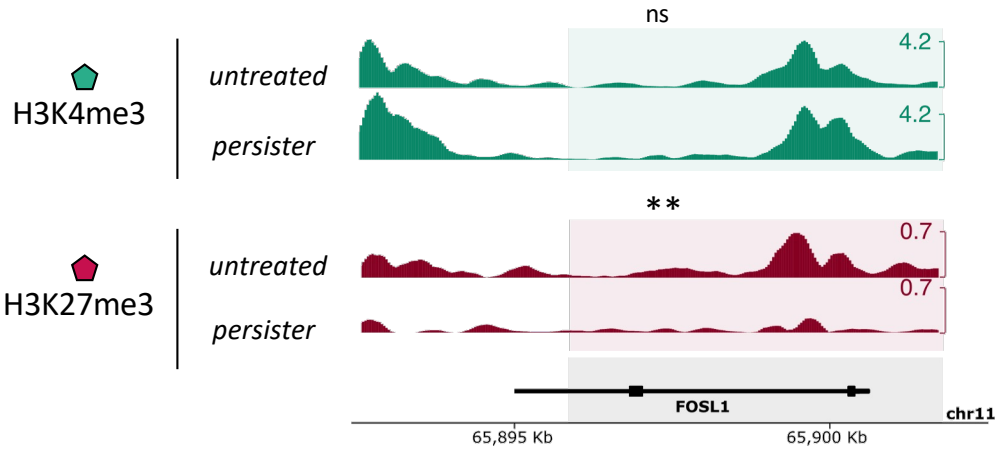
Activation of the persister expression program?

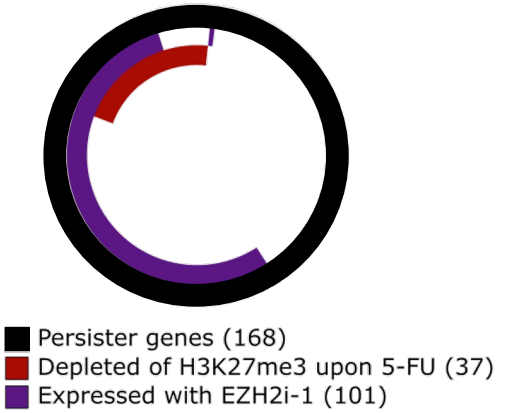
Marsolier & Prompsy et al., 2022

Persister expression program is locked by H3K27me3



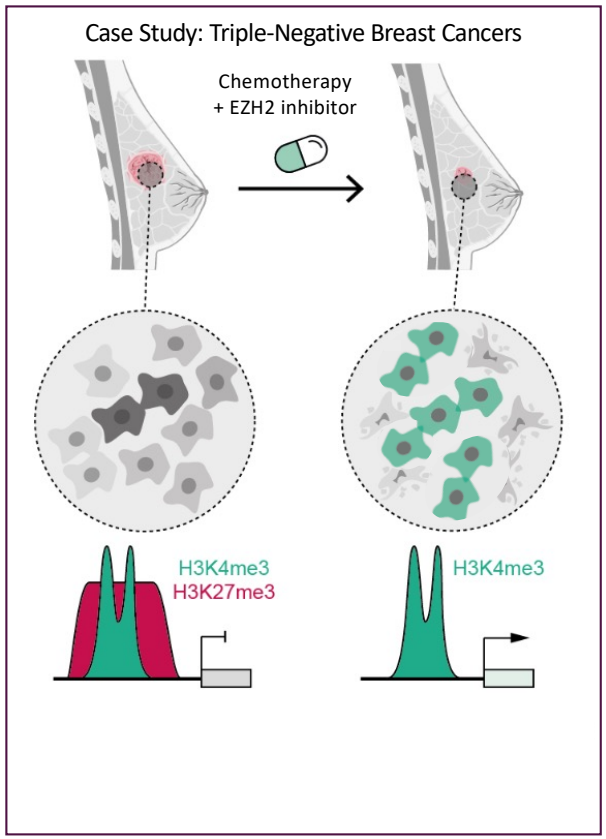
Histone marks profiles



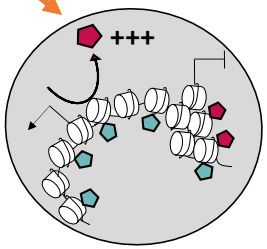


Marsolier & Prompsy et al., 2022

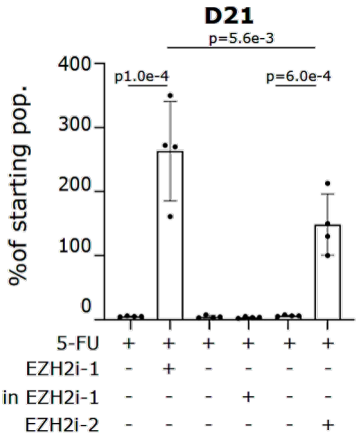
Erasing H3K27me3 increases the chemopersistence



H3K27me3
histone methyltransferase
inhibitor
(**EZH2i**)

