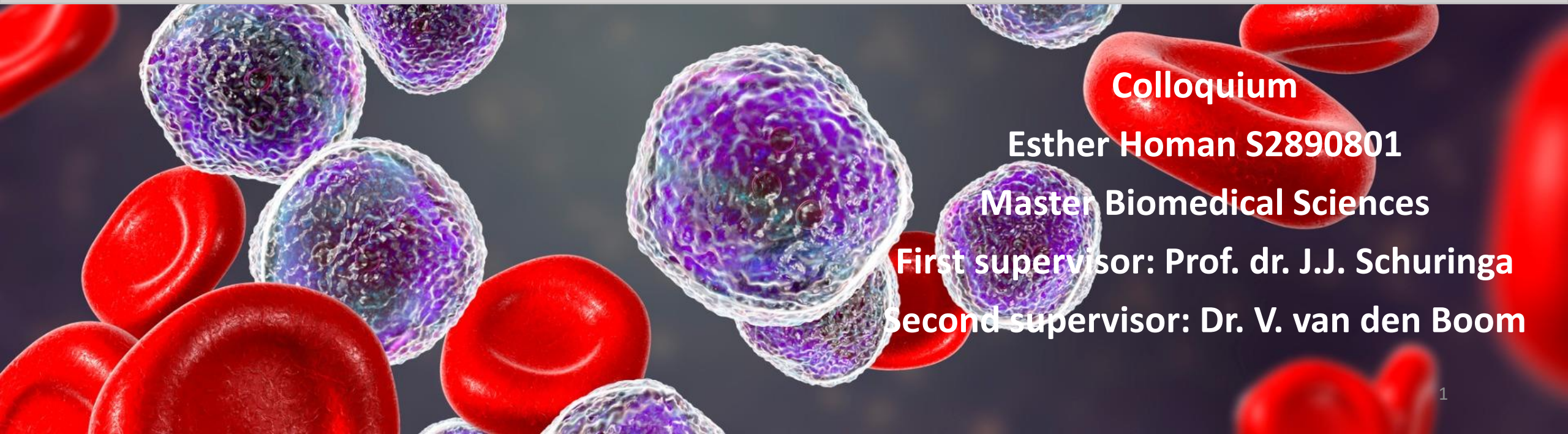
rijksuniversiteit groningen

05-10-2021

Single cell techniques to reveal clonal heterogeneity in acute myeloid leukemia



Colloquium

Esther Homan S2890801

Master Biomedical Sciences

First supervisor: Prof. dr. J.J. Schuringa

Second supervisor: Dr. V. van den Boom

Outline

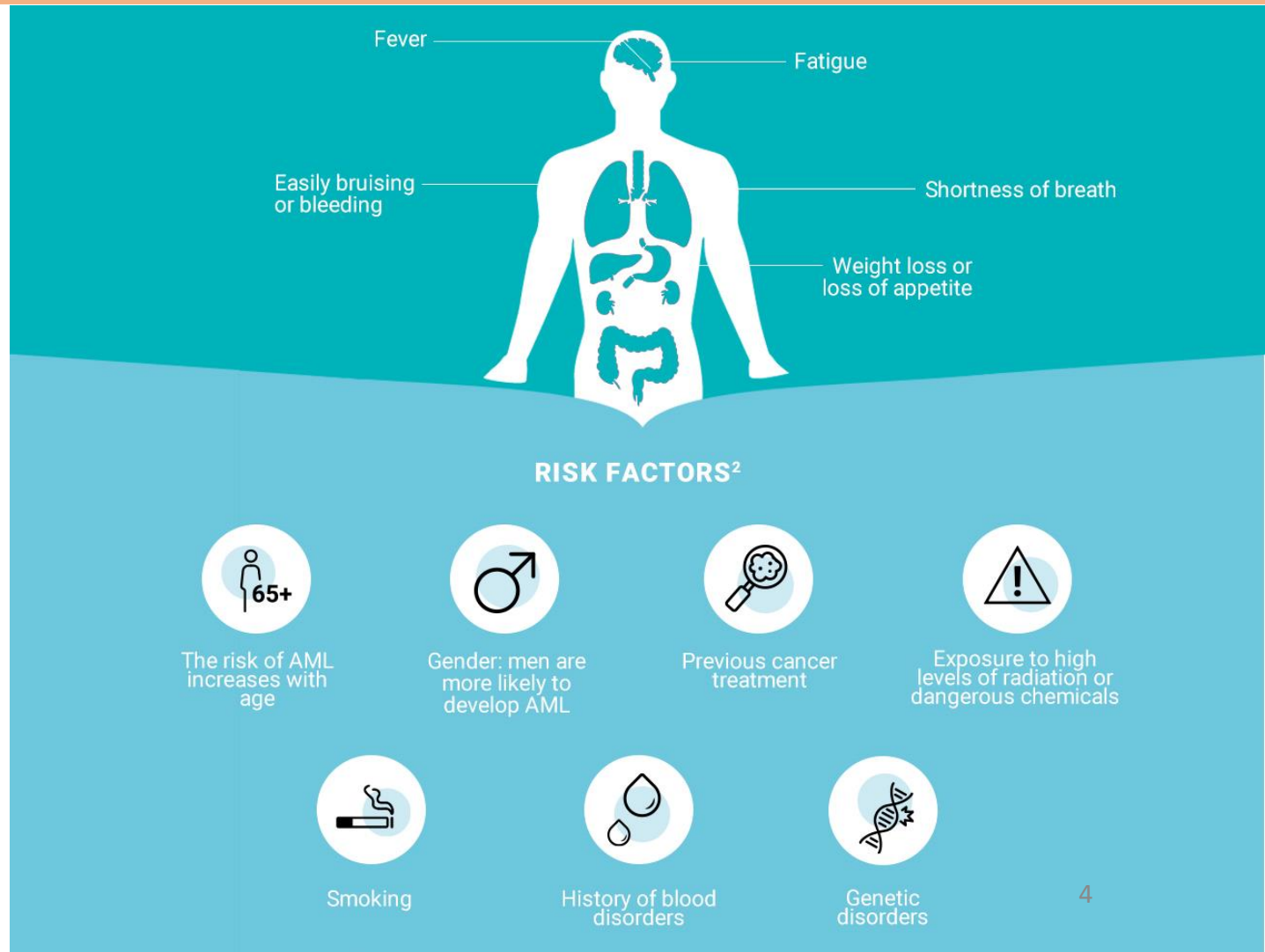
- Introduction AML
- Current therapies
- Single cell sequencing
- Types of single cell sequencing
- Future

Outline

- Introduction AML
- Current therapies
- Single cell sequencing
- Types of single cell sequencing
- Future

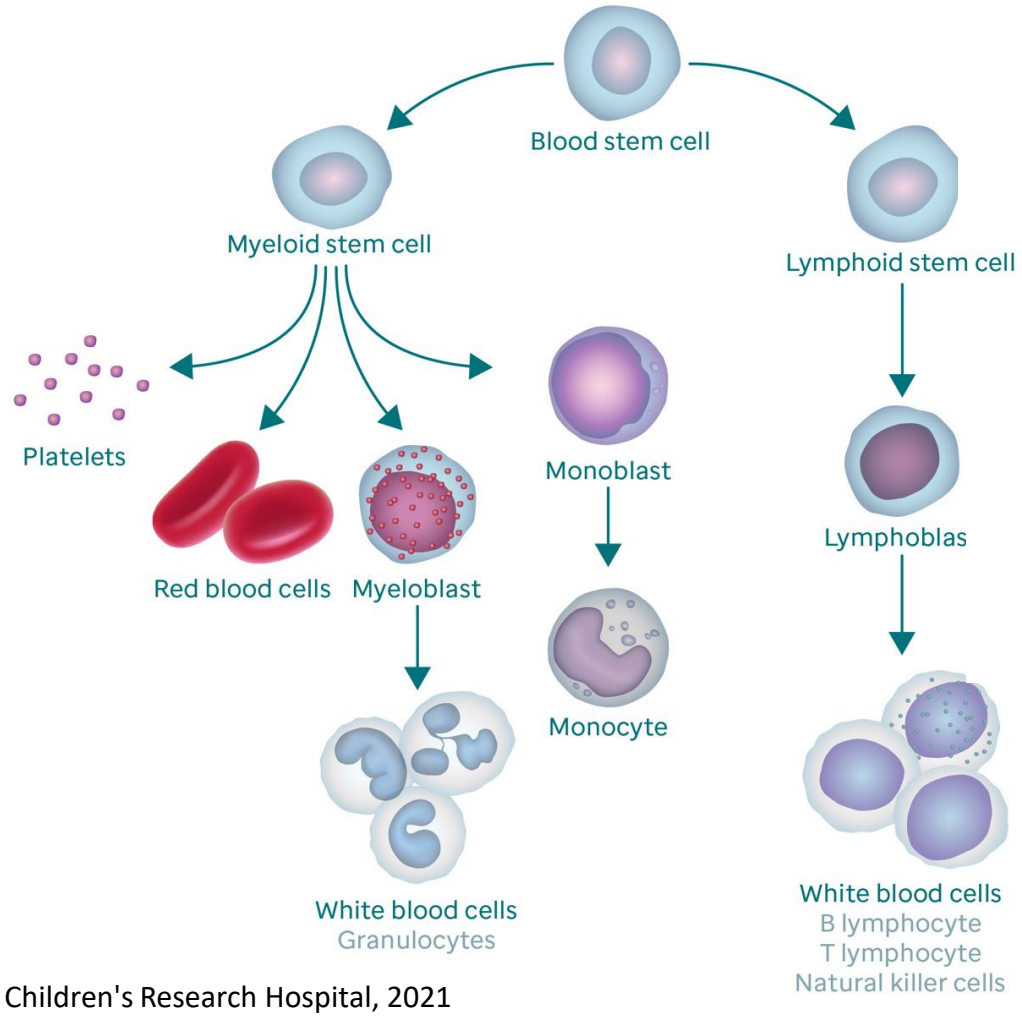
Introduction acute myeloid leukemia (AML)

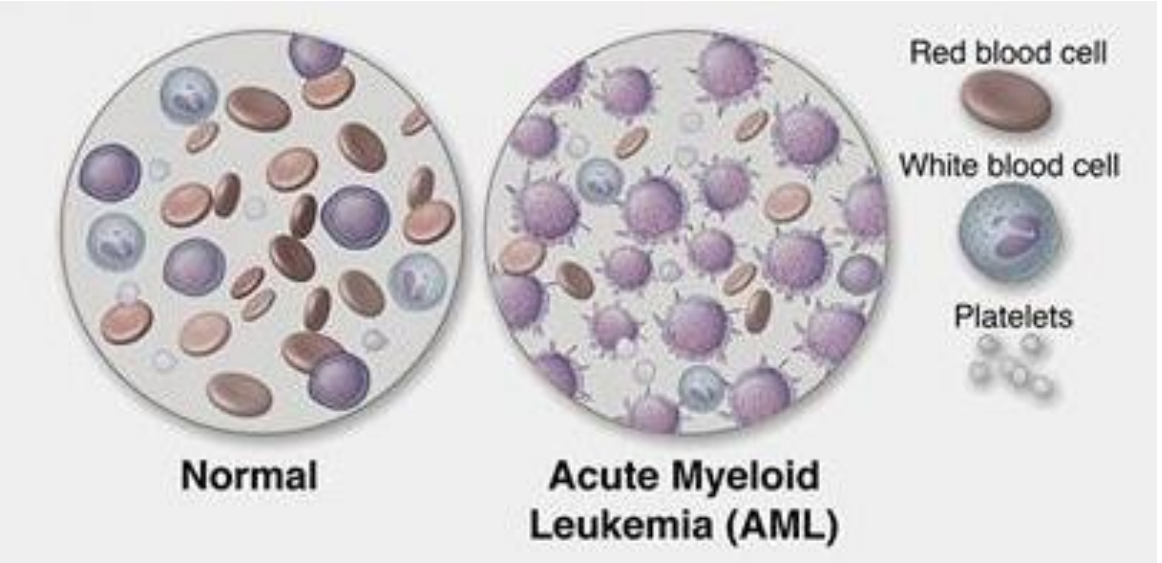
- Cancer: **abnormal blood cells i**n the bone marrow
- Blood and bone marrow **tests to diagnose**
- 5-year **survival**: 15-70%
- Elderly



Servier, 2020

Introduction acute myeloid leukemia (AML)





20 out of every 100 white blood cells are blast cells

St. Jude Children's Research Hospital, 2021
https://cancer.osu.edu/for-patients-and-caregivers/learn-about-cancers-and-treatments/cancers-conditions-and-treatment/cancer-types/acute-myeloid-leukemia

Outline

- Introduction AML
- Current therapies
- Single cell sequencing
- Types of single cell sequencing
- Future

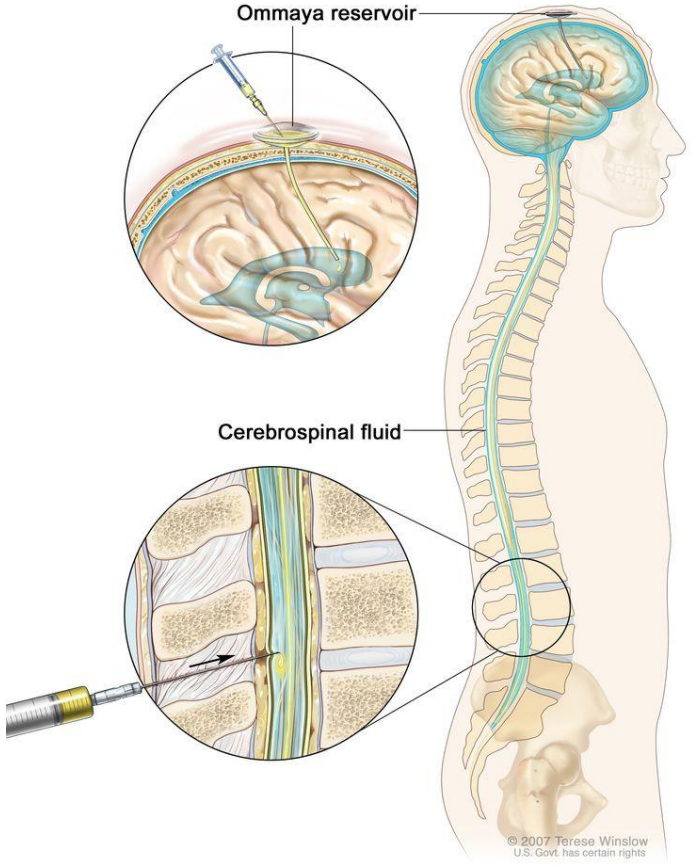
Current therapies AML

- First line treatment = Chemotherapy
- Bone marrow/stem cell transplantation
- Immunotherapy

Current therapies AML

- **First line treatment = Chemotherapy**
- Bone marrow/stem cell transplantation
- Immunotherapy

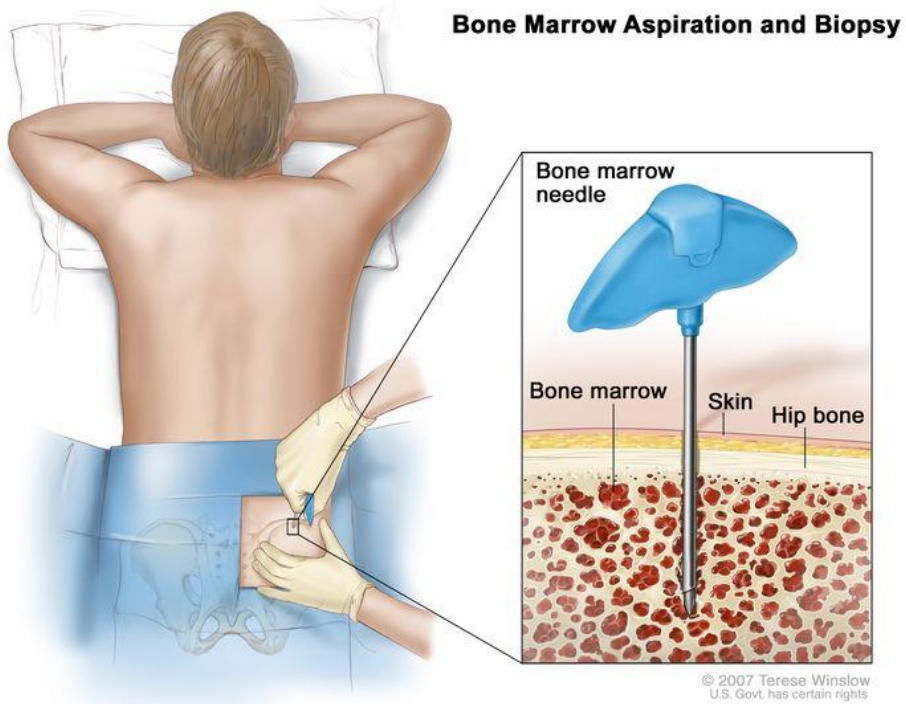
Intrathecal chemotherapy



https://www.cancer.gov/types/leukemia/patient/adult-aml-treatment-pdq

Current therapies AML

- First line treatment = Chemotherapy
- **Bone marrow/stem cell transplantation**
- Immunotherapy



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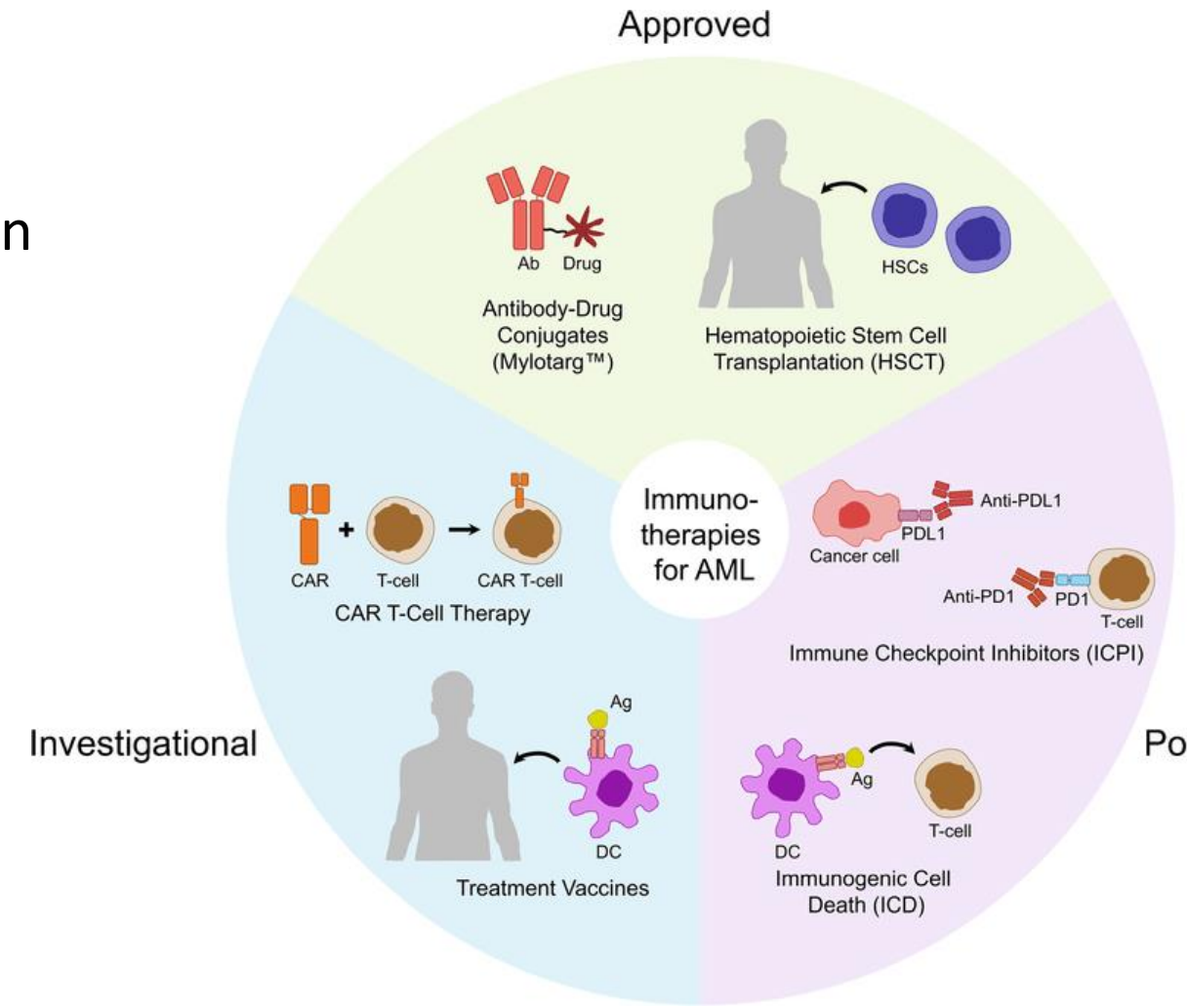
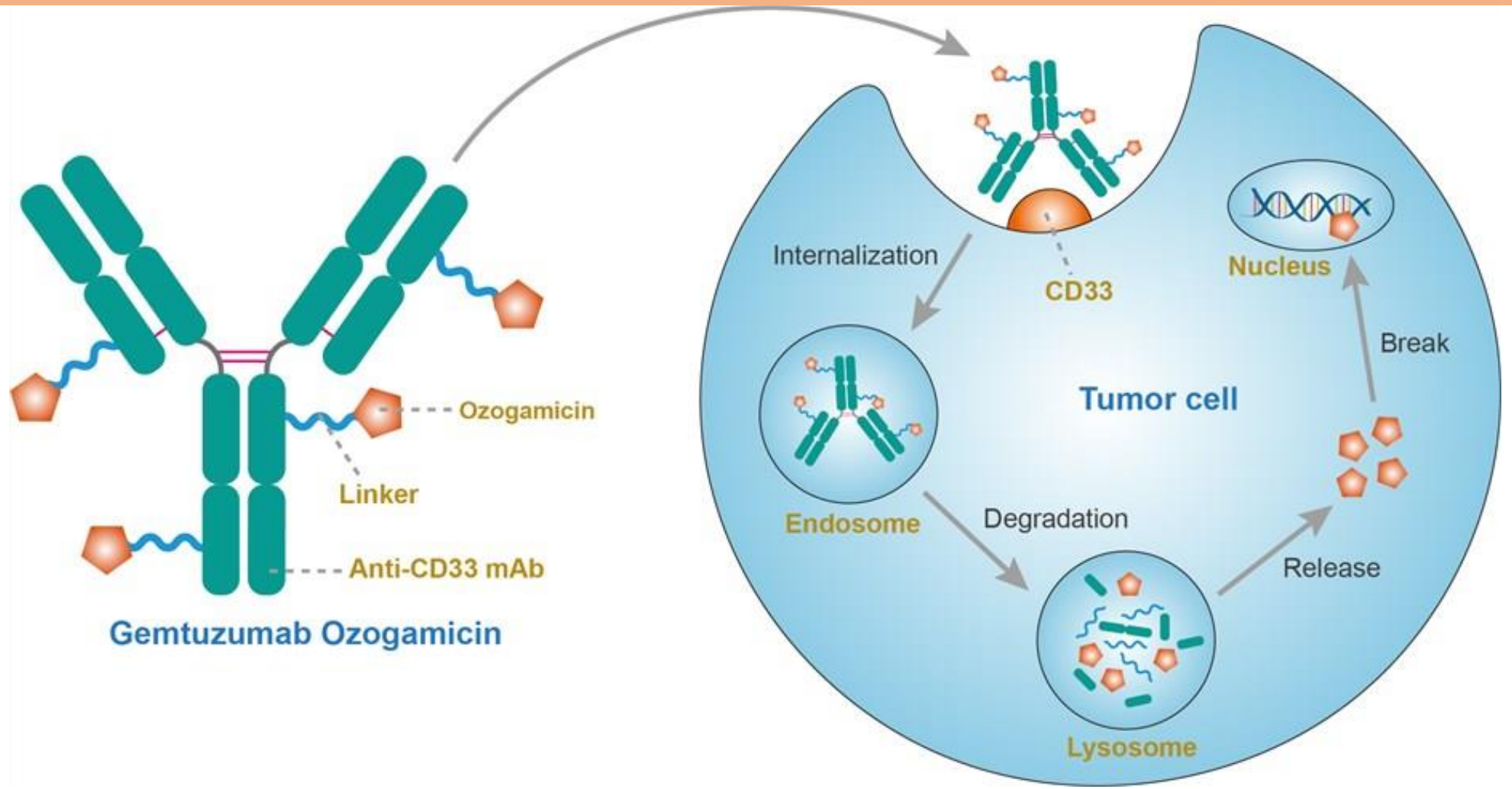
Current therapies AML

- First line treatment = Chemotherapy
- Bone marrow/stem cell transplantation
- **Immunotherapy**



Chen K.T.J. et al, 2019

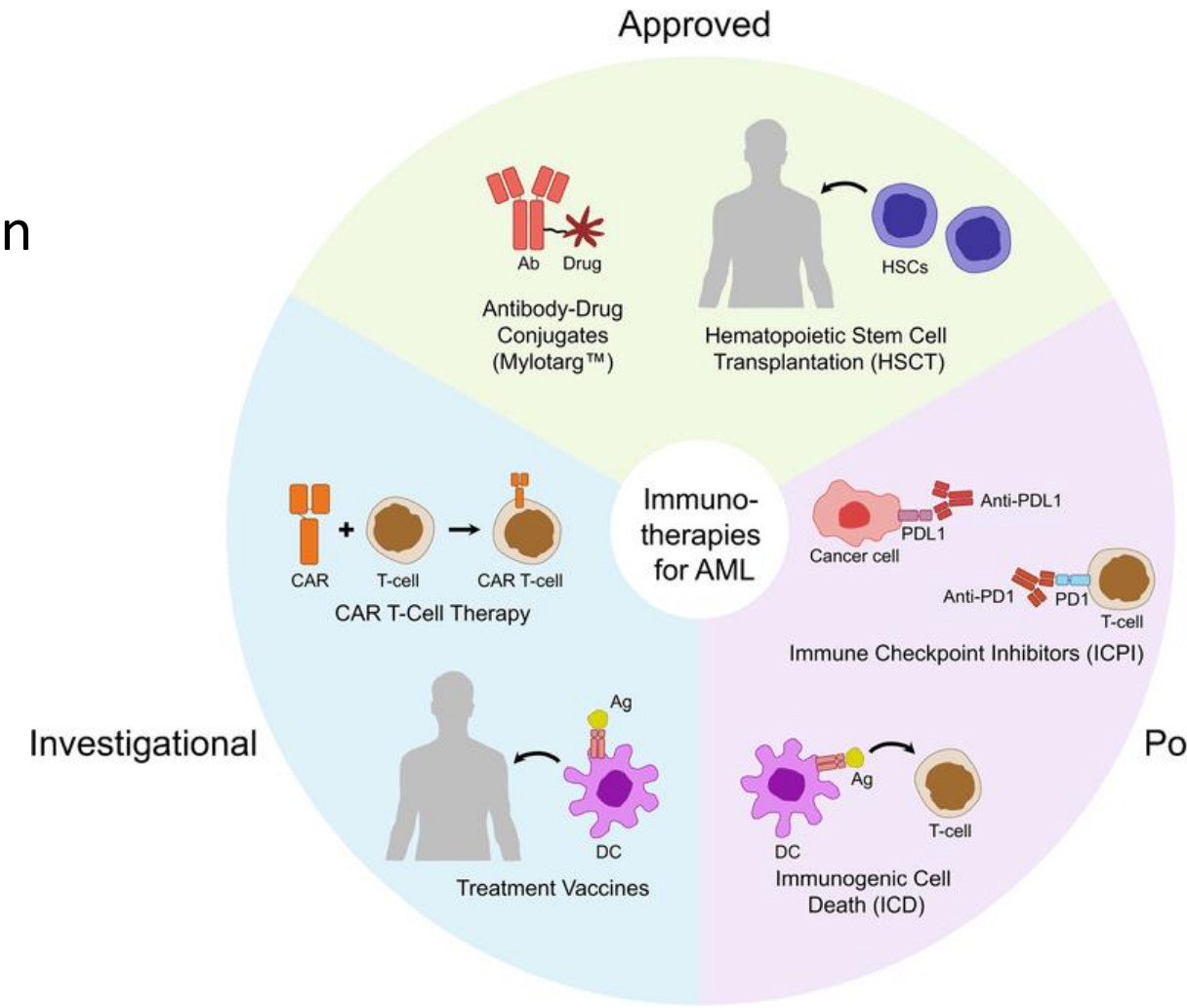
Targeted therapy drug: Gemtuzumab Ozogamicin (Mylotarg)



Creative BioLabs, 2021

Current therapies AML

- First line treatment = Chemotherapy
- Bone marrow/stem cell transplantation
- **Immunotherapy**



Chen K.T.J. et al, 2019

Current therapies AML

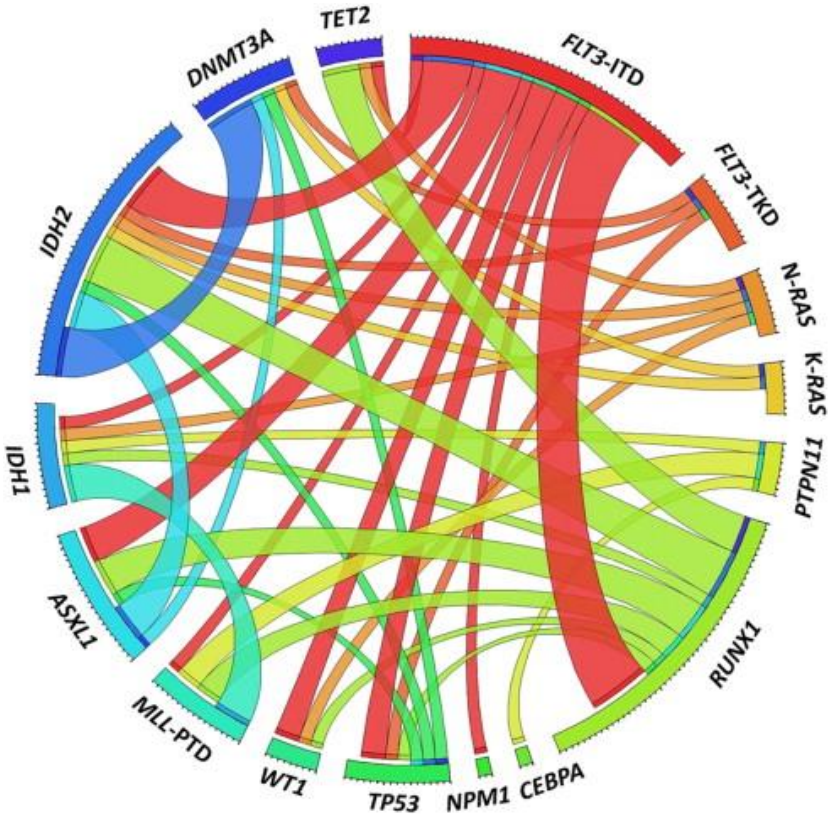
- First line treatment = Chemotherapy
- Bone marrow/stem cell transplantation
- Immunotherapy

- Overall surival ~70% but still ~30% of patients experience relapse

Somatic mutations AML

Young abnormal blood cell

Somatic mutations

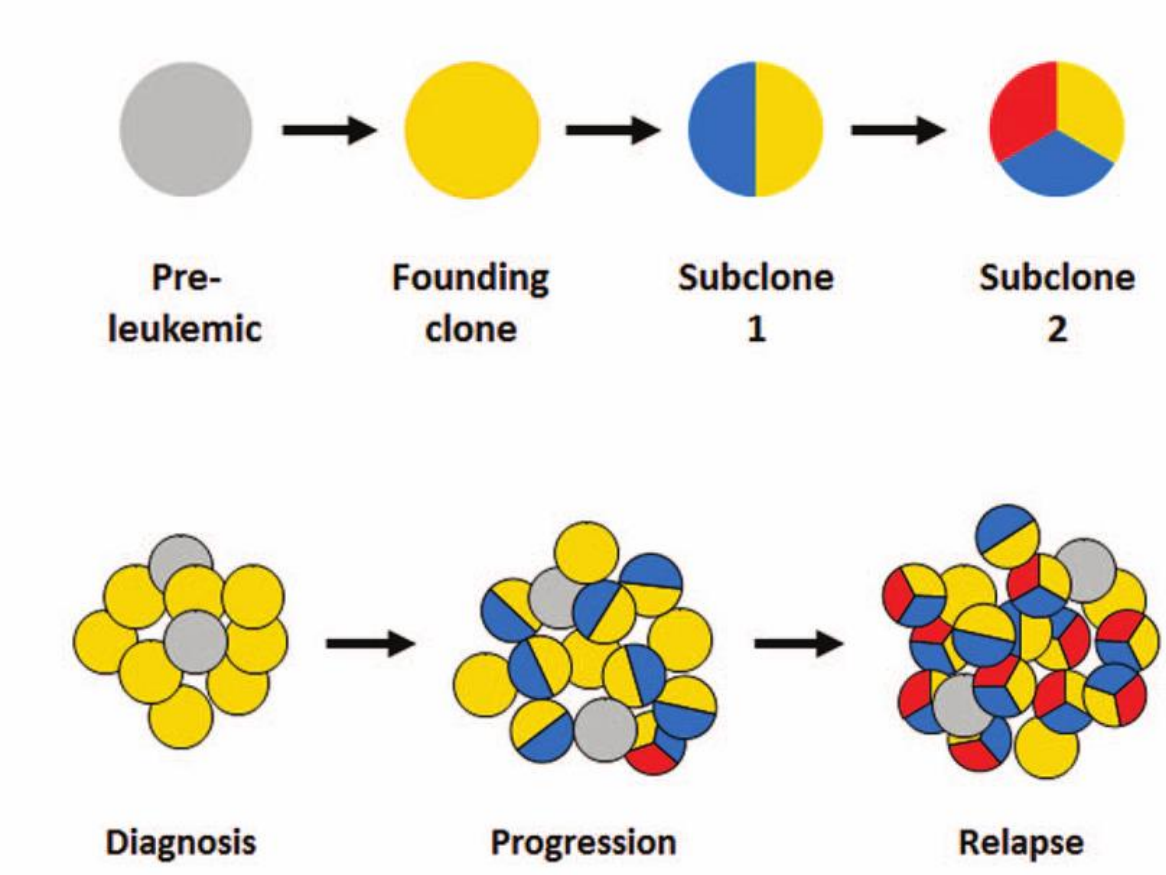


Kao H. et al, 2014

Clonal hematopoiesis (CH)

Premalignant clonal state induced by somatic mutations in hematopoietic stem cells.

- Timing and order during clonal evolution (population based)
- First acquisition of CH mutation(s): such as DNMT3A, TET2, ASXL1 and JAK2.
- Second acquisition additional mutation(s): such as IDH1/2, NPM1, RAS, FLT3, TP53.



Graubert, T.A. et al, 2014

Current therapies AML

- First line treatment = Chemotherapy
- Bone marrow/stem cell transplantation
- Immunotherapy

- Overall surival ~70% but still ~30% of patients experience relapse
- Bulk sequencing already suggests clonal heterogeneity

Aim of this colloquium

Main question: How can clonal heterogeneity in AML be mapped at the cell biological level?

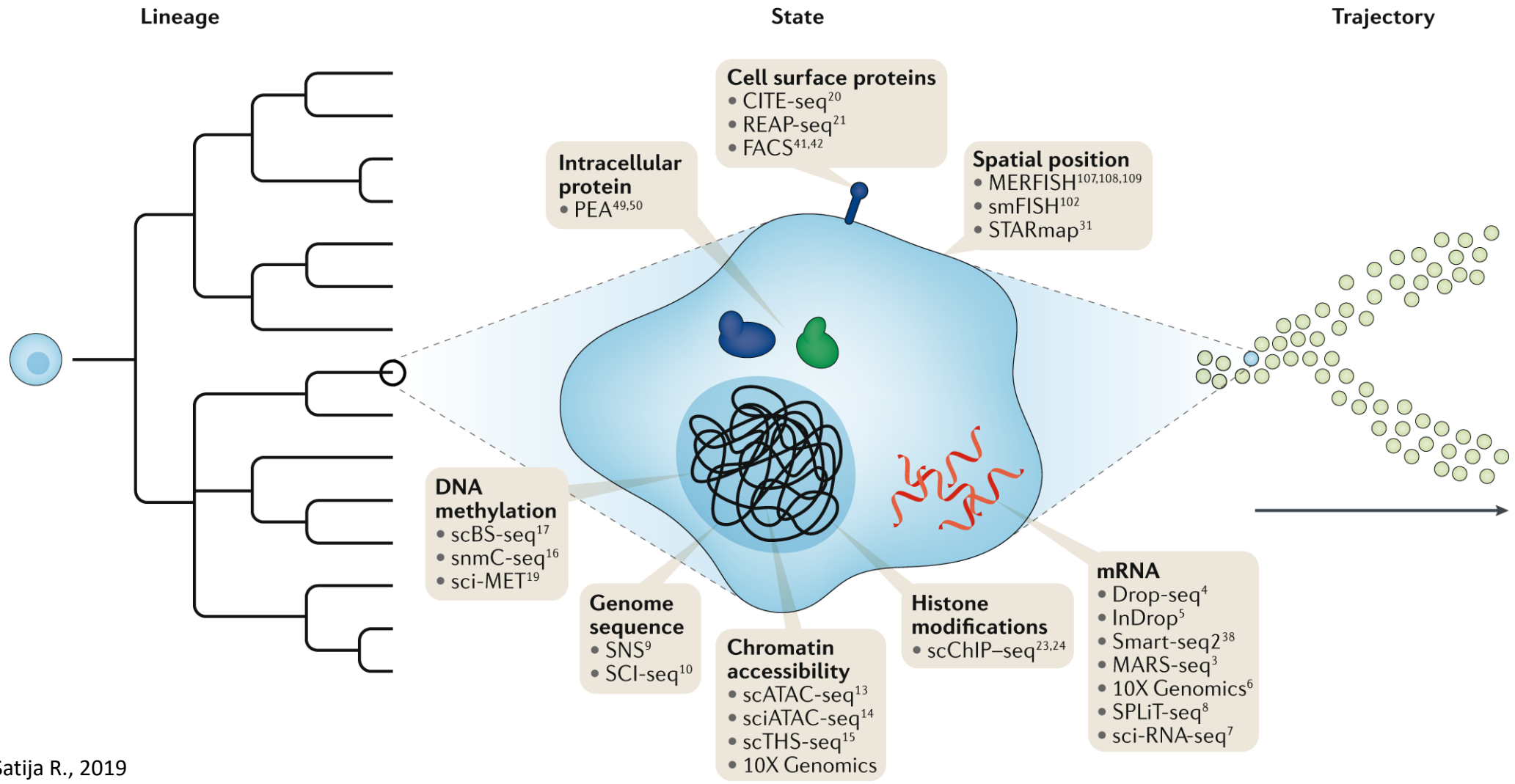
Additional questions:

- What are the sequential events that progress to AML?
- What are the differences between the AML subclones?
- How does the environment of the cells change the fitness of the clones?

Outline

- Introduction AML
- Current therapies
- **Single cell sequencing**
- Types of single cell sequencing
- Future

Next-generation sequencing at single-cell level



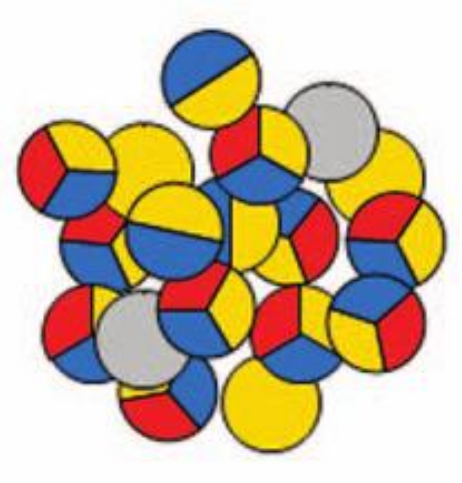
Stuart T. & Satija R., 2019

Characterization of clonal heterogeneity

Contribution to acute myeloid leukemia **progression and relapse.**

Specific combination of mutations promote clonal dominance.