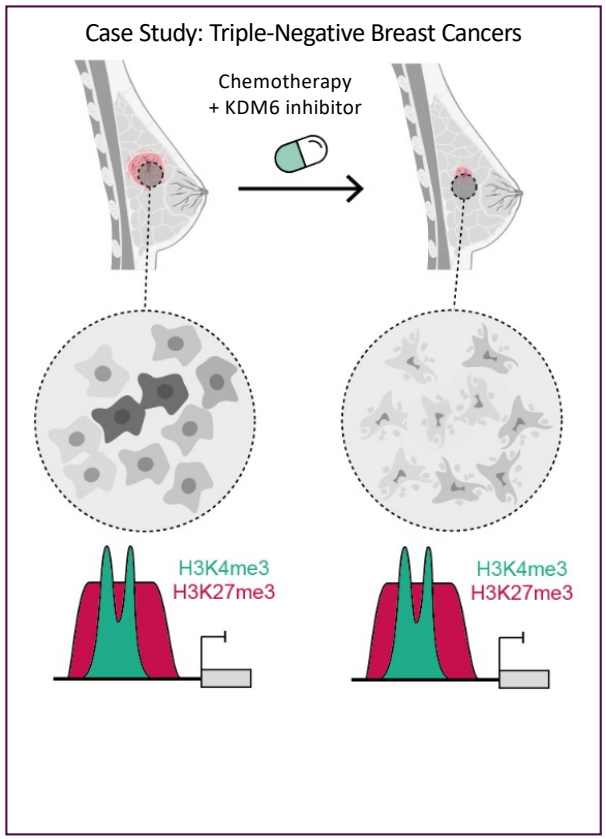


Number of persister cells

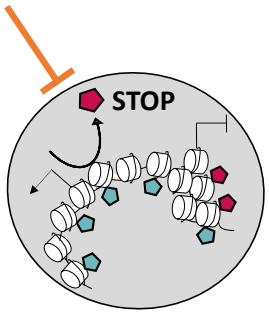


Marsolier & Prompsy et al., 2022

Co-treatment with KDM6A/Bi delays the emergence of persister cells

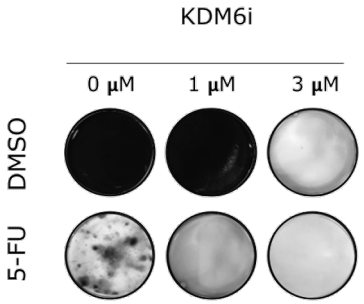


H3K27me3
histone demethylase inhibitor
(**KDM6A/Bi**)

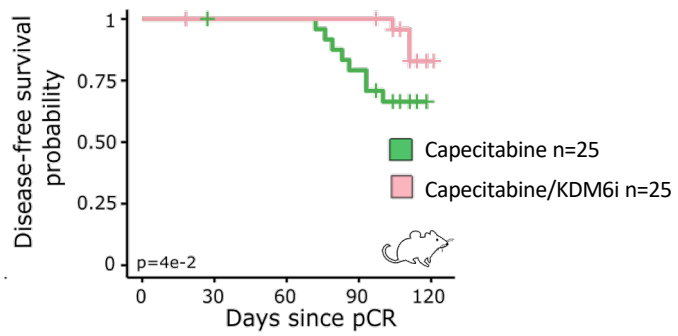


Number of persister cells

Colony forming assay

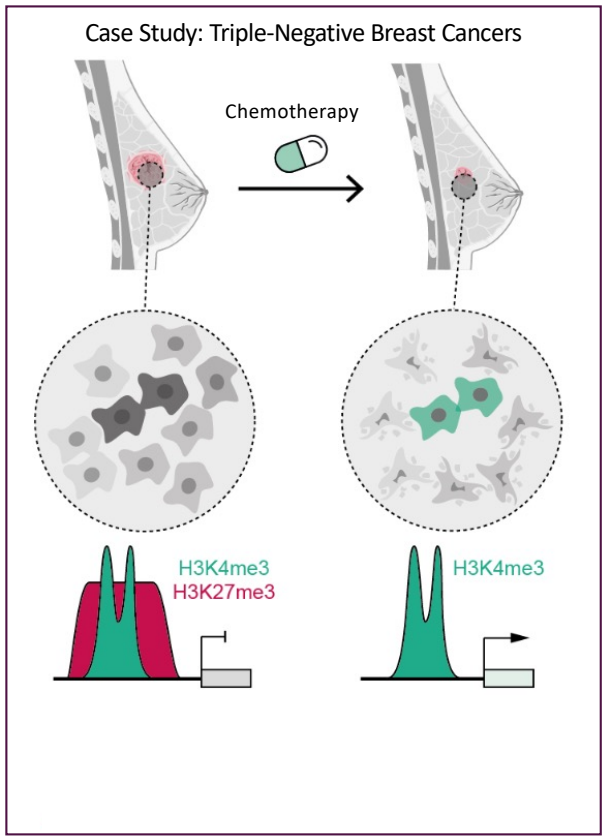


Recurrence

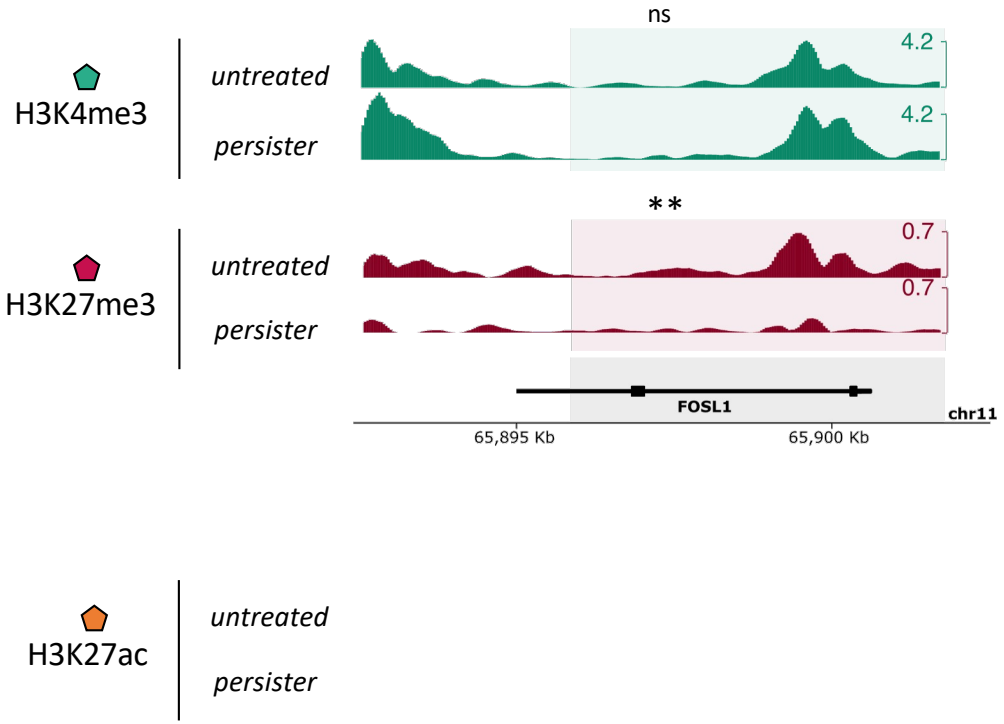


Marsolier & Prompsy et al., 2022

Profiling of additional histone marks in response to chemotherapy treatment

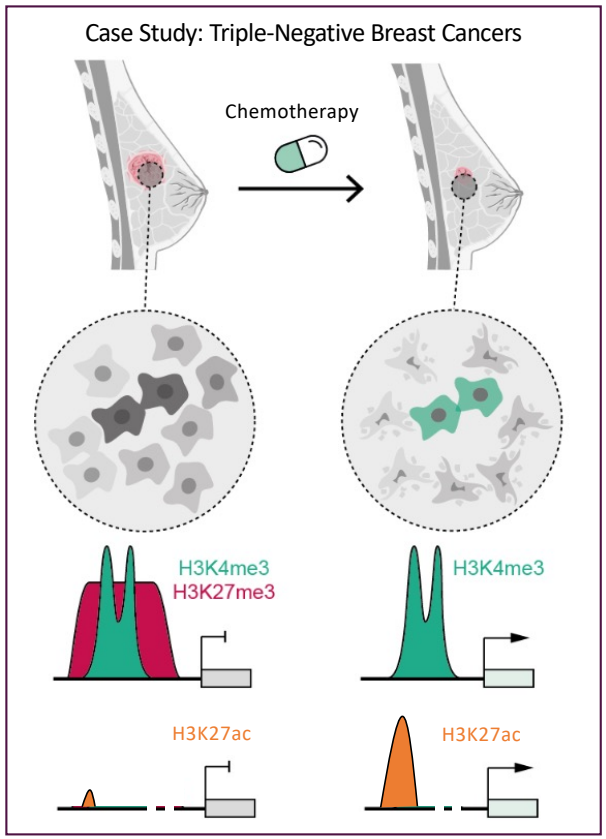


Histone marks profiles