Small number of mutated genes in AML.



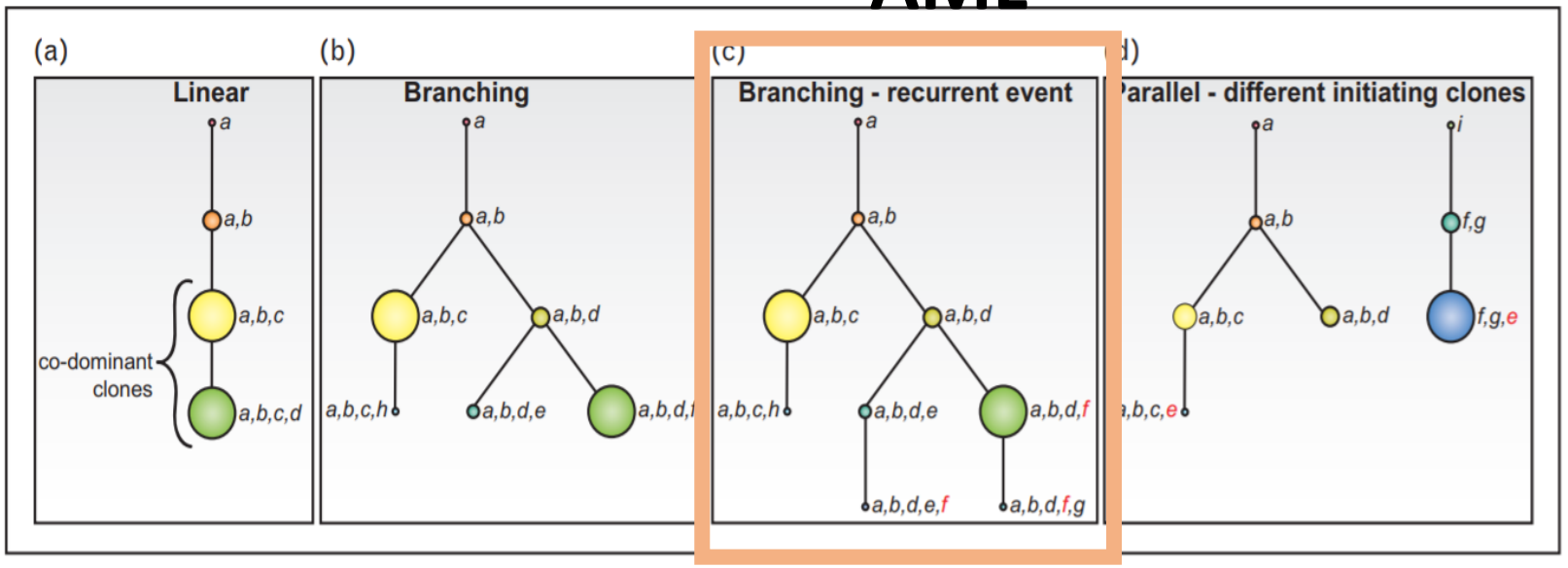
Significant **challenge** in development of new effective therapies **for treatment**. Improbability of ‘one size fits all’.

Distribution subclones variation under the **pressure of immune response.**

Pressure of conventional chemotherapy drugs or targeted **therapies.**

Single-cell level: statements

AML



DNMT3A, IDH1/2

RAS genes, *FLT3*

Epigenetic factors

Mutations signaling genes

Clonal heterogeneity:

1. Not static entity
2. Changes as consequence of treatment

(Romer-Seibert & Meyer, 2021)

Outline

Introduction AML

Current therapies

Single cell sequencing

Types of single cell sequencing

Future

Types of single-cell techniques

Method name	Data type
Single-cell RNA sequencing (scRNA-seq)	mRNA
CITE-seq and REAP-seq	mRNA + cell surface protein
Single-cell DNA sequencing (scDNA-seq)	Whole genome
TARGET-seq	mRNA + genome

Types of single-cell techniques

Method name	Data type
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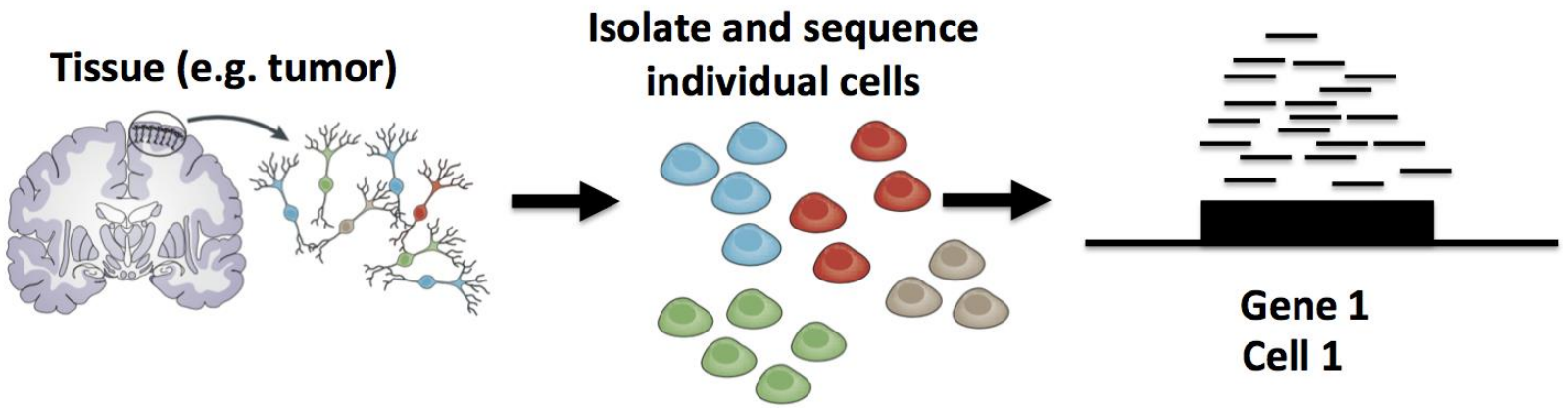
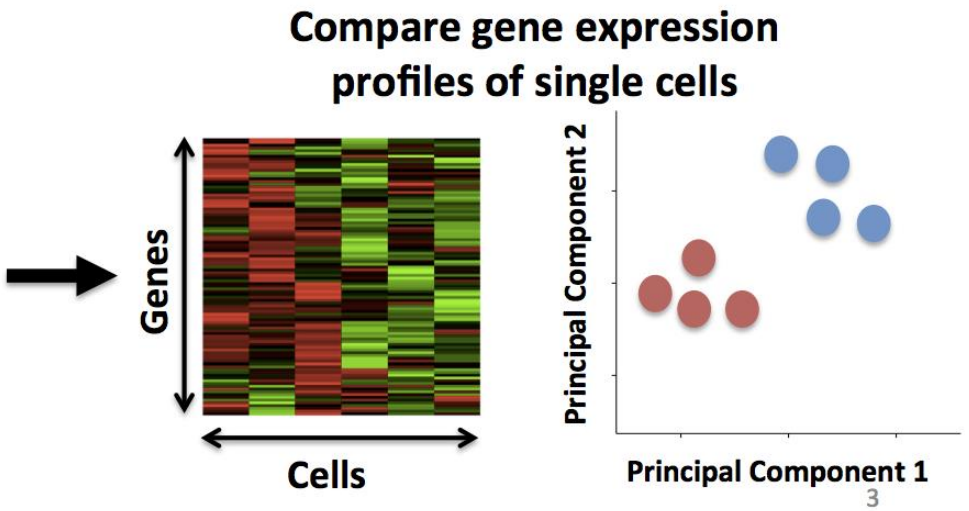
Single-cell RNA sequencing



Read Counts

	Cell 1	Cell 2	…
Gene 1	18	0	
Gene 2	1010	506	
Gene 3	0	49	
Gene 4	22	0	
…			



scRNA-seq

PROS

01

Different cell types
Better analyses and understanding of different cell types in BM samples

02

Transcriptional heterogeneity
Better analyses and understanding of clonal evolution and tumor heterogeneity

CONS

01

Specific detection
Only detection of small part of the transcript, due to small amount of available material

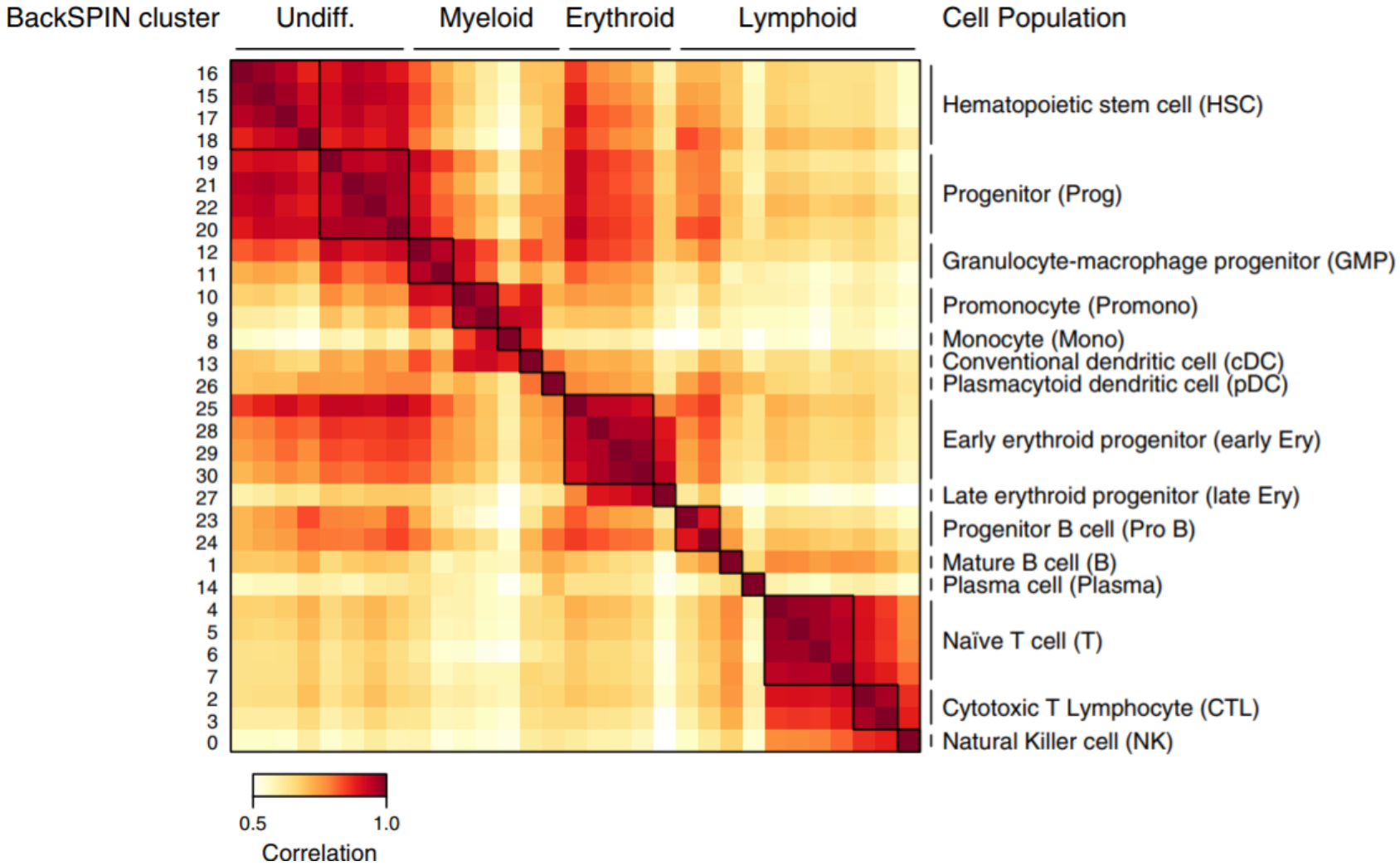
02

Cell death
The to be detected cell will die after mRNA is taken out

03

Cost-intense
More reagents per cell are needed, costs are 10-20 times higer than for bulk sequencing

scRNA-seq: cellular diversity in bone marrow



Van Galen P. et al., 2019

Types of single-cell techniques

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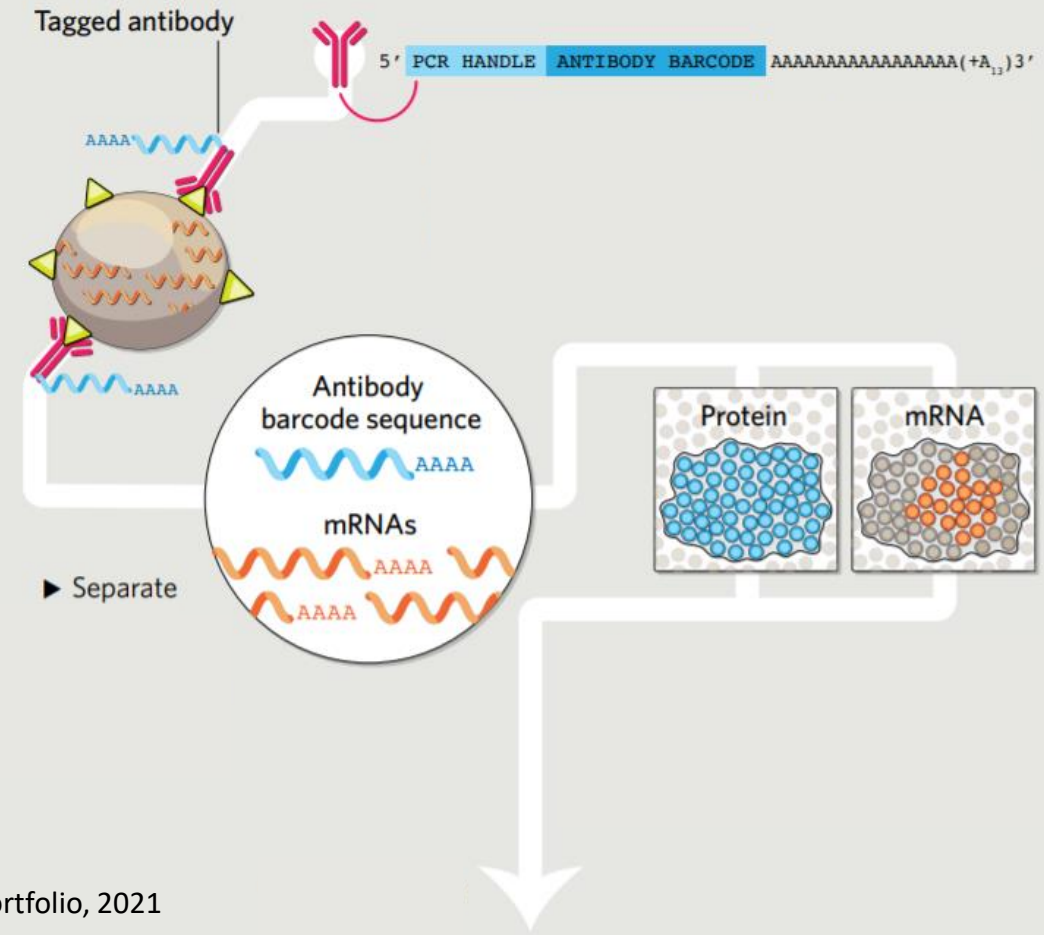
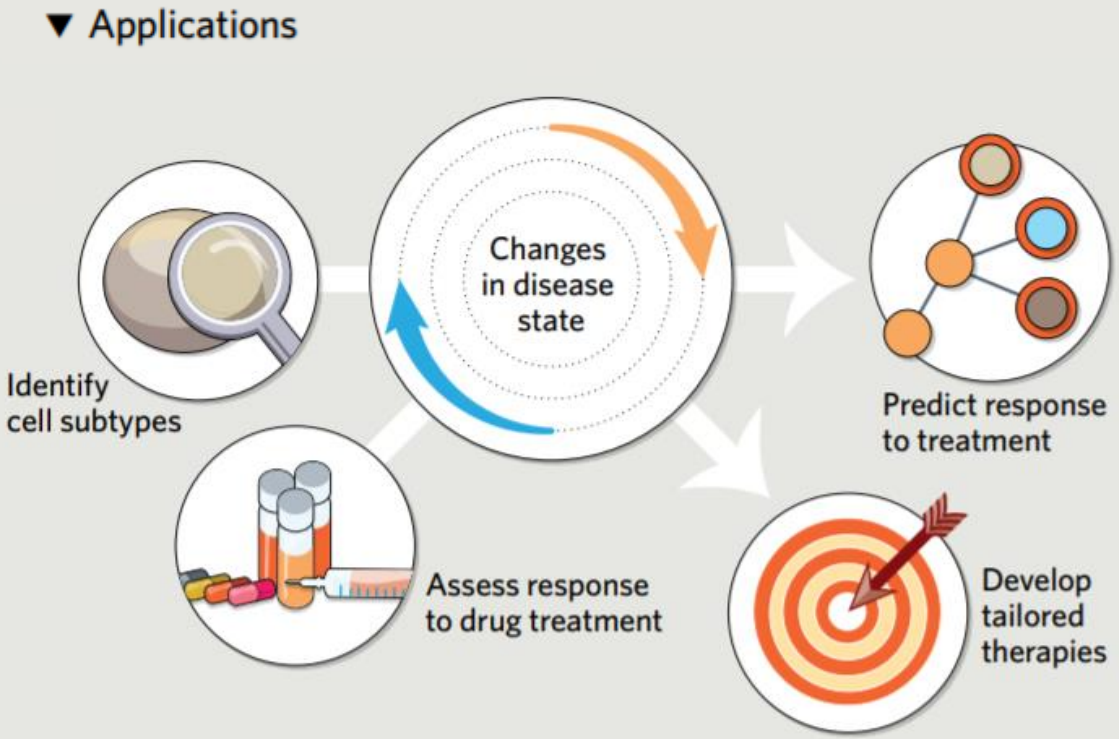
CITE-seq & REAP-seq





Nature portfolio, 2021

CITE-seq & REAP-seq

PROS

01

Low amount RNA
Detection of protein of interest even if RNA is in low abundance

02

Availability antibodies
More than 200 oligo-conjugated antibodies have become available

03

Characterization subtype
Enhancement of characterization of known subtypes and identification of the role of subsets of cell types in immune response