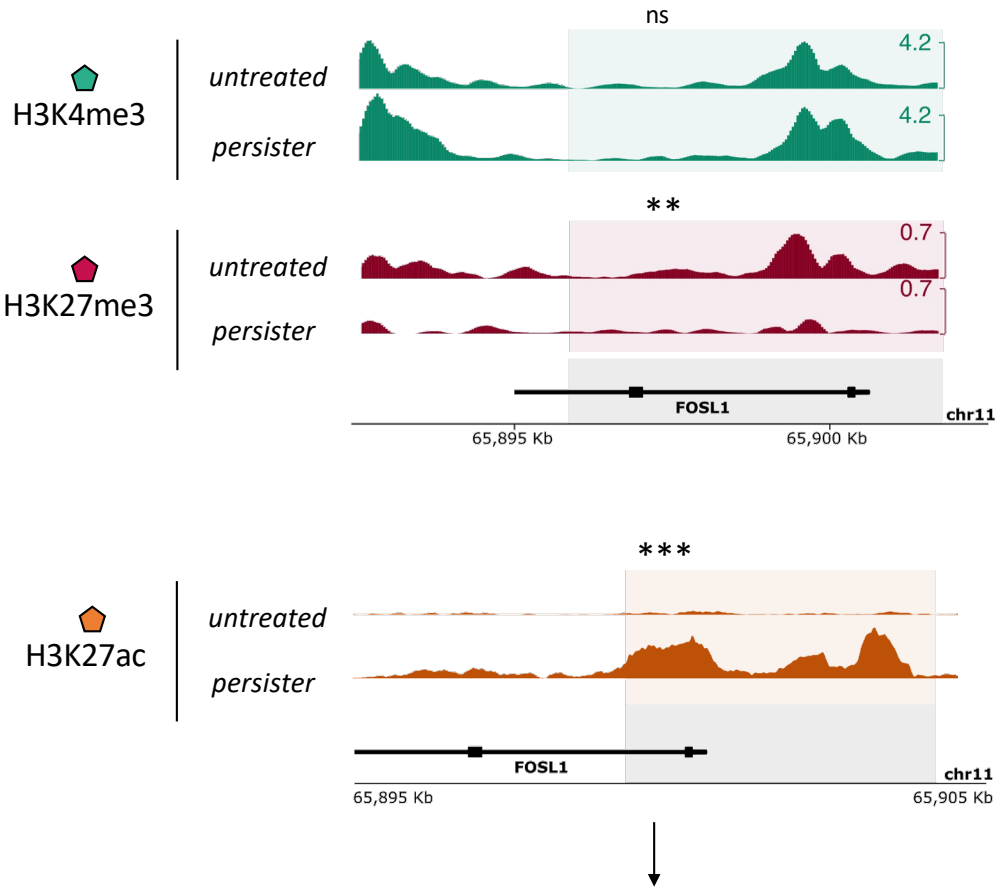


Baudre & Jouault et al., 2025

Rewiring of the H3K27ac landscape in persister cells

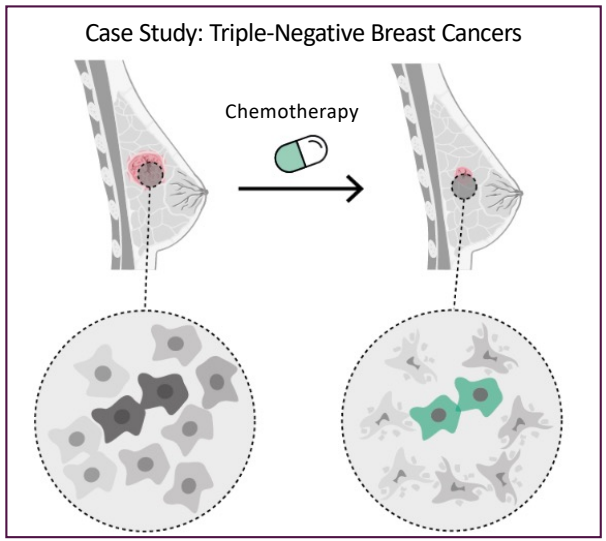


Histone marks profiles



Localized gains of H3K27ac

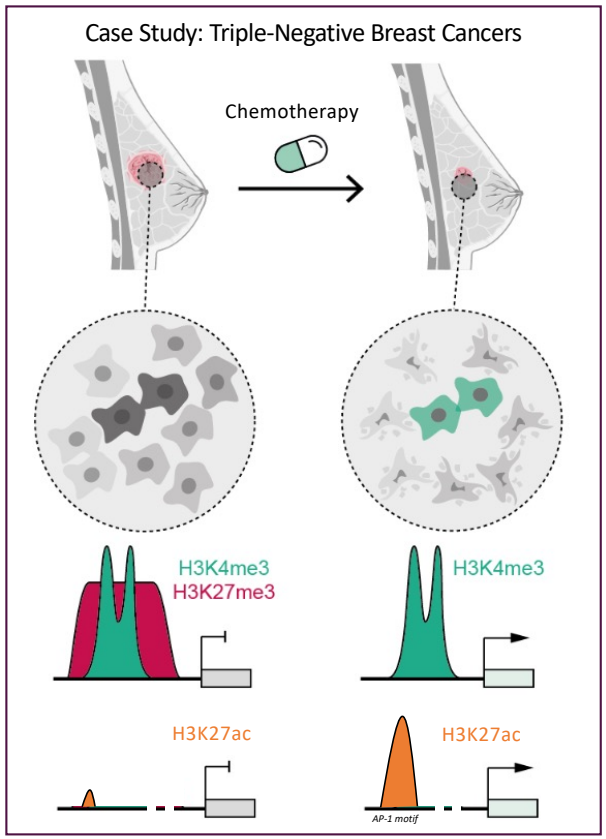
Baudre & Jouault et al., 2025



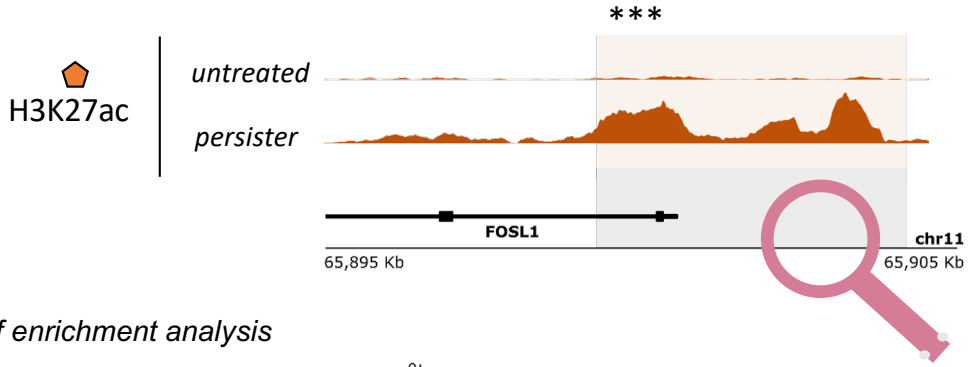
Mechanisms regulating the activation of persister genes?