CONS

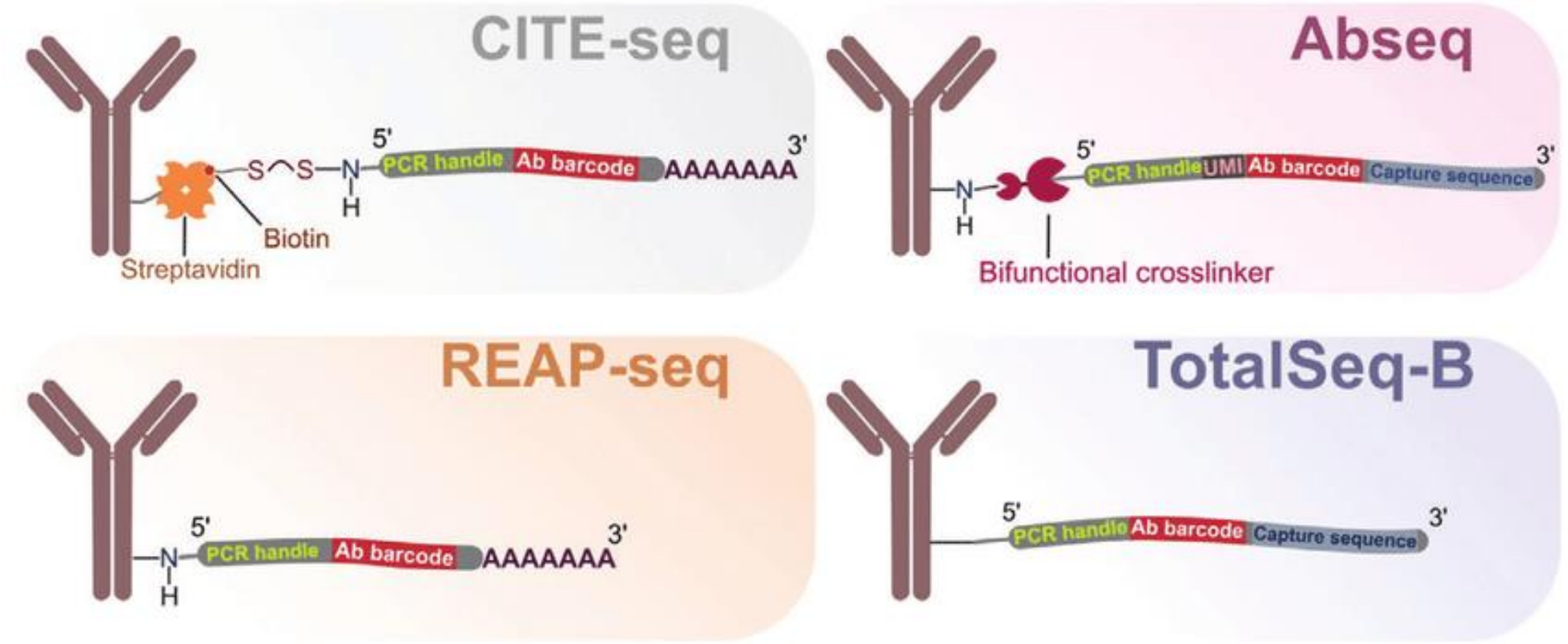
01

Cell death
The to be detected cell will die after mRNA is taken out

02

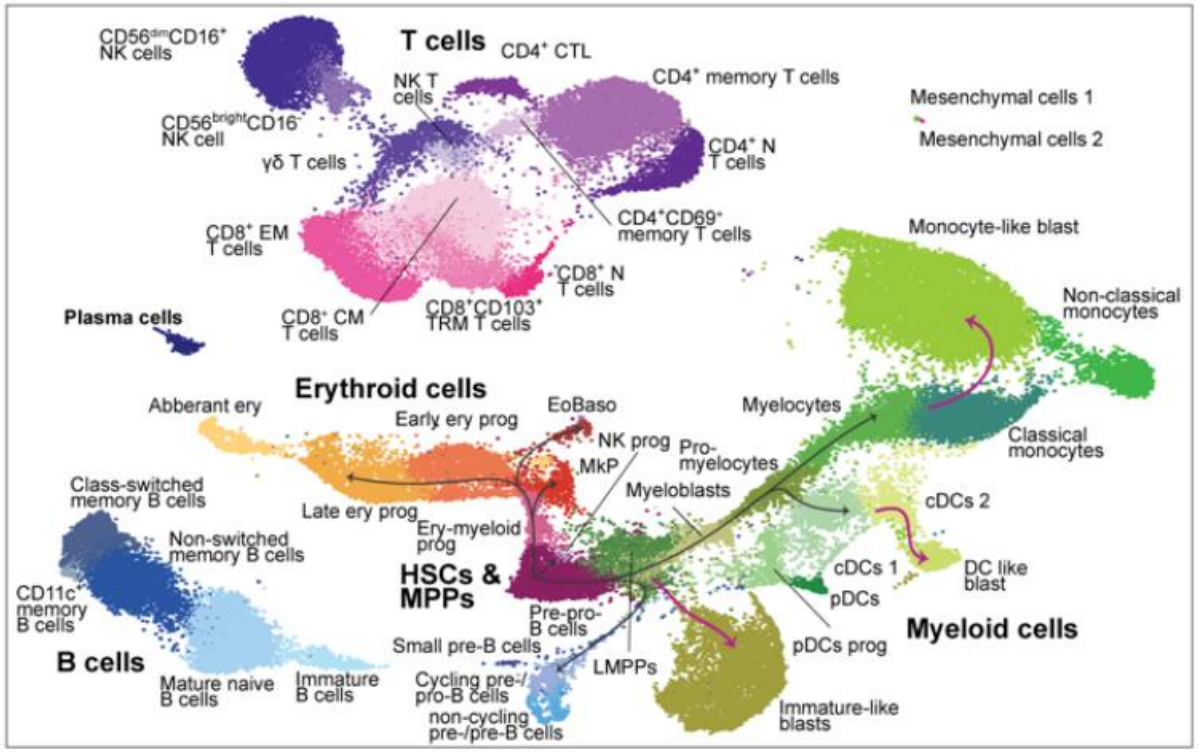
Cost-intense
More reagents per cell are needed, costs are 10-20 times higer than for bulk sequencing

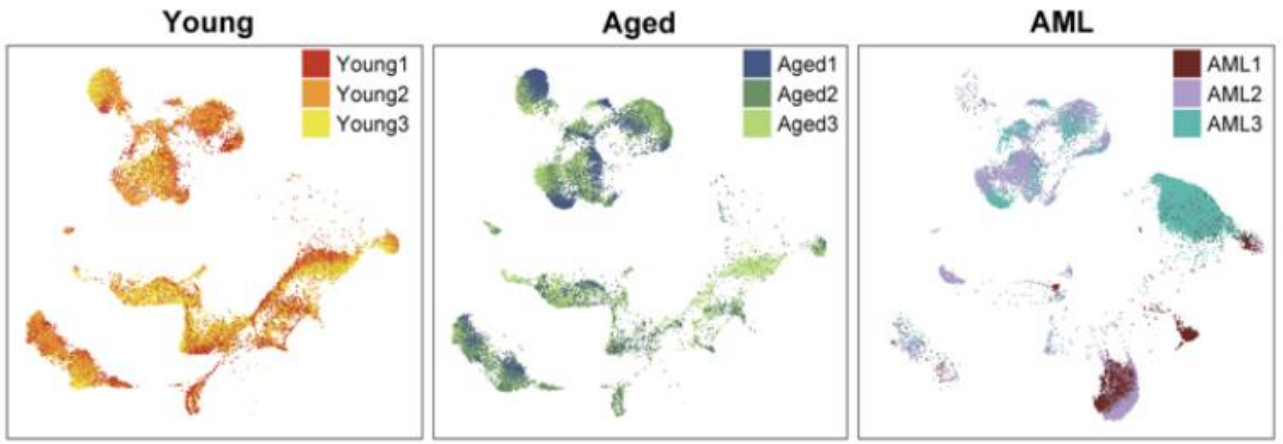
Antibody-oligonucleotides



https://www.researchgate.net/figure/Comparison-of-the-labeled-antibodies-used-for-single-cell-proteome-analysis-protocols_fig6_337548474

Abseq + scRNAseq: reference map of the hematopoietic system





-> Explanation of cell type identities, differentiation stages and biological processes.

Triana S.H. et al., 2021

Types of single-cell techniques

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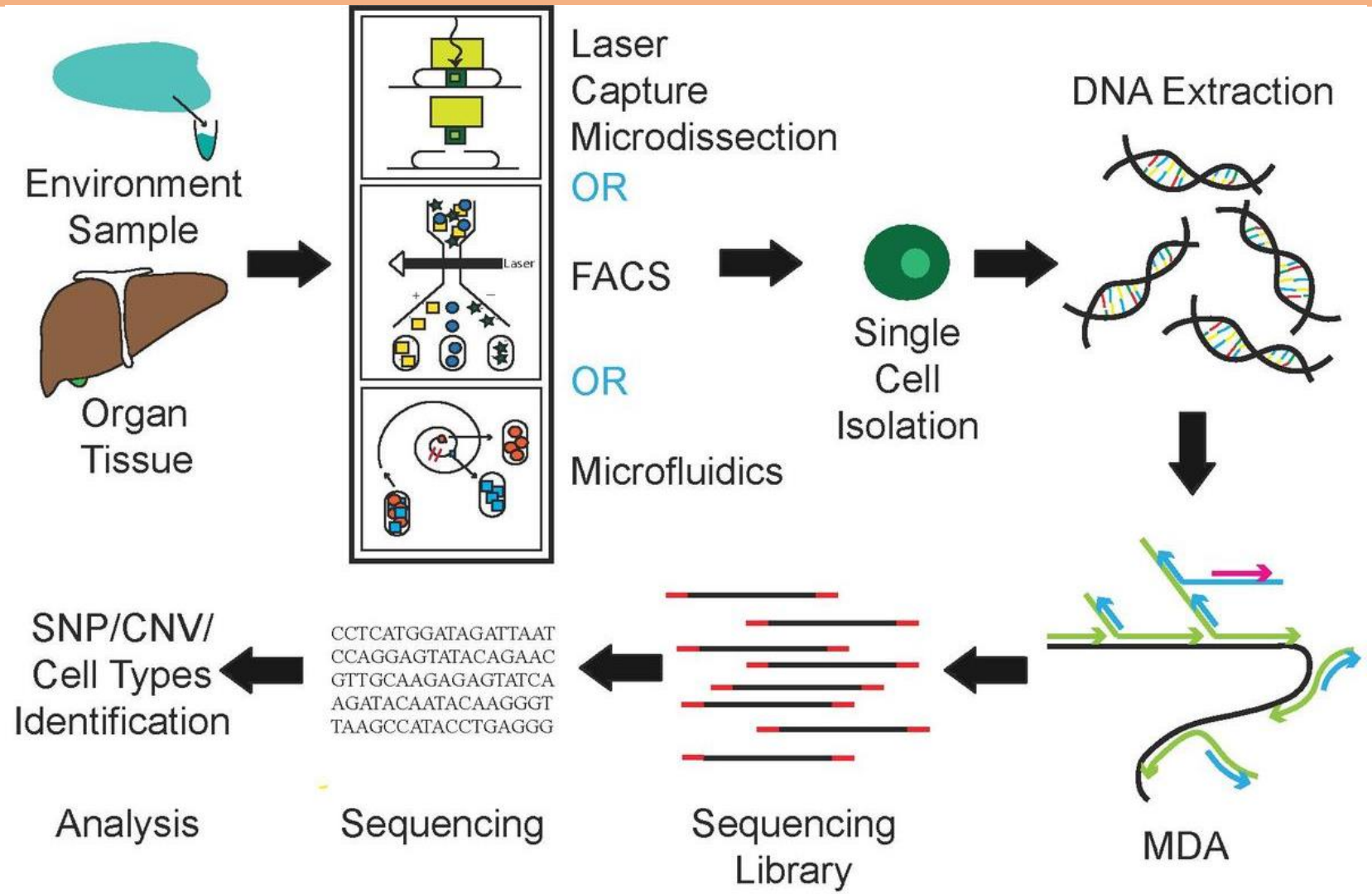
TARGET-seq	Transcriptome + genome

Single-cell DNA sequencing



Wikipedia, 2014

ScDNA-seq

PROS

01

Identify mutations
Identify true mutation co-occurrence in clonal populations

02

Zygosity state
Separates heterozygous and homozygous mutations from each other

03

Clonal heterogeneity
Provides the true measure of clonal heterogeneity and clonal architecture in a tumor.

CONS

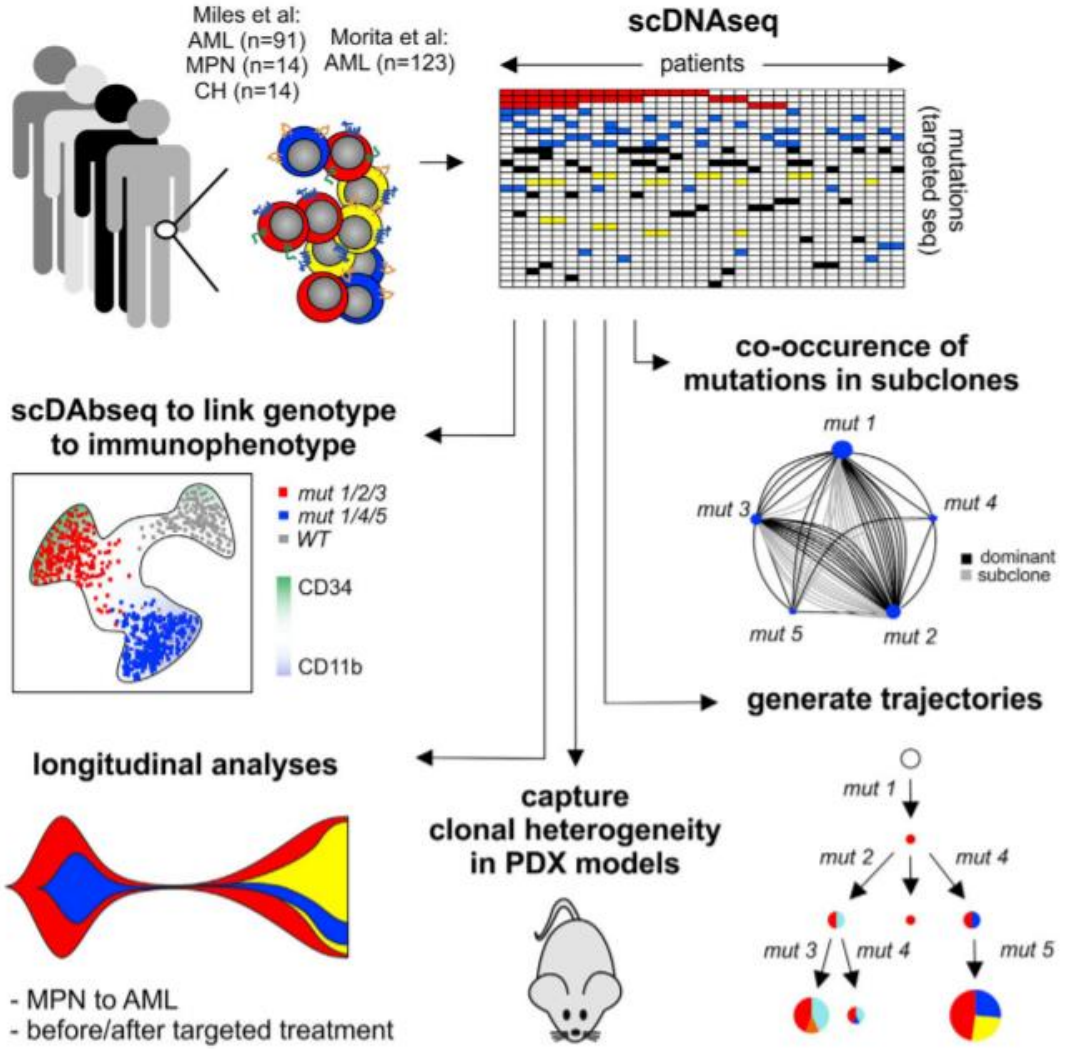
01

Cell death
The to be detected cell will die after DNA is taken out

02

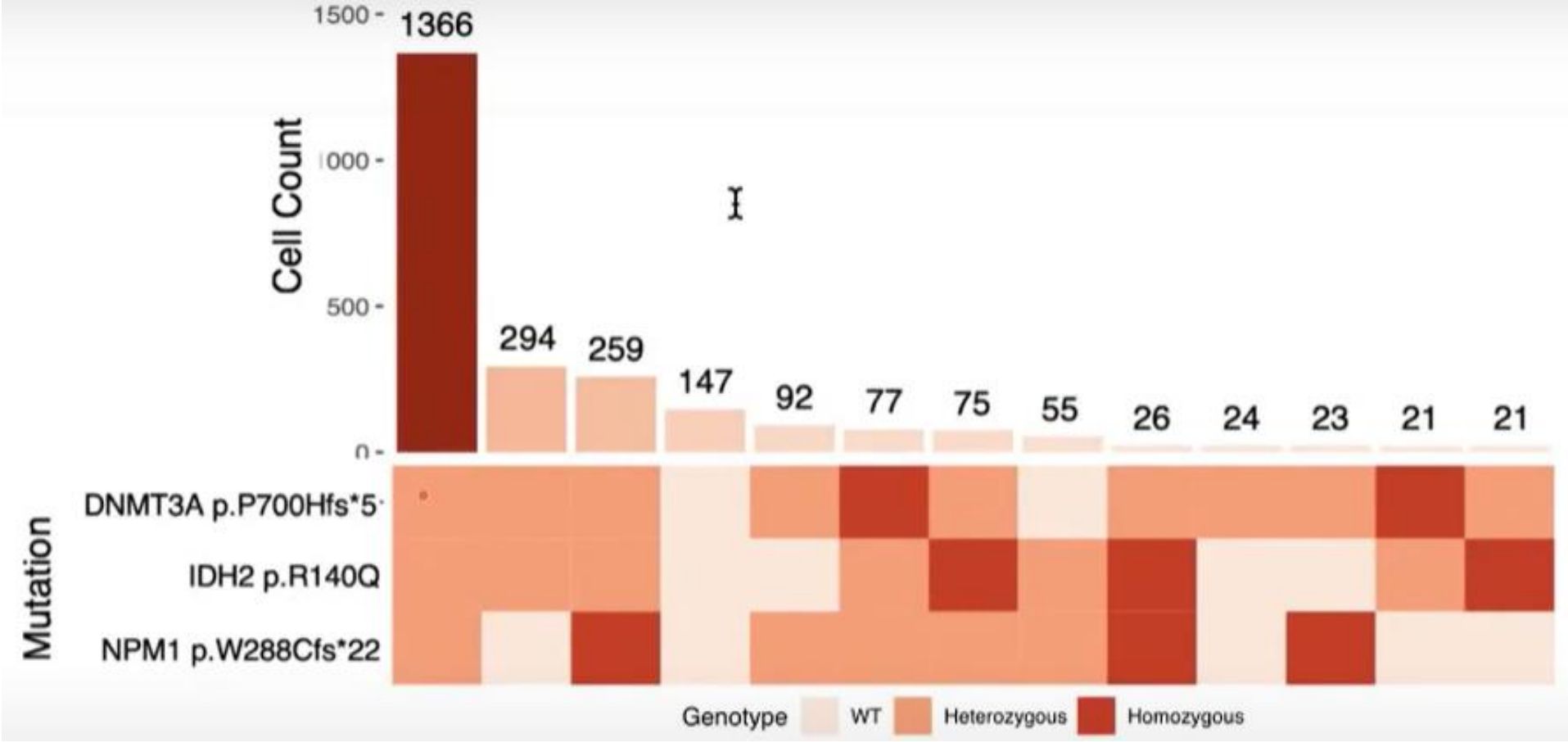
Cost-intense
More reagents per cell are needed, costs are 10-20 times higer than for bulk sequencing

Applications scDNA-seq



Schuringa J.J. & Bonifer C., 2020

Application scDNA-seq: clonal dominance

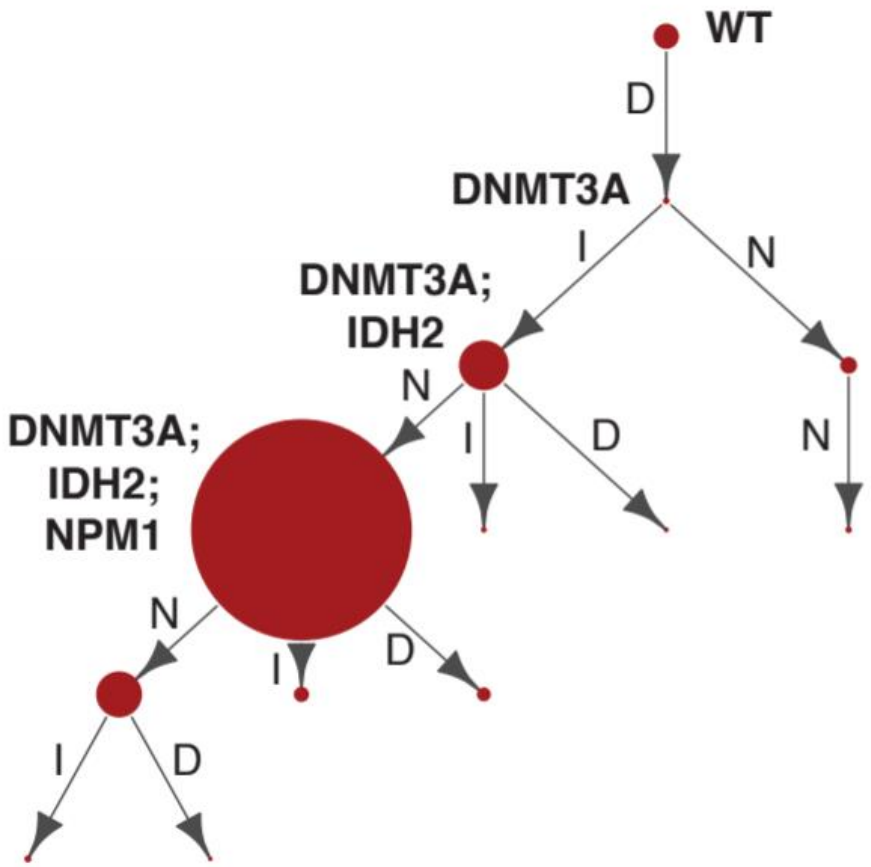


-> Specific mutational combinations lead to competitive advantage/increased fitness.