Transcription factors

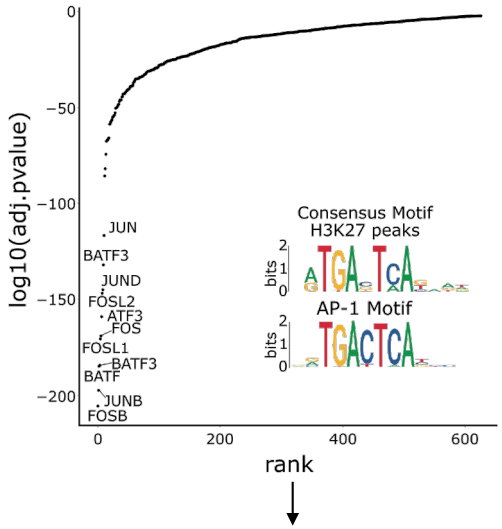
Epigenomic remodeling of enhancers in persister cells is dedicated to AP-1 sites



Histone marks profiles



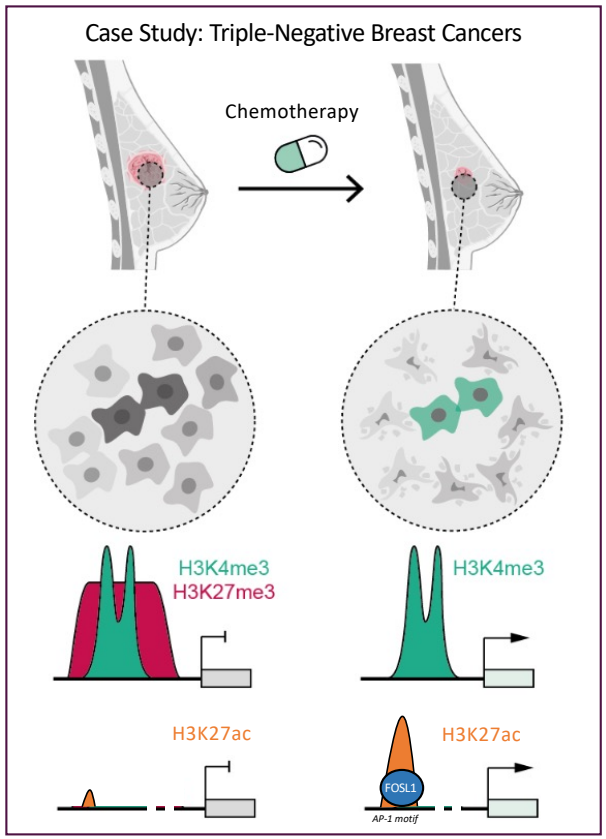
Motif enrichment analysis



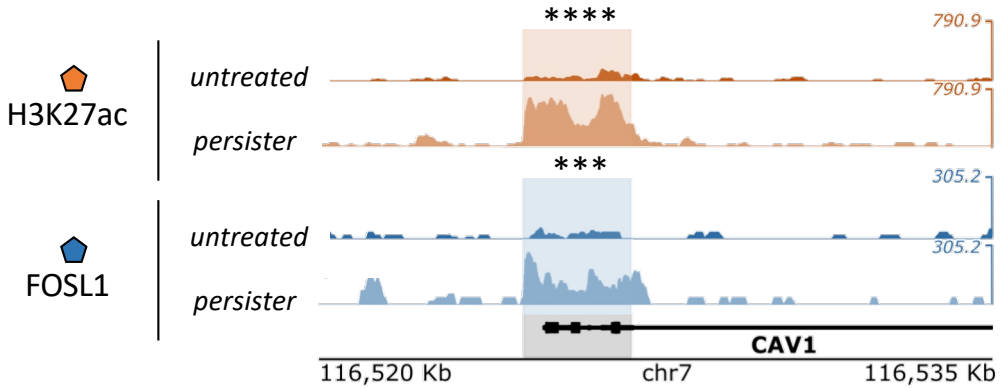
AP-1 binding motif

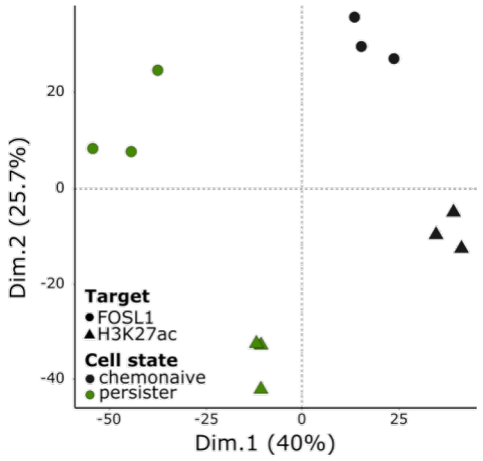
Baudre & Jouault et al., 2025

FOSL1 is overexpressed upon treatment and binds activated enhancers