Miles L.A. et al, 2021

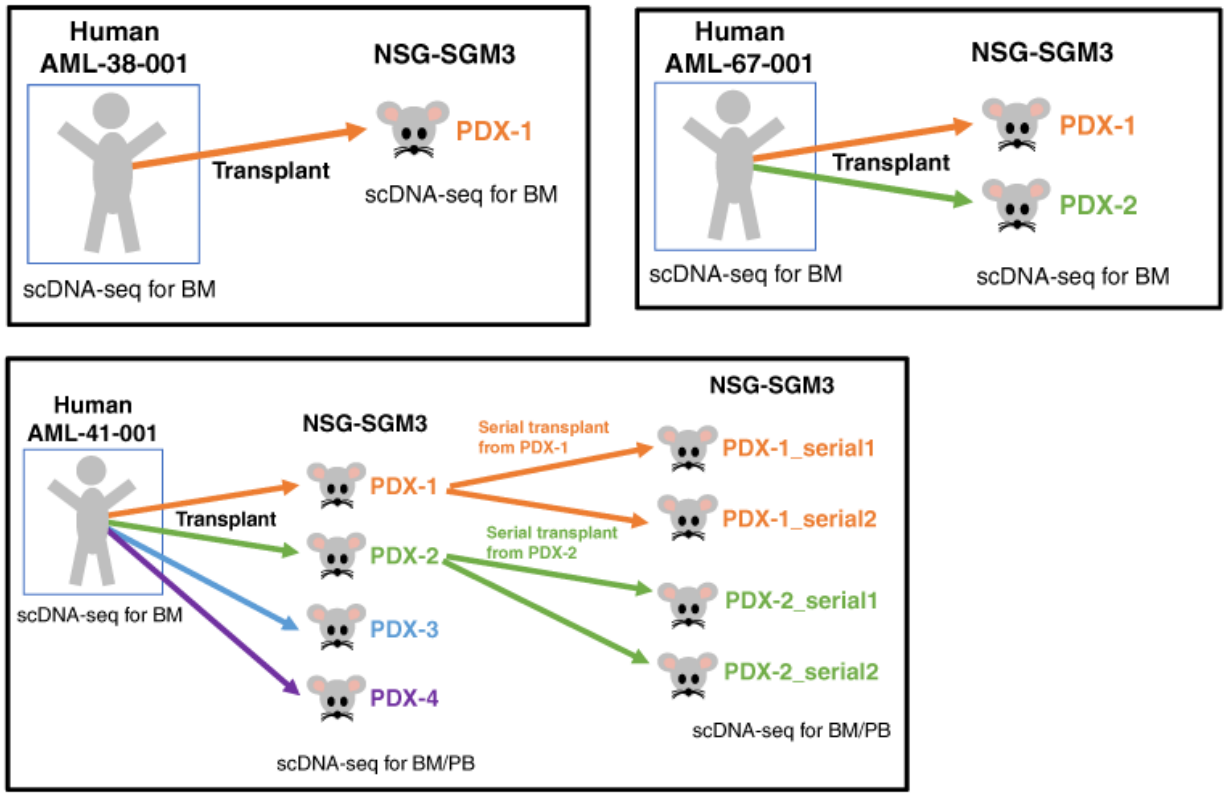
Application scDNA-seq: relative fitness

Variant	Abbr.
DNMT3A P700Hfs*5	D
IDH2 R140Q	I
NPM1 W288Cfs*12	N



Miles L.A. et al, 2021

Application scDNA-seq: PDX models

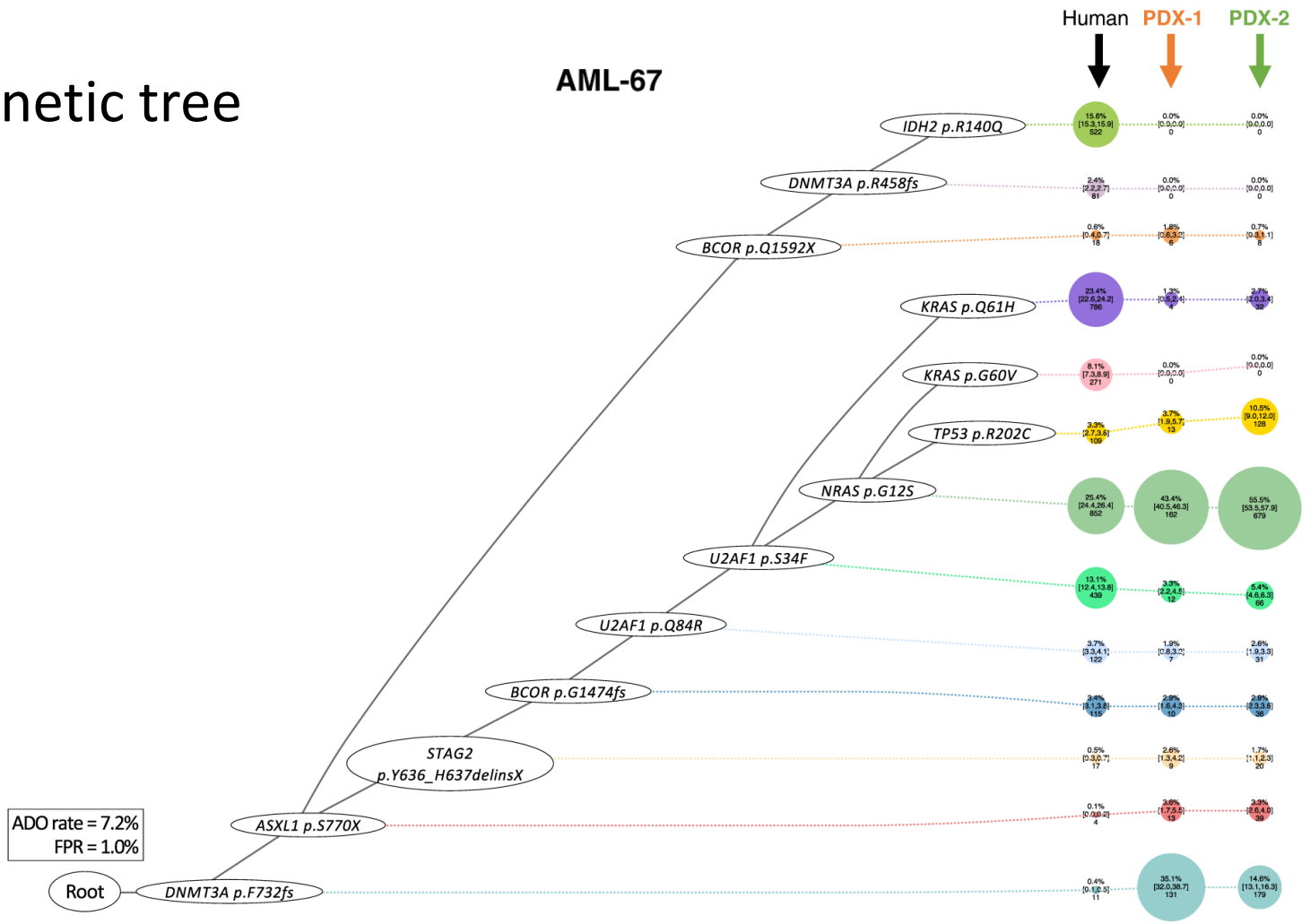


In vivo!

Morita K. et al., 2020

Application scDNA-seq: PDX models

Phylogenetic tree



Morita K. et al., 2020

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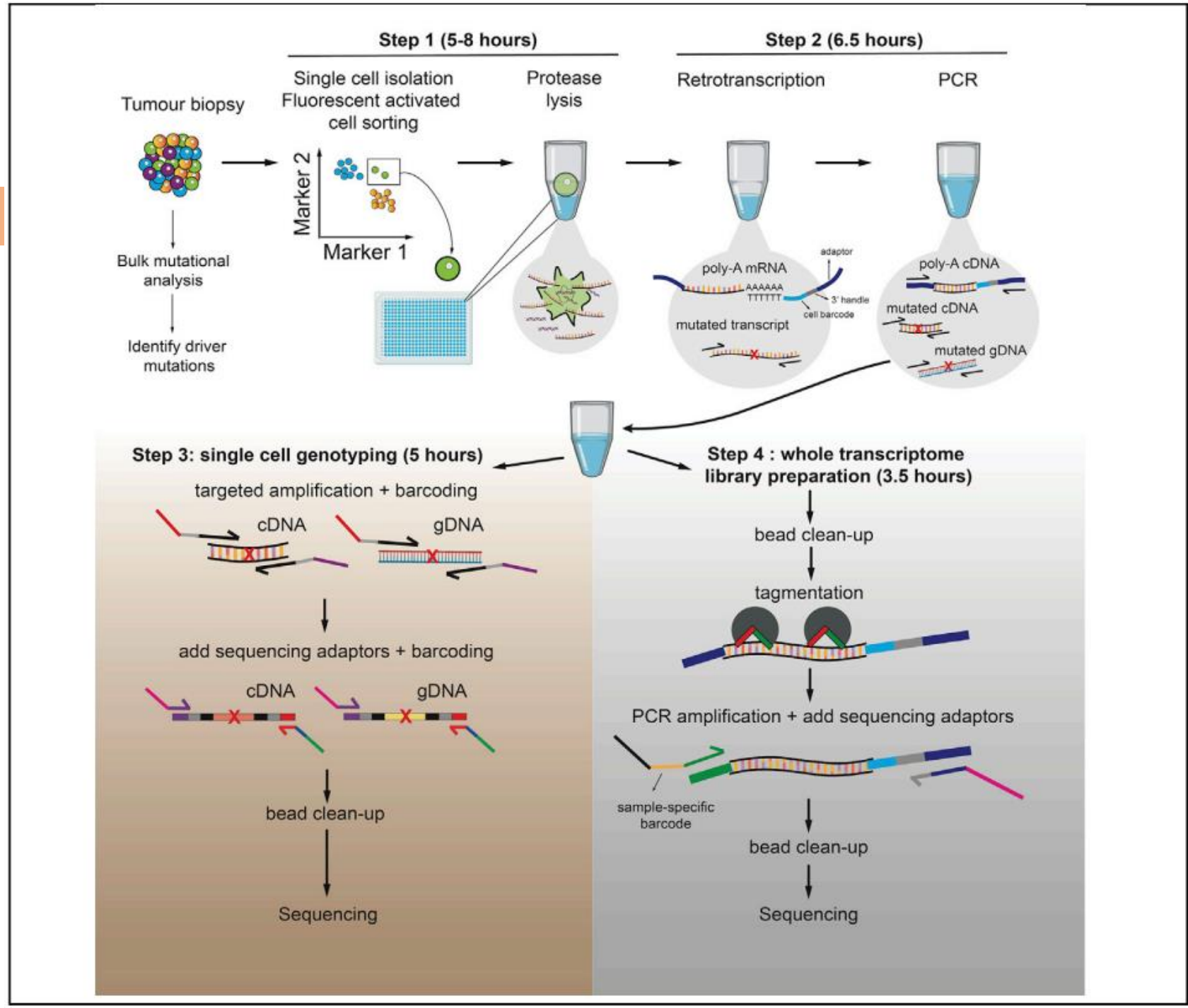
TARGET-seq	Transcriptome + genome

TARGET-seq

- Combination of mRNA and genome sequencing



Rodriguez-Meira A. et al., 2020

TARGET-seq

PROS

01

Transcriptome + genome
Simultaneously detection of whole transcriptomes and genomic DNA

02

Combination technique
All advantages of scRNA-seq and scDNA-seq

CONS

01

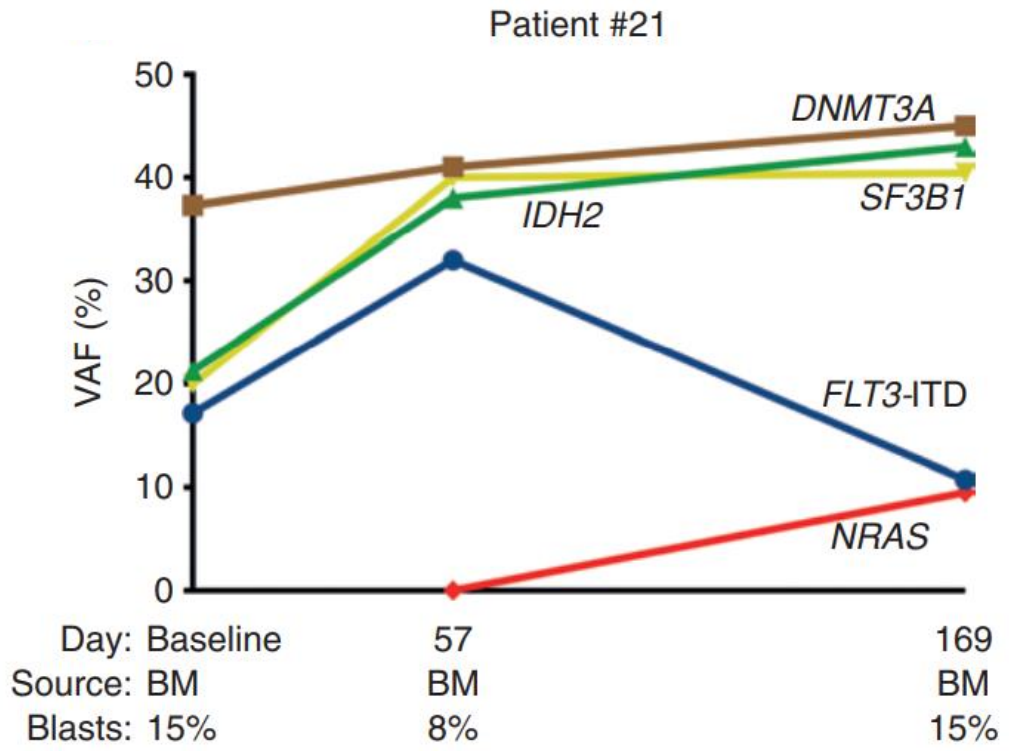
Cell death
The to be detected cell will die after both DNA and mRNA is taken out

02

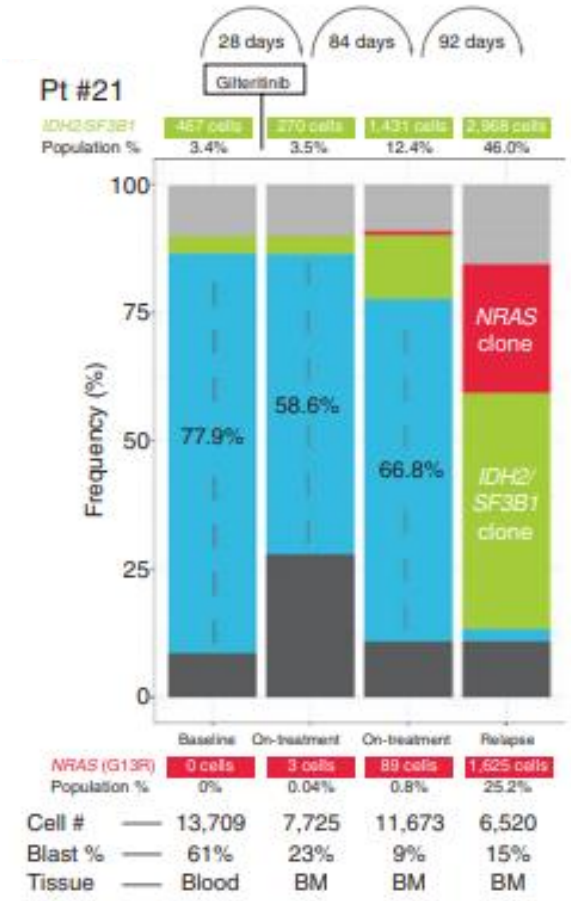
Cost-intense
More reagents per cell are needed, costs are 10-20 times higer than for bulk sequencing

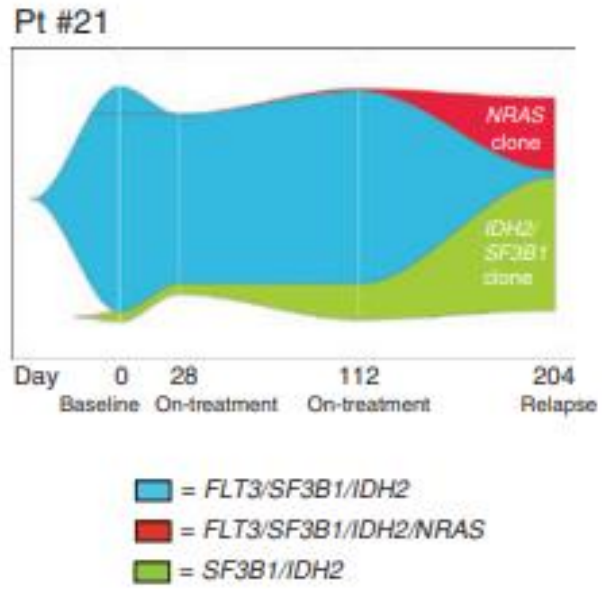
Therapy resistance: Gilteritinib

Bulk sequencing



scDNA-seq

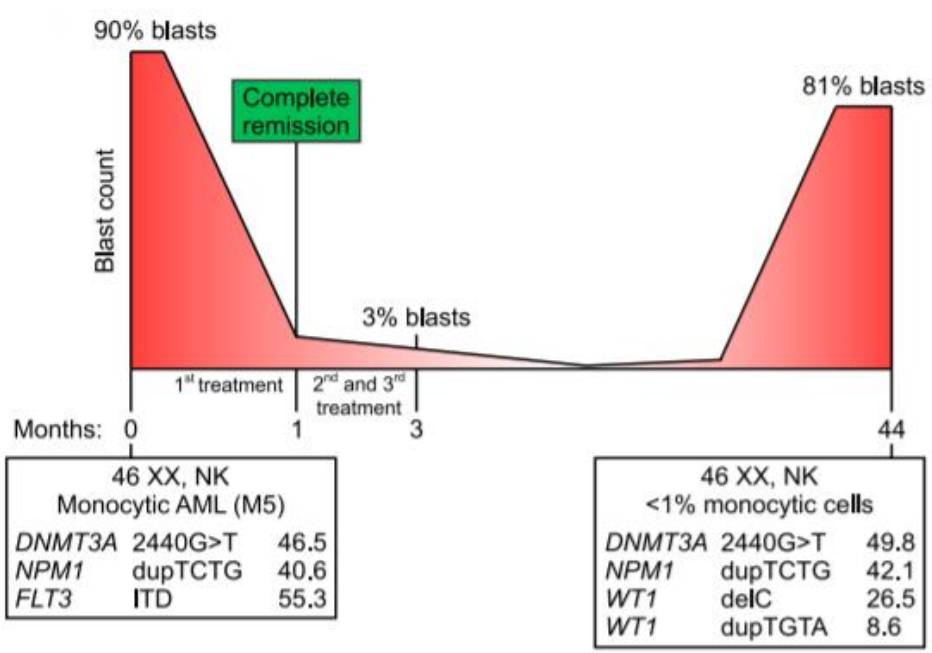


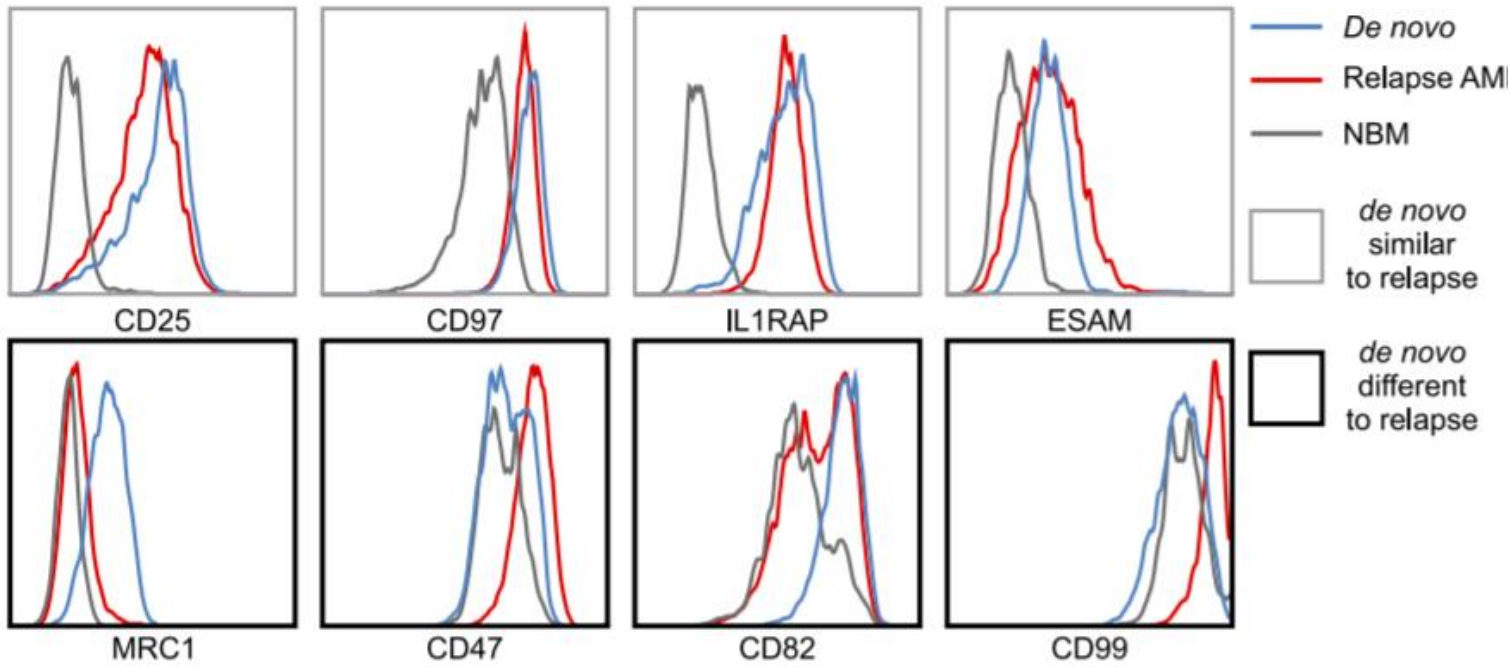


McMahon C.M. et al, 2019

Relapse: PM marker expression

Leukemic clones change genetically over time, uncovered by plasma membrane (PM) marker expression.





De Boer B. et al., 2018

Relapse and drug resistance

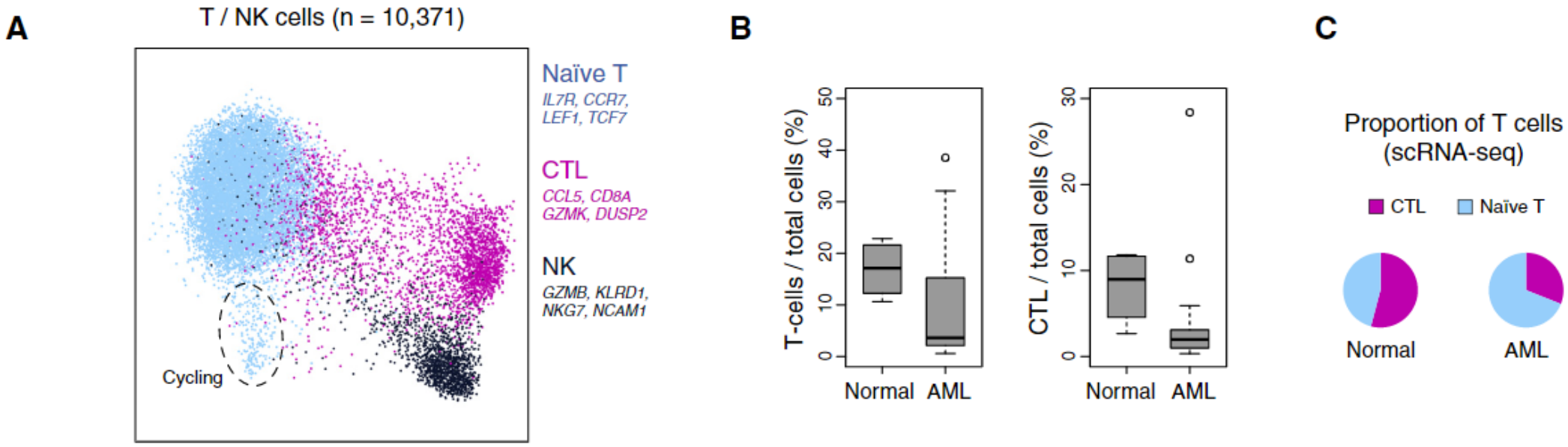
Hypotheses

- Crosstalk between the amount of space in the bone marrow niche and the amount of leukemic stem cells.

- Chemotherapy or drug therapy can eridacate the dominant subclones, but leaves the minor subclones behind which can become dominant after treatment.

-> Microenvironment?

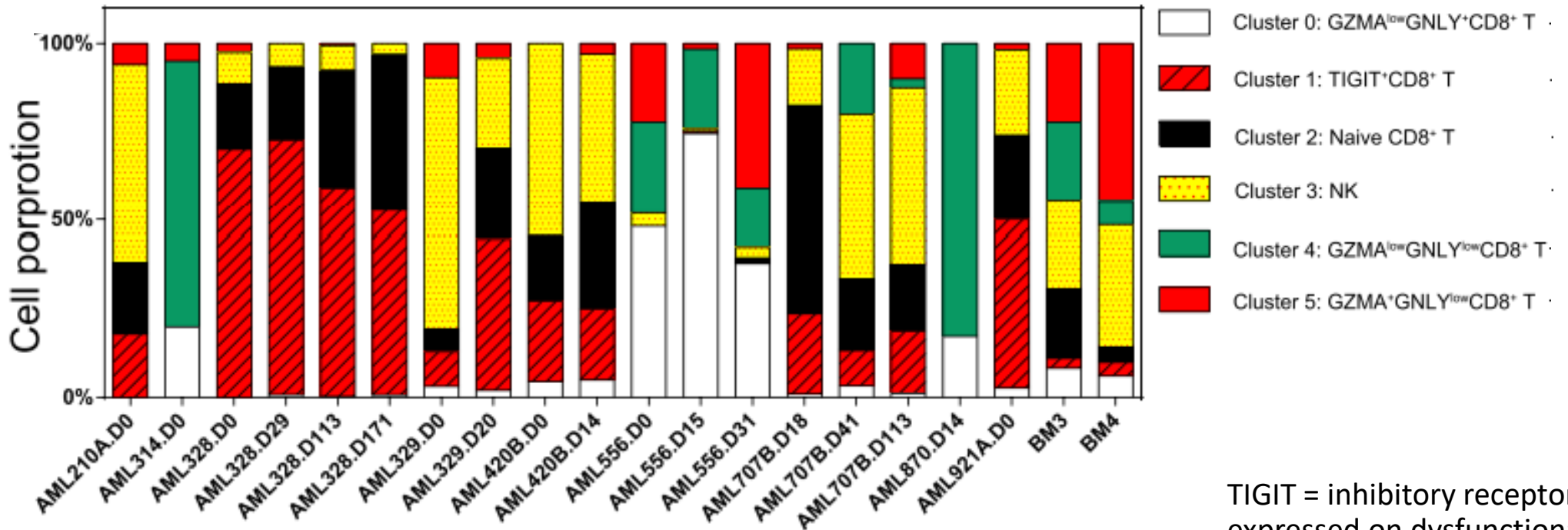
The microenvironment: T cell exhaustion



Van Galen P. et al., 2019

The microenvironment: CD8^{+}-T/NK subsets

scRNA-seq



TIGIT = inhibitory receptor expressed on dysfunctional T cells

Guo R. et al., 2021

Summary

Main question: How can clonal heterogeneity in AML be mapped at the cell biological level?

-> Multiple types of single-cell techniques

-> Unravel many aspects of clonal heterogeneity

Outline

Introduction AML

Current therapies

Single cell sequencing

Types of single cell sequencing

Future

Future

- Personalized therapy
- TARGET-seq promising
- Prevention relapse
- Role of the microenvironment

Thank you!

- Questions?

Current therapies AML

Immunotherapy

AML Vaccines are being tested for humans.

Vaccination is an attractive strategy for patients who are not eligible for HSCT or who relapse following HSCT. To date, three main types of vaccines are being tested in humans for AML: peptide, granulocyte macrophage colony stimulating factor (GM-CSF), and dendritic cell (DC) vaccines.

Immune Checkpoint Inhibitors (ICPIs)

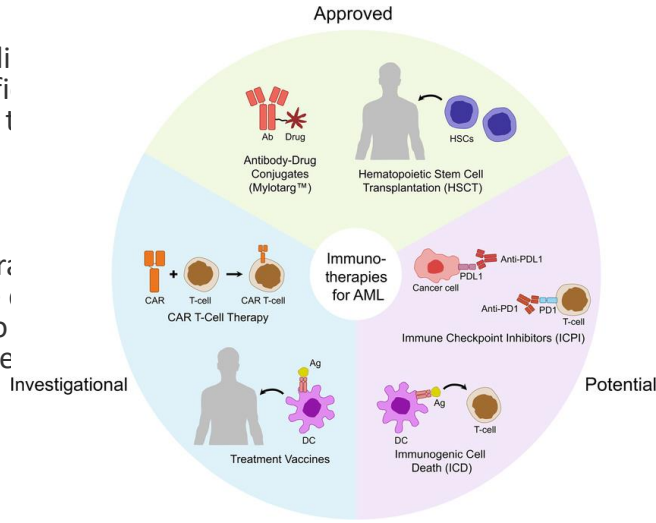
ICPIs have been associated with remarkable treatment outcomes in various solid tumours including NSCLC and melanoma (57). ICPIs involve the removal of immunosuppressive signals that are often used as a mechanism by cancer cells to evade immune detection. The role of these agents will become more clear from ongoing clinical trials.

Immunomodulators for Immunogenic Cell Death Induction

Immunogenic cell death (ICD) has emerged in recent years as a popular immunotherapeutic concept that is being investigated pre-cli
ICD is a form of cell death wherein cancer cells, in the process of treatment-induced death, emit certain molecular signals in a specifi
result in the recruitment of immune cells, presentation of tumour-specific antigens, and activation of an adaptive immune response t
eradication and generation of immunological memory against future re-challenges (93,94,95)

Chimeric Antigen Receptor T (CAR-T) Cell Therapy

CAR-T therapy has been greatly successful in the treatment of some hematologic malignancies but this accomplishment has yet to tra
are patient-derived T cells that have been genetically modified to recognize antigens expressed on the cancer cell's surface (82). The
AML, however, has been much more challenging compared to that for B cell malignancies due to poor immunogenicity stemming fro
of AML-specific antigens leading to the risk of generating on-target off-leukemia toxicities, and the heterogeneous biology of the dise
progenitors



Chen K.T.J. et al, 2019

Variant allele frequency (VAF)

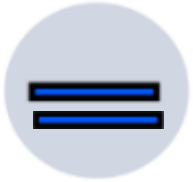
- Heterozygous vs homozygous mutations

Example

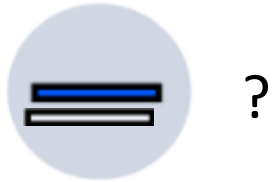
DNTM3a
VAF = 0,5 (Heterozygous)

FLT3
VAF = 0,3

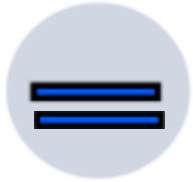
?



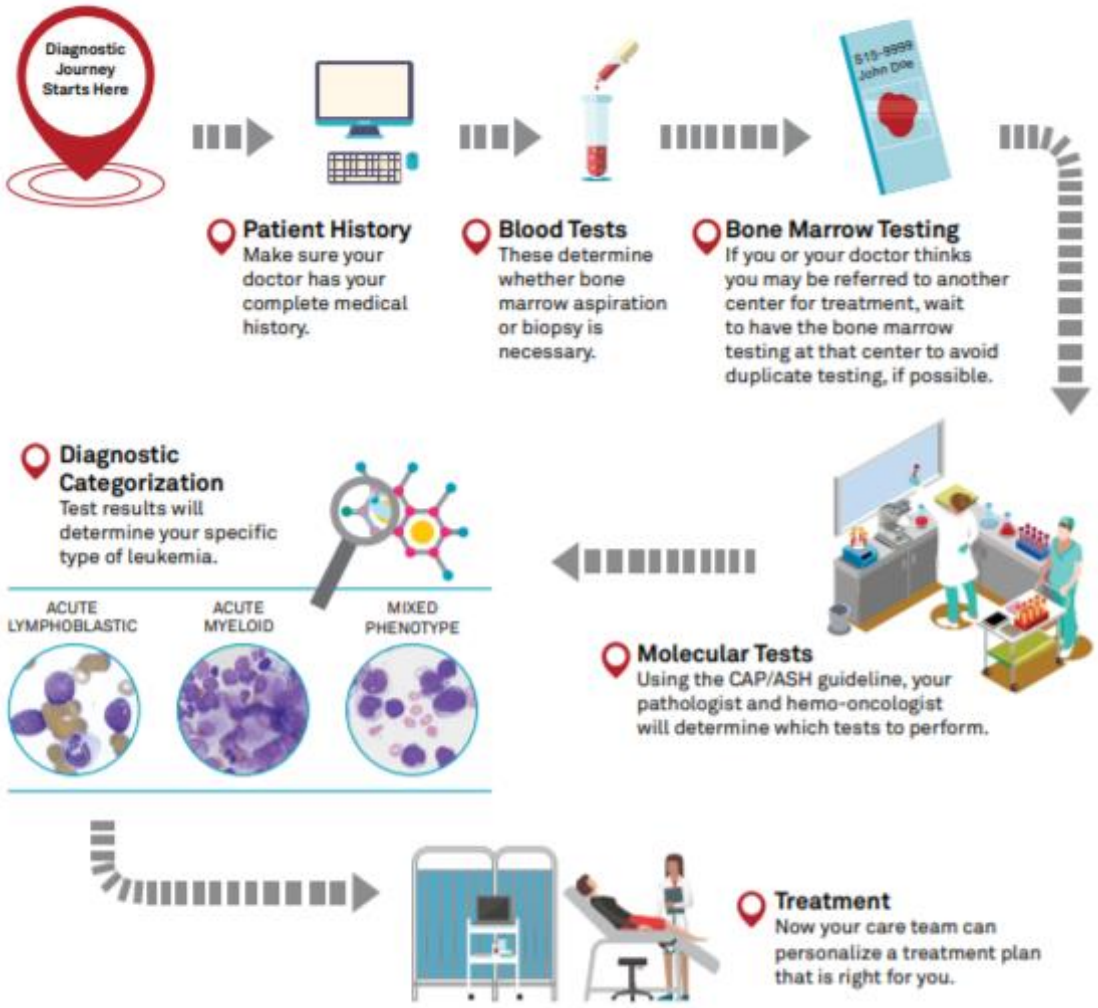
Additional mutation
VAF = 0,2



?



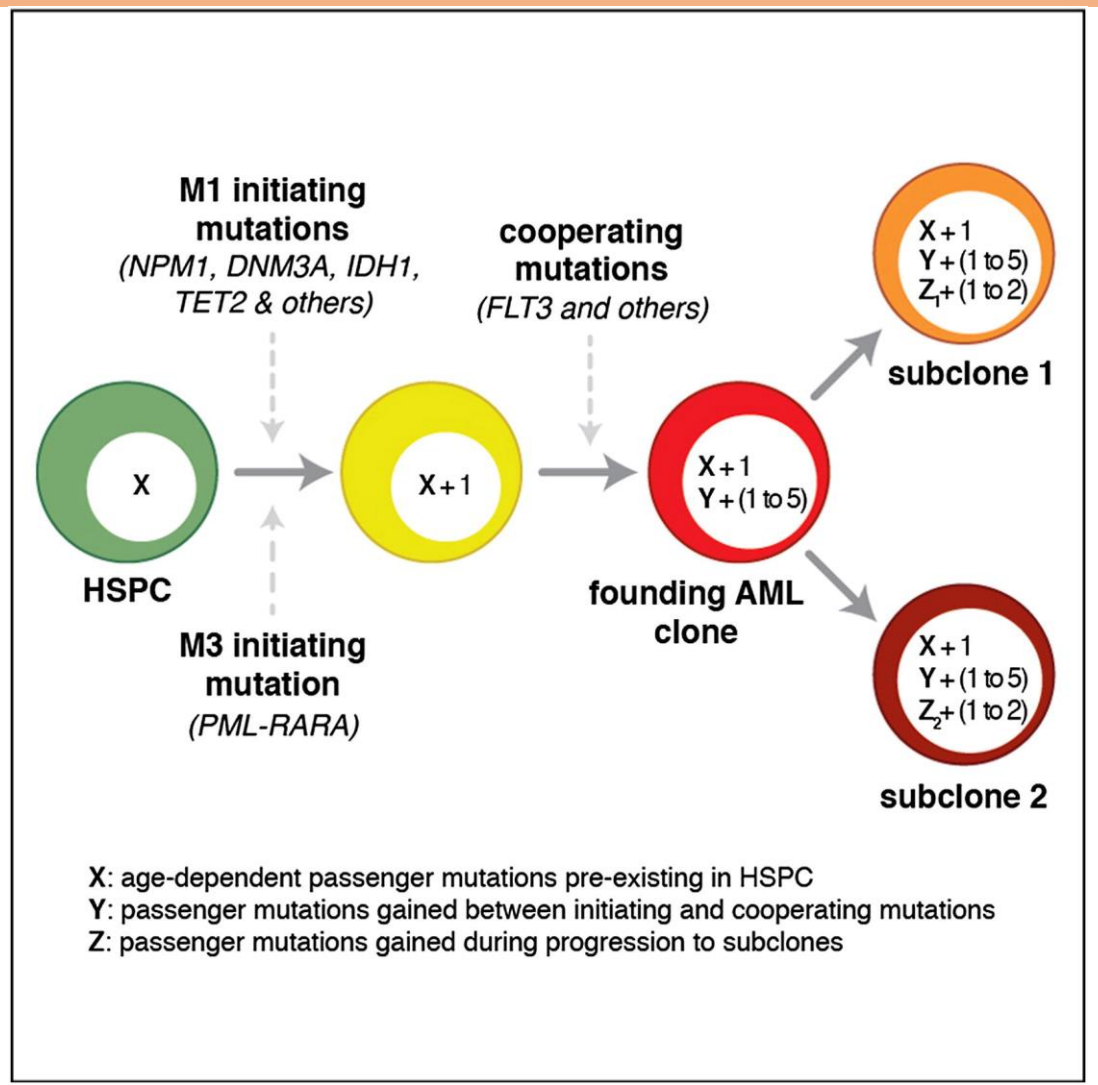
Diagnosis AML



https://www.lls.org/leukemia/[illegible]
leukemia/diagnosis

Clonal evolution in AML

- Timing and order during clonal evolution (population based)
- First acquisition of CH mutation(s): such as DNMT3A, TET2, ASXL1 and JAK2.
- Second acquisition additional mutation(s): such as IDH1/2, NPM1, RAS, FLT3, TP53.