H3K27ac & FOSL1 genomic profiling

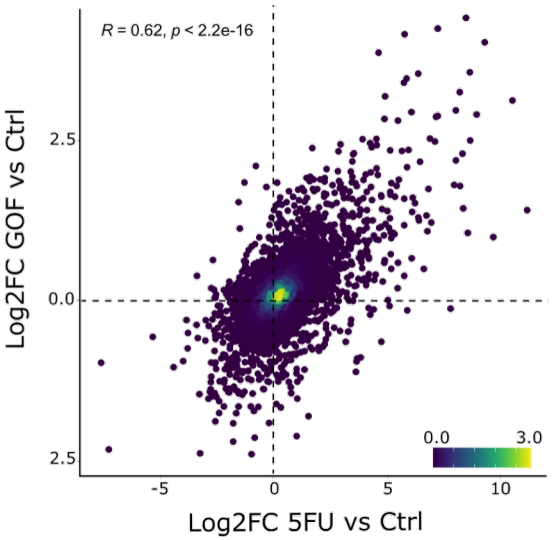




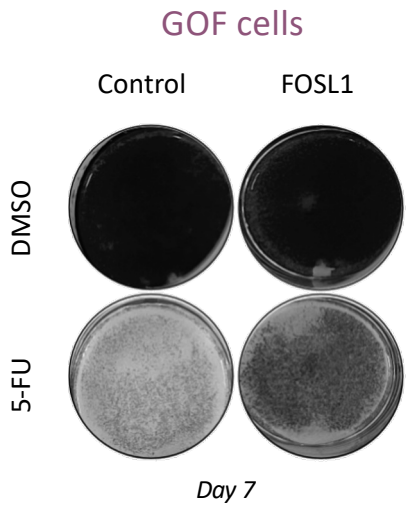
Baudre & Jouault et al., 2025

FOSL1 is sufficient to reach the persister state

Transcriptome correlation



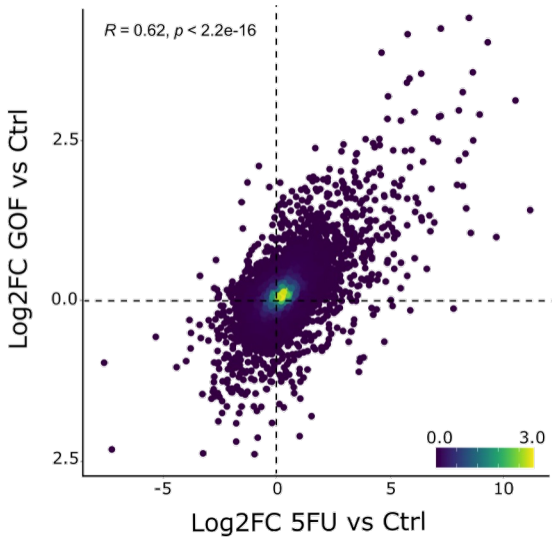
Colony forming assay



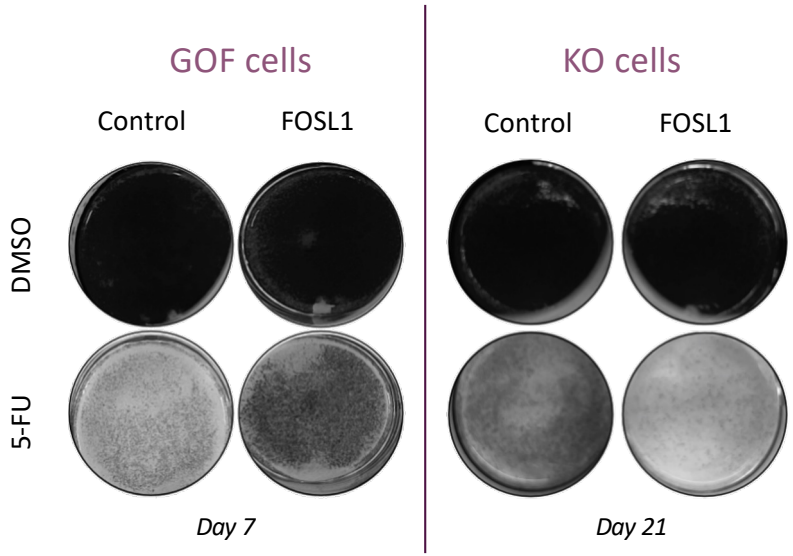
Baudre & Jouault et al., 2025

Preventing the activation of FOSL1 expression upon treatment leads to a decrease of persister cells