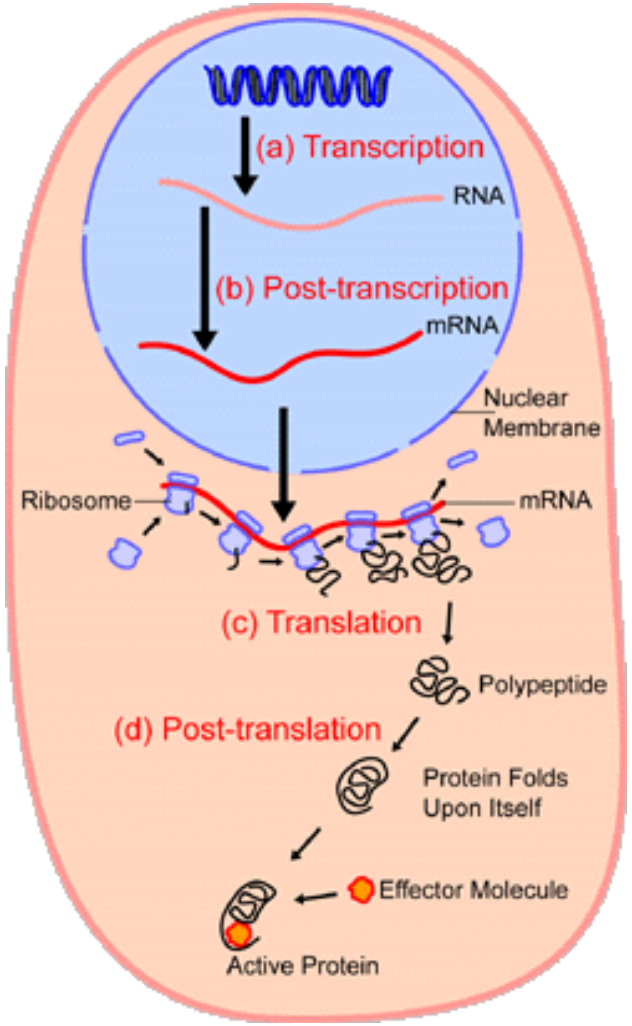


Welch J.S. et al., 2012

Targeted Therapy Drugs AML

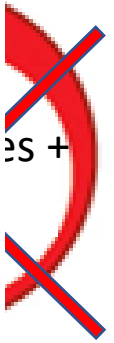
Drug	Example
FLT3 inhibitors	Gilteritinib (Xospata)
IDH inhibitors	Ivosidenib (Tibsovo)
Antibody-drug conjugate	Gemtuzumab ozogamicin (Mylotarg)
BCL-2 inhibitor	Venetoclax (Venclexta)
Hedgehog pathway inhibitor	Glasdegib (Daurismo)

Single cell


(a) Transcription
RNA
(b) Post-transcription
mRNA
Nuclear Membrane
Ribosome
mRNA
(c) Translation
Polypeptide
(d) Post-translation
Protein Folds Upon Itself
Effector Molecule
Active Protein

Single-cell level: statements

FIGURE 1. Clonal evolution trajectories and clonal diversity. Tree diagrams depict examples of linear (a) and branching (b–d) acute myeloid leukemia evolution trajectories. The size of each clonal node corresponds to the overall fraction of cells in the clone of the total cells sequenced. (a–c) Each tree starts with the acquisition of mutation in gene '*a*'. (a) In the linear model, sequential acquisition of mutations '*b*', '*c*', and then '*d*' in the linear evolution trajectory results in comutational cooperation as the clone size expands with new mutations. (b) In the branching model, cells from clone '*a,b*' acquire two different mutations which expand to make clones '*a,b,c*' and '*a,b,d*' which evolve through separate clonal trajectories, and one additional branch evolves from clone '*a,b,d*'. Although there are four clones in the linear model (a) and seven clones in the branching model (c) illustrating greater clonal complexity in the branching model, there is similar clonal diversity in both models as each has two codominant clones (yellow and green) that comprise the majority of each tumor. (c) Clone '*a,b,d,f*' and clone '*a,b,d,e,f*' are in two separate branched clonal trajectories and have acquired the same gene '*f*' mutation (red) independently late in disease evolution. Clone '*a,b,d,f,g*' descendent from '*a,b,d,f*' also carries the gene '*f*' mutation. (d) Two independent clonal trajectories are evolving in parallel from two distinct initiating mutations in '*a*' and '*i*' then subsequently evolving through in branched and linear trajectories, respectfully. Mutation '*e*' (red) is acquired late in both trajectories.



(Römer-Seibert & Meyer, 2021)

DNMT3A, IDH1/2 — RAS genes, *FLT3*

Epigenetic factors — Mutations signaling genes

Clonal heterogeneity:

1. Not static entity
2. Changes as consequence of treatment

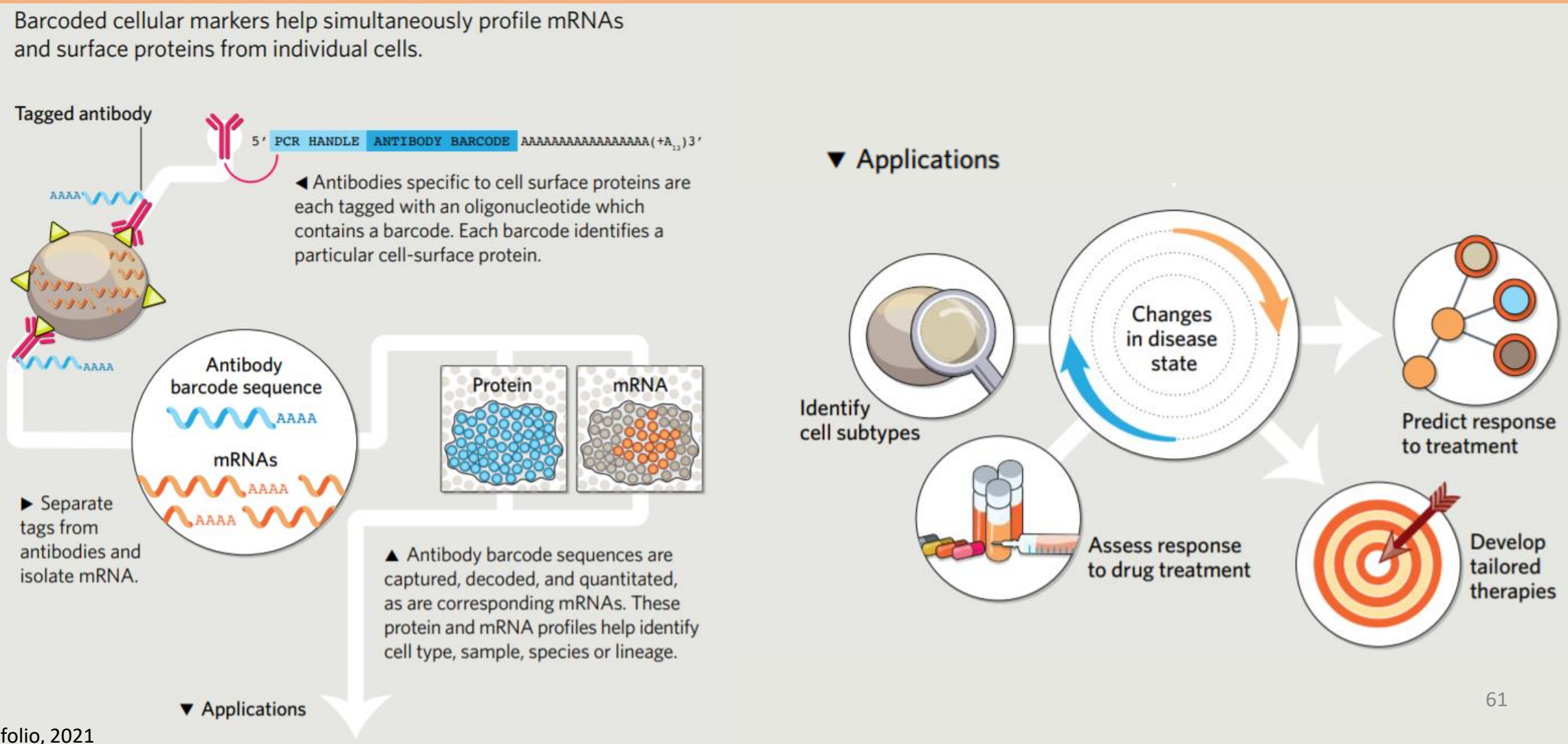
Types single-cell sequencing

Data types	Method name	Feature throughput
Unimodal		
mRNA	Drop-seq	Whole transcriptome
	InDrop	Whole transcriptome
	10X Genomics	Whole transcriptome
	Smart-seq2	Whole transcriptome
	MARS-seq	Whole transcriptome
	CEL-seq	Whole transcriptome
	SPLiT-seq	Whole transcriptome
	sci-RNA-seq	Whole transcriptome
Genome sequence	SNS	Whole genome
	SCI-seq	Whole genome
Chromatin accessibility	scATAC-seq	Whole genome
	sciATAC-seq	Whole genome
	scTHS-seq	Whole genome
DNA methylation	scBS-seq	Whole genome
	snmC-seq	Whole genome
	sci-MET	Whole genome
	scRRBS	Reduced representation genome
Histone modifications	scChIP-seq	Whole genome + single modification
Chromosome conformation	scHi-C-seq	Whole genome

Data types	Method name	Feature throughput
Multimodal		
Histone modifications + spatial	NA	Single locus + single modification
mRNA + lineage	scGESTALT	Whole transcriptome
	ScarTrace	Whole transcriptome
	LINNAEUS	Whole transcriptome
Lineage + spatial	MEMOIR	NA
mRNA + spatial	osmFISH	10–50 RNAs
	STARmap	20–1,000 RNAs
	MERFISH	100–1,000 RNAs
	seqFish	125–250 RNAs
mRNA + cell surface protein	CITE-seq	Whole transcriptome + proteins
	REAP-seq	Whole transcriptome + proteins
mRNA + chromatin accessibility	sci-CAR	Whole transcriptome + whole genome
mRNA + DNA methylation	scM&T-seq	Whole genome
mRNA + genomic DNA	G&T-seq	Whole genome + whole transcriptome
mRNA + intracellular protein	NA	96 mRNAs + 38 proteins
		82 mRNAs + 75 proteins
DNA methylation + chromatin accessibility	scNOMe-seq	Whole genome