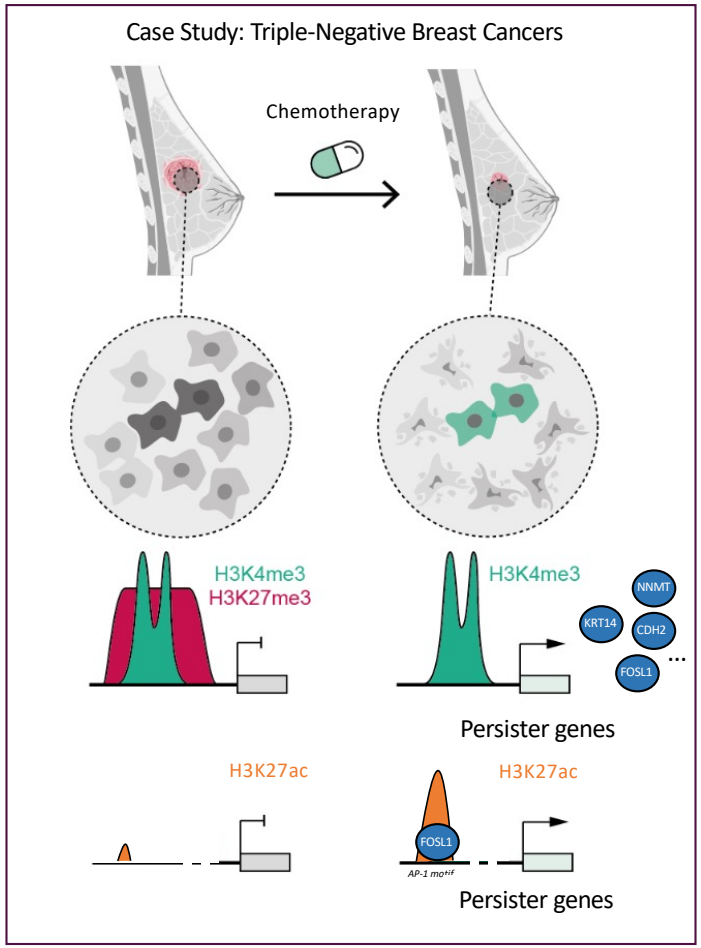
Transcriptome correlation



Colony forming assay



Baudre & Jouault et al., 2025

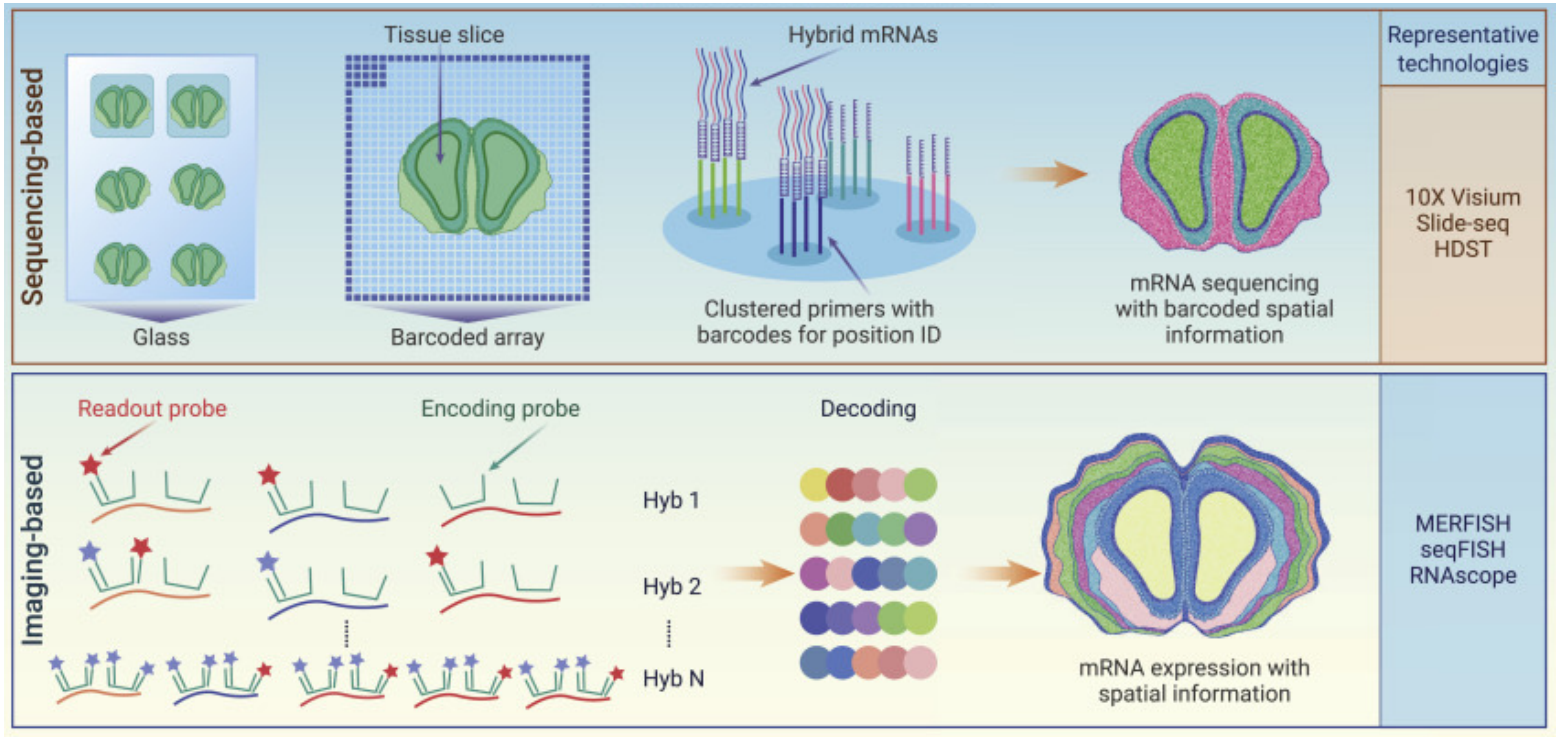


Marsolier & Prompsy et al., 2022
Baudre & Jouault et al., 2025

Single-cell multi-omics