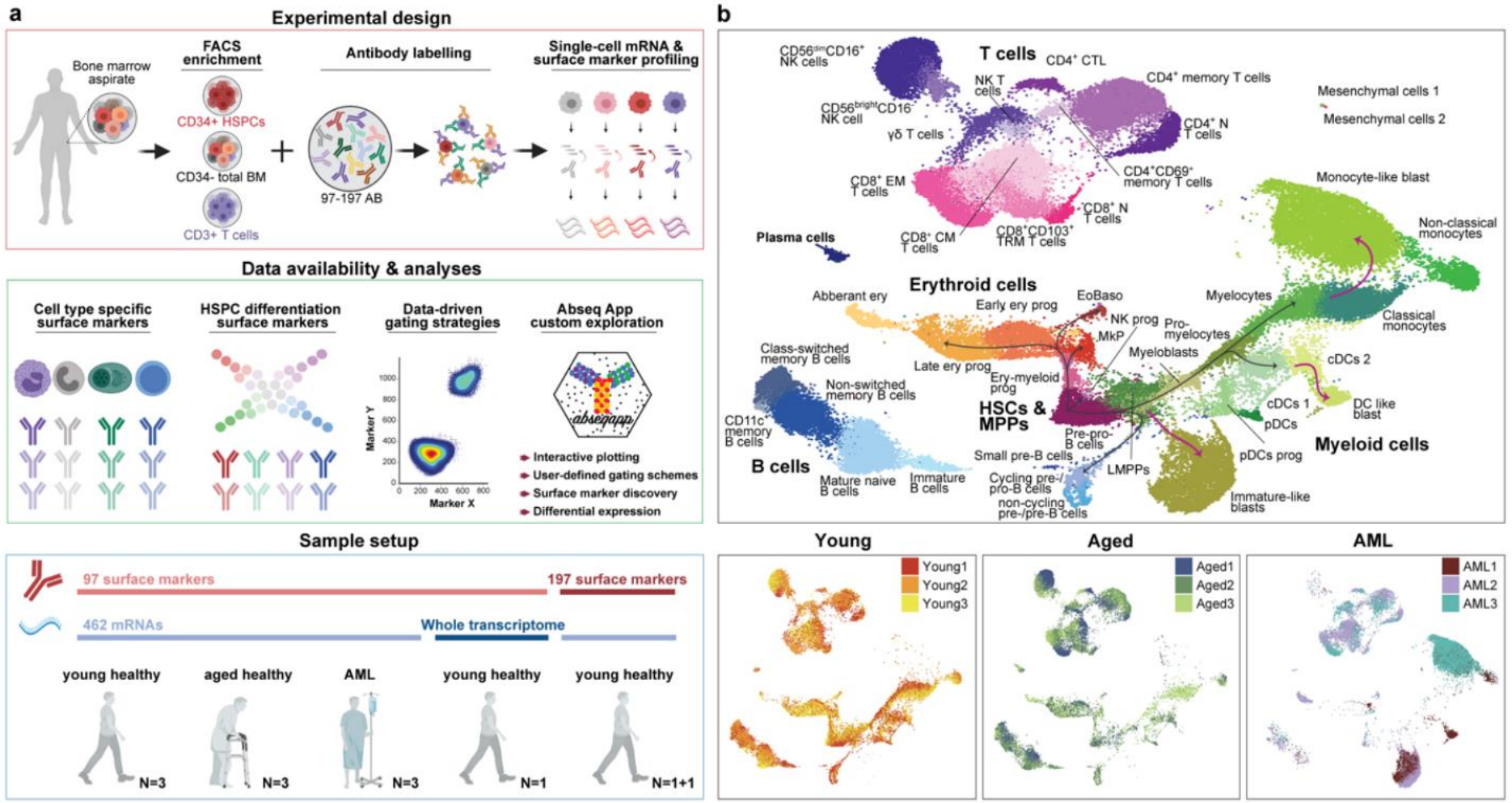
Stuart T. & Satija R., 2019

CITE-seq & REAP-seq



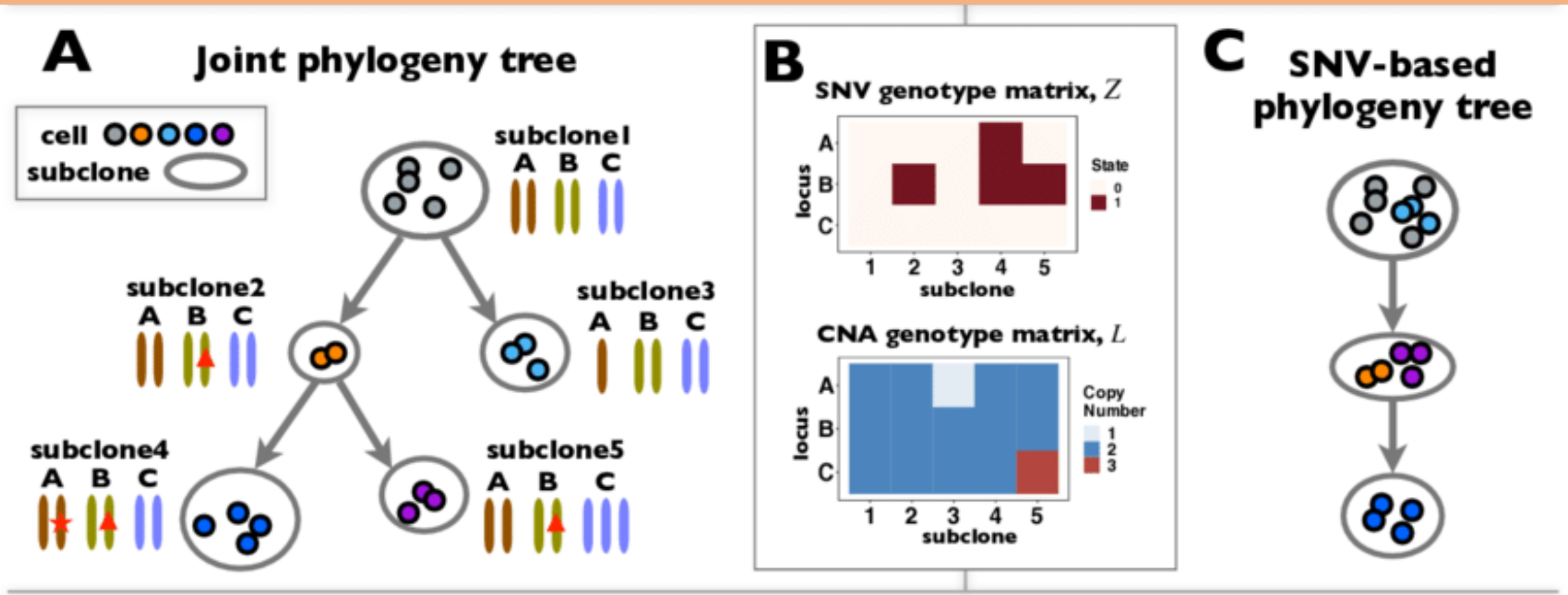
Nature portfolio, 2021

Abseq + scRNAseq: reference map of the hematopoietic system



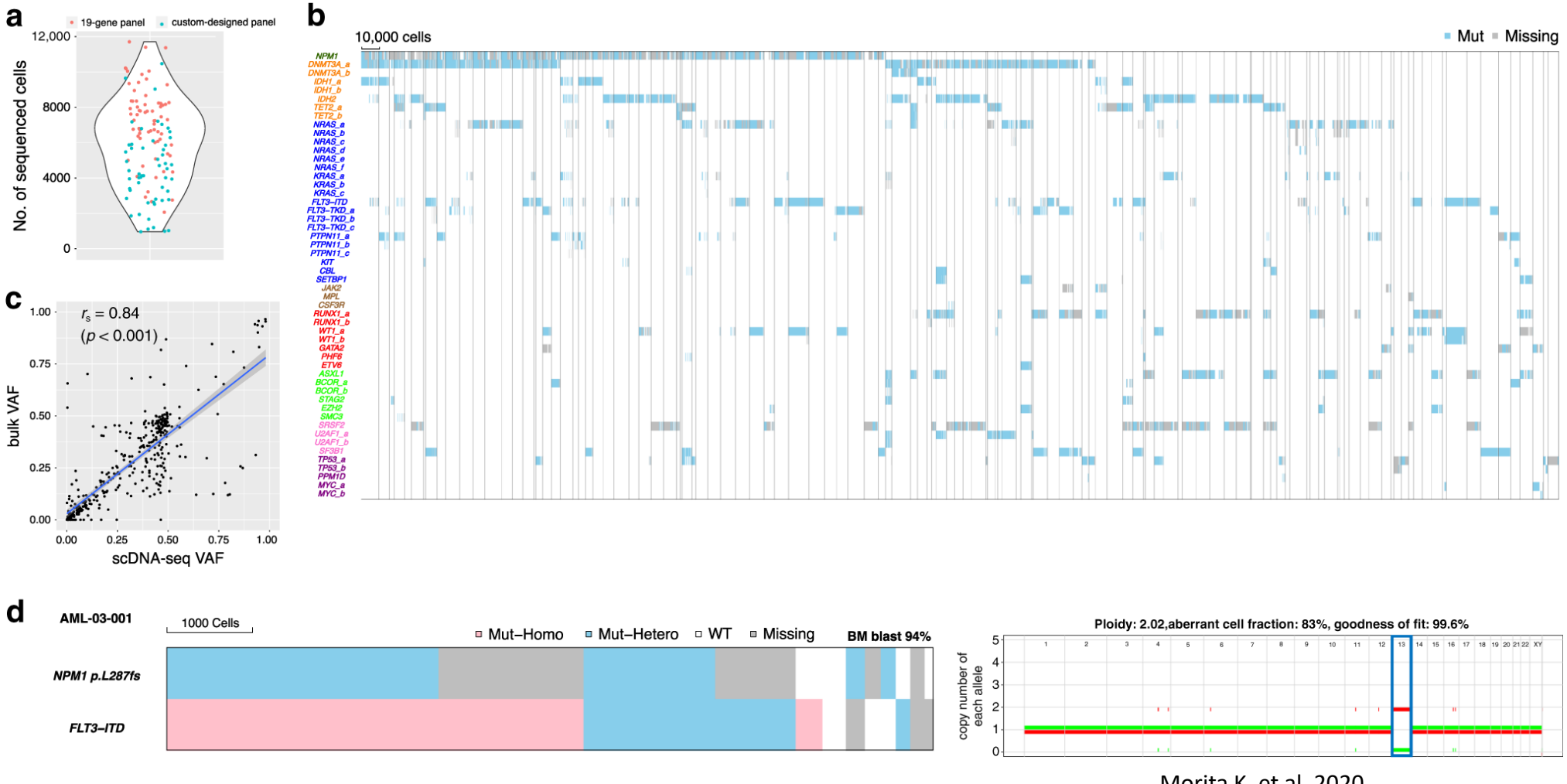
Triana S.H. et al., 2021

Single cell genome sequencing workflow



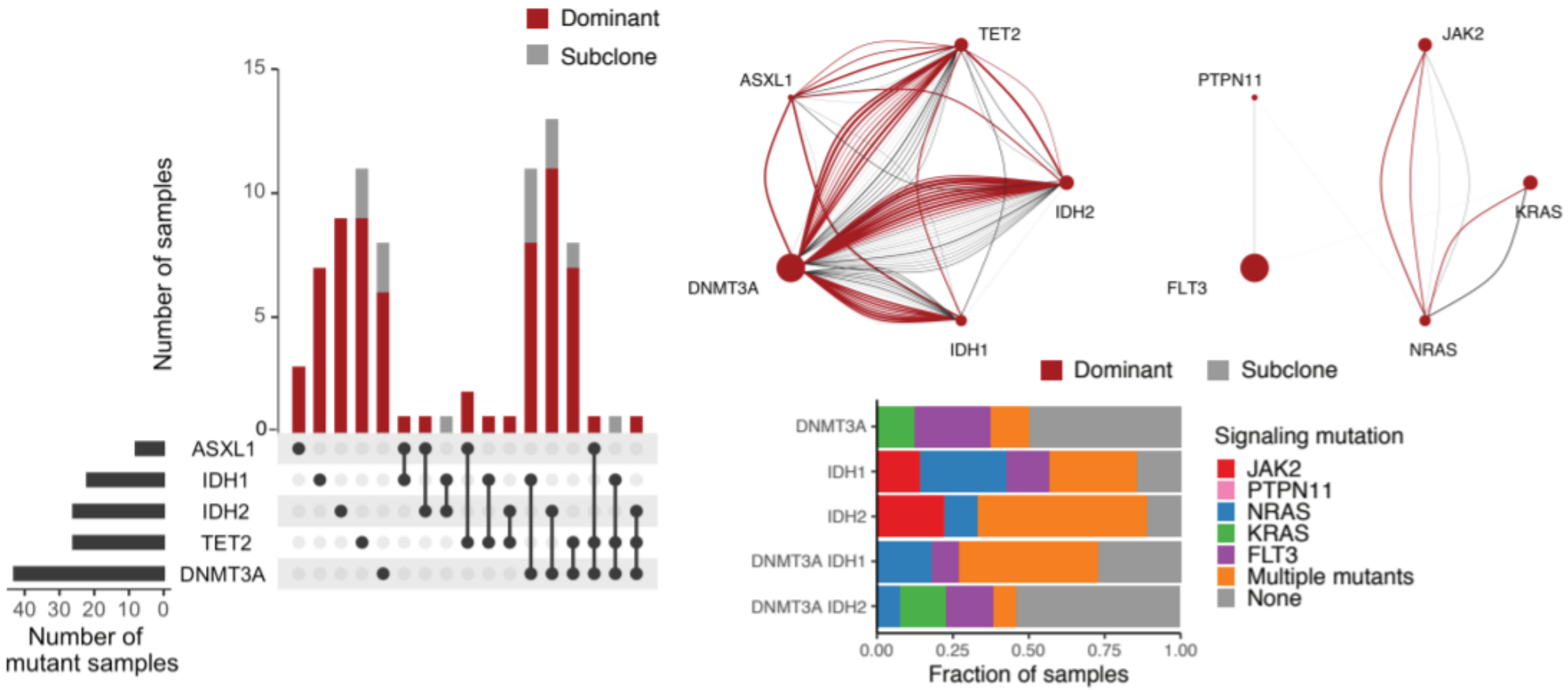
https://www.researchgate.net/figure/ScDNA-seq-data-display-tumor-heterogeneity-A-Joint-tumor-phylogeny-tree-by-SNV-and_fig1_347466280

Application scDNA-seq: genetic landscape



Morita K. et al, 2020

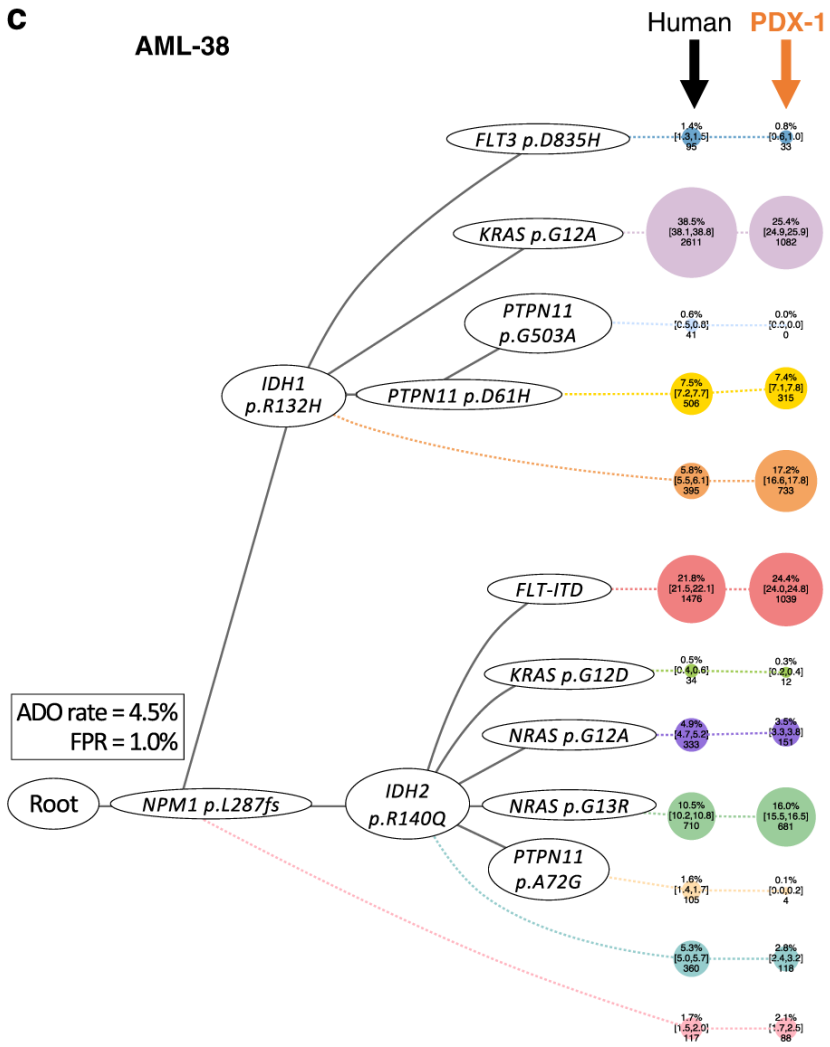
Application scDNA-seq: clonal dominance



Miles L.A. et al, 2021

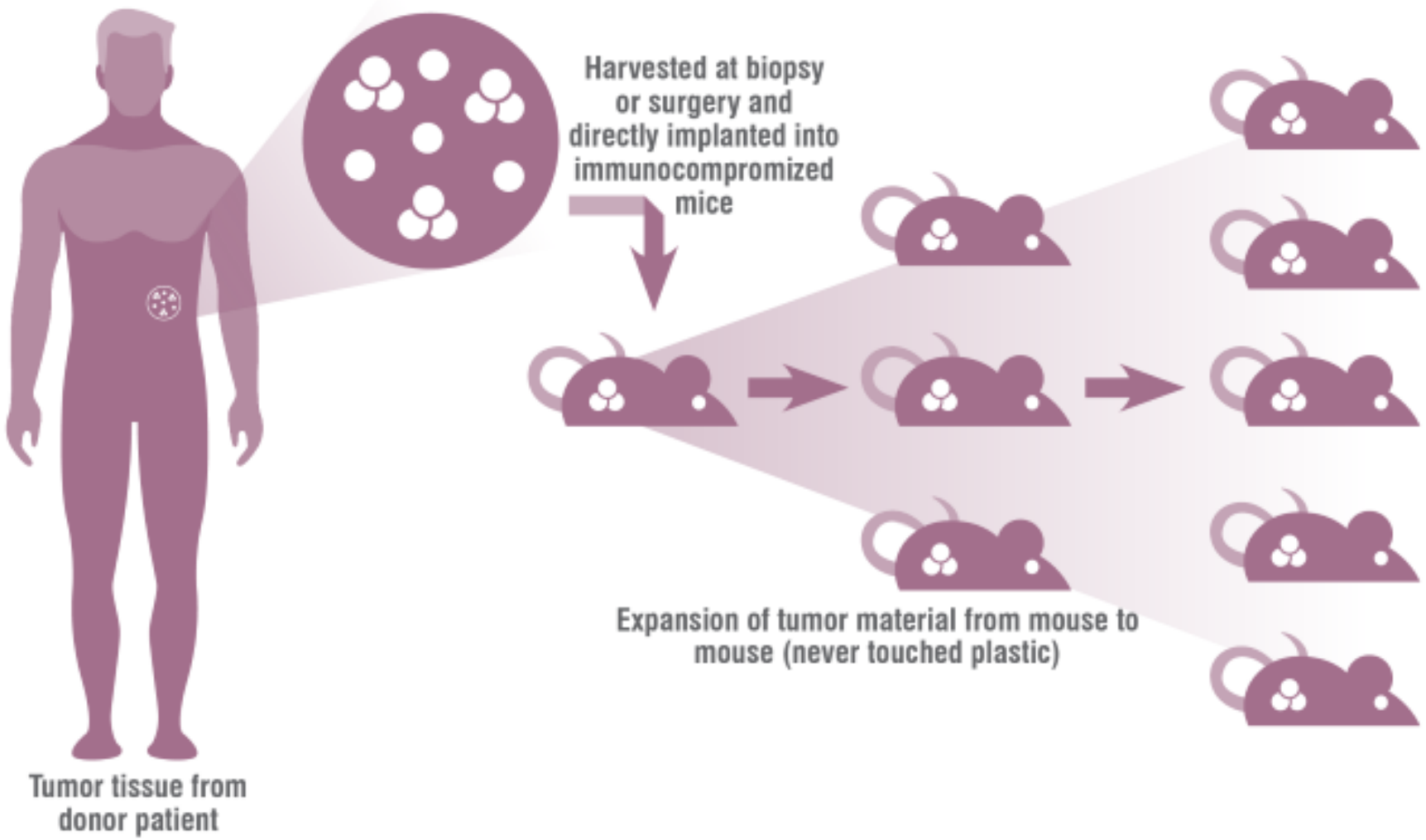
Application scDNA-seq: PDX models

Phylogenetic tree



Morita K. et al., 2020

Xenograft



https://www.criver.com/products-services/discovery-services/pharmacology-studies/oncology-immuno-oncology-studies/oncology-study-models/patient-derived-xenografts-pdx-models?region=3696

Relapse

Newly diagnosed (untreated) AML

- In untreated AML, the disease is newly diagnosed. It has not been treated except to relieve signs and symptoms such as fever, bleeding, or pain, and the following are true:
- The complete blood count is abnormal.
- At least 20% of the cells in the bone marrow are blasts (leukemia cells) or there are certain gene changes.
- There are signs or symptoms of leukemia.

AML in remission

- In AML in remission, the disease has been treated and the following are true:
- The complete blood count is normal.
- Less than 5% of the cells in the bone marrow are blasts (leukemia cells).
- There are no signs or symptoms of leukemia in the brain and spinal cord or elsewhere in the body.

The microenvironment: T cell exhaustion

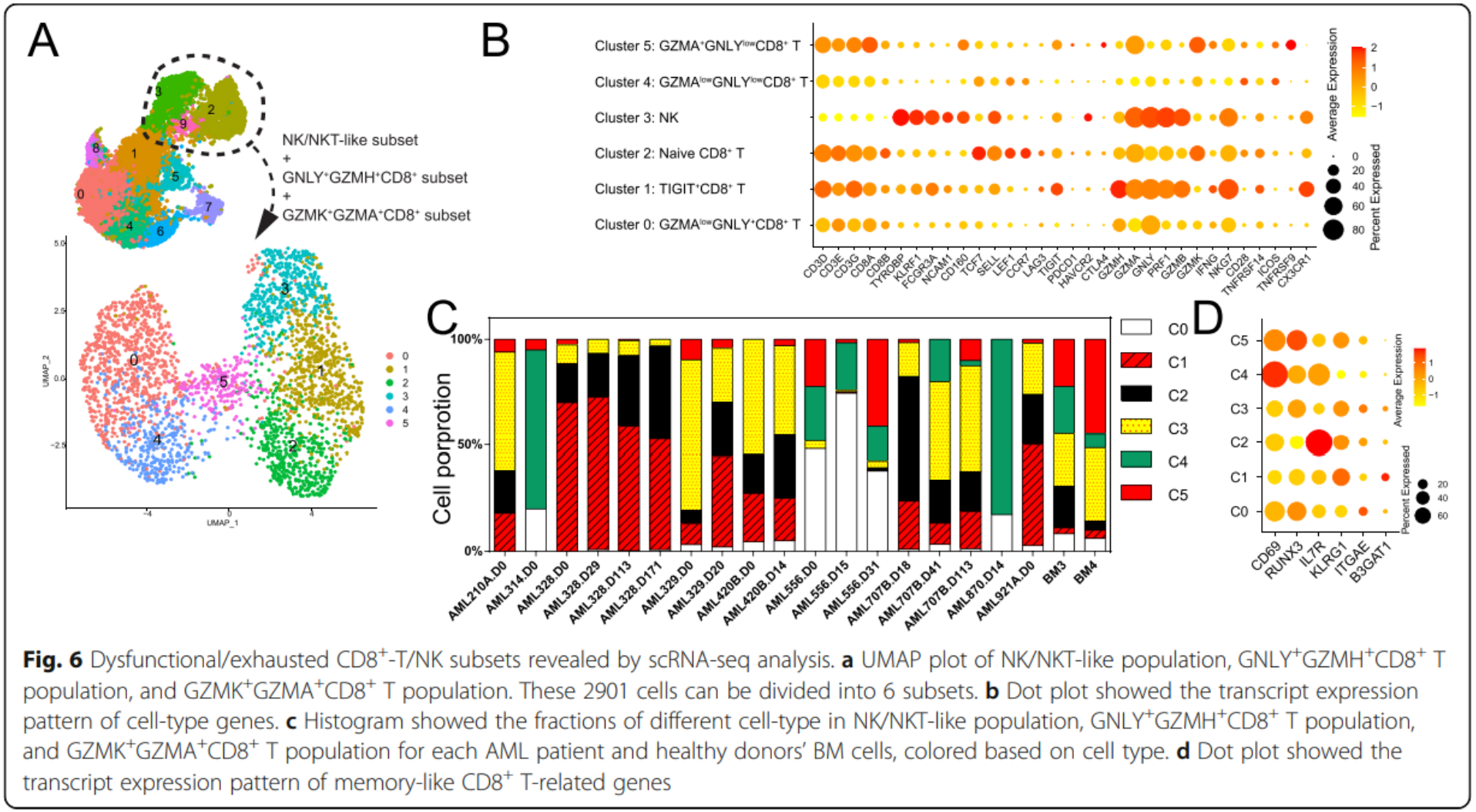
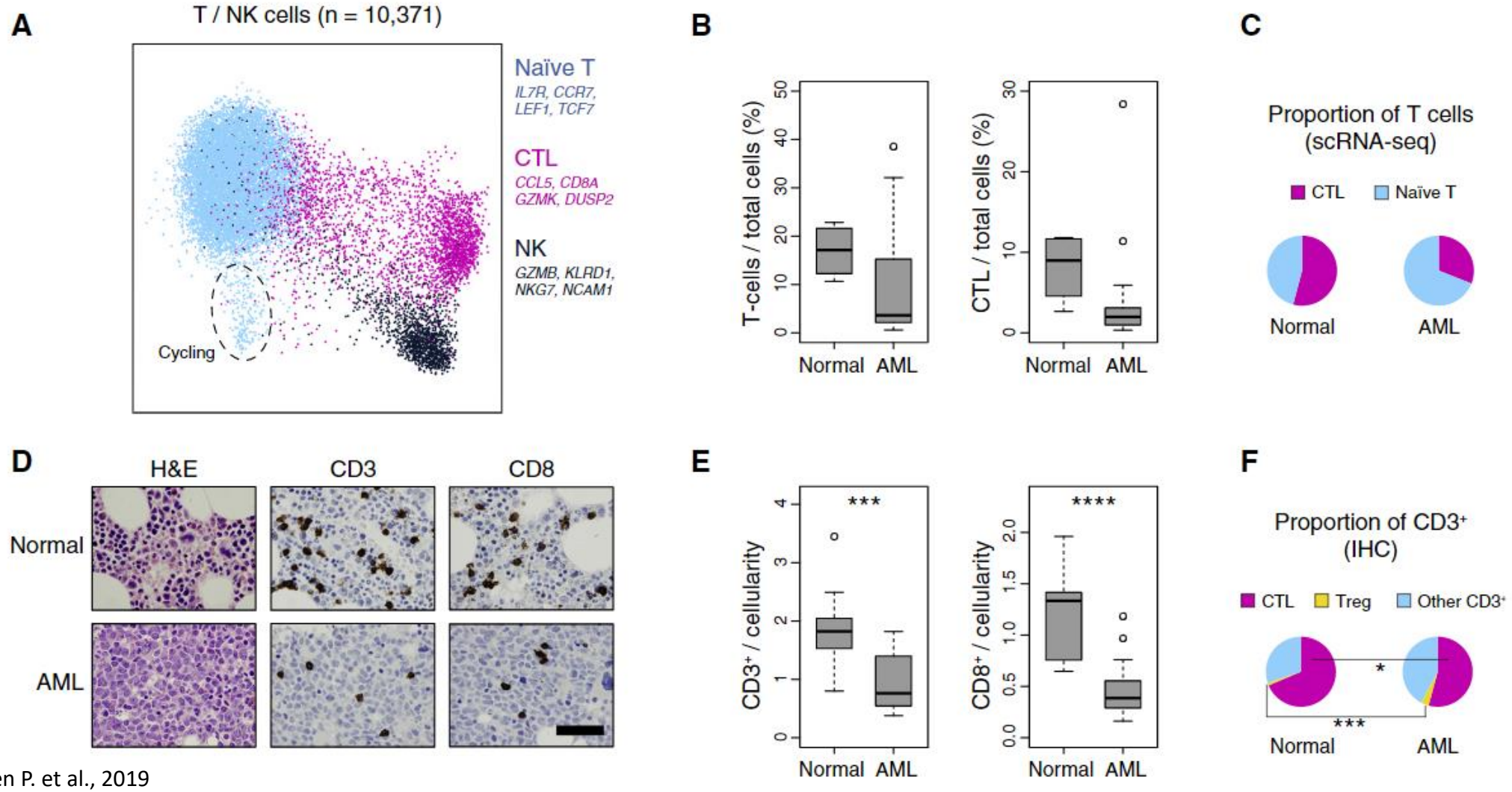


Fig. 6 Dysfunctional/exhausted CD8$^+$-T/NK subsets revealed by scRNA-seq analysis. **a** UMAP plot of NK/NKT-like population, GNLY$^+$GZMH$^+$CD8$^+$ T population, and GZMK$^+$GZMA$^+$CD8$^+$ T population. These 2901 cells can be divided into 6 subsets. **b** Dot plot showed the transcript expression pattern of cell-type genes. **c** Histogram showed the fractions of different cell-type in NK/NKT-like population, GNLY$^+$GZMH$^+$CD8$^+$ T population, and GZMK$^+$GZMA$^+$CD8$^+$ T population for each AML patient and healthy donors' BM cells, colored based on cell type. **d** Dot plot showed the transcript expression pattern of memory-like CD8$^+$ T-related genes

Guo R. et al., 2021

The microenvironment: T cell exhaustion



Van Galen P. et al., 2019

Future

- What do we know and what not?
- What did we learn?
- Best techniques?
- Steps for the future

The role of the microenvironment

- How can gene regulatory networks (GRN) and cell-extrinsic factors derived from bone marrow niche impact clone fitness and the trajectory of clonal diversity over time?

- Which immune cell types exist near AML, what is the immune status and molecular mechanisms of AML patient BM microenvironment?

The microenvironment: Stromal/mesenchymal cells

Immunophenotype