https://youtu.be/rjke1BWcyBA

Spatial transcriptomics



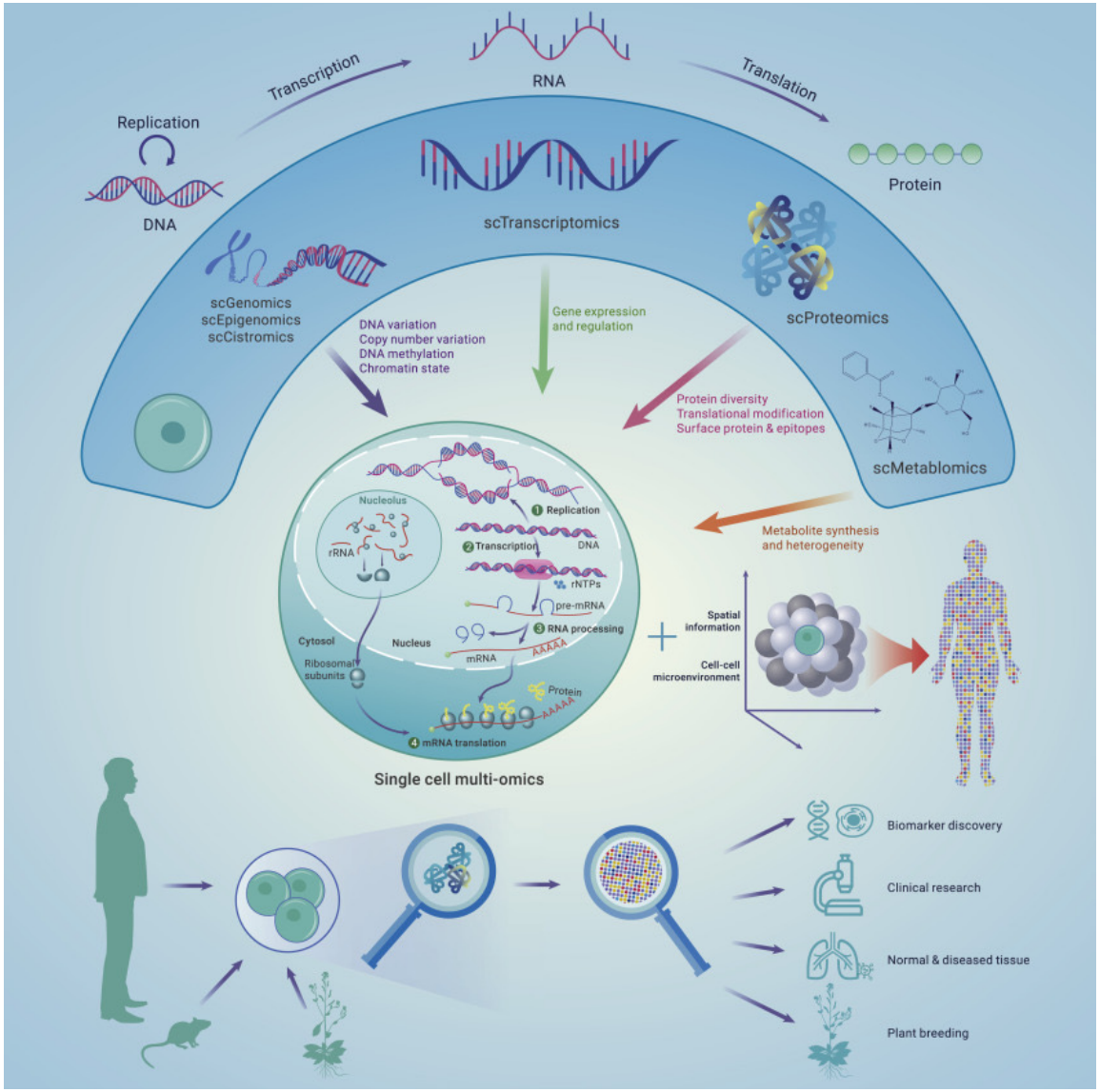
Weng et al.,2022

Spatial transcriptomics: sequencing-based approach

https://youtu.be/VwNk4d-0RJc

https://youtu.be/O0QekKSscjA?si=zKvWvGTSZoD7HzaF

Future directives



Weng et al.,2022