Biology of Richter Syndrome
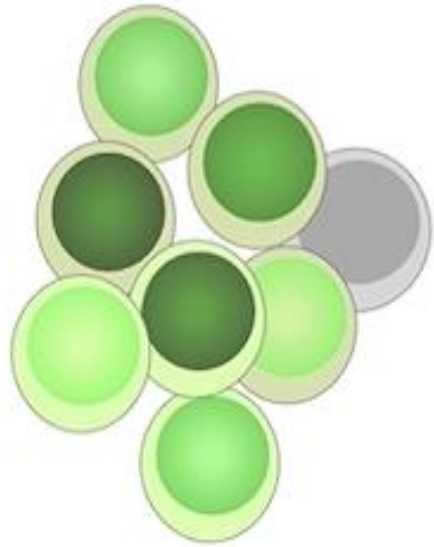
Erin M. Parry, MD PhD

October 19, 2023
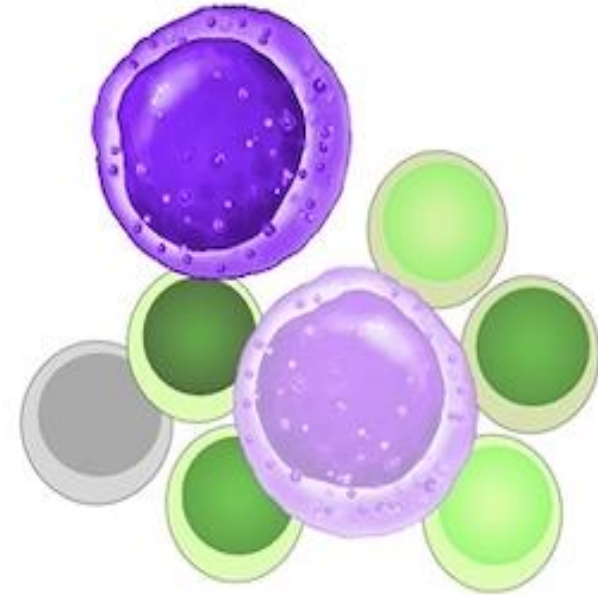
Journées du FILO

Dana-Farber Cancer Institute

Histologic transformation:
Evolution to aggressive lymphoma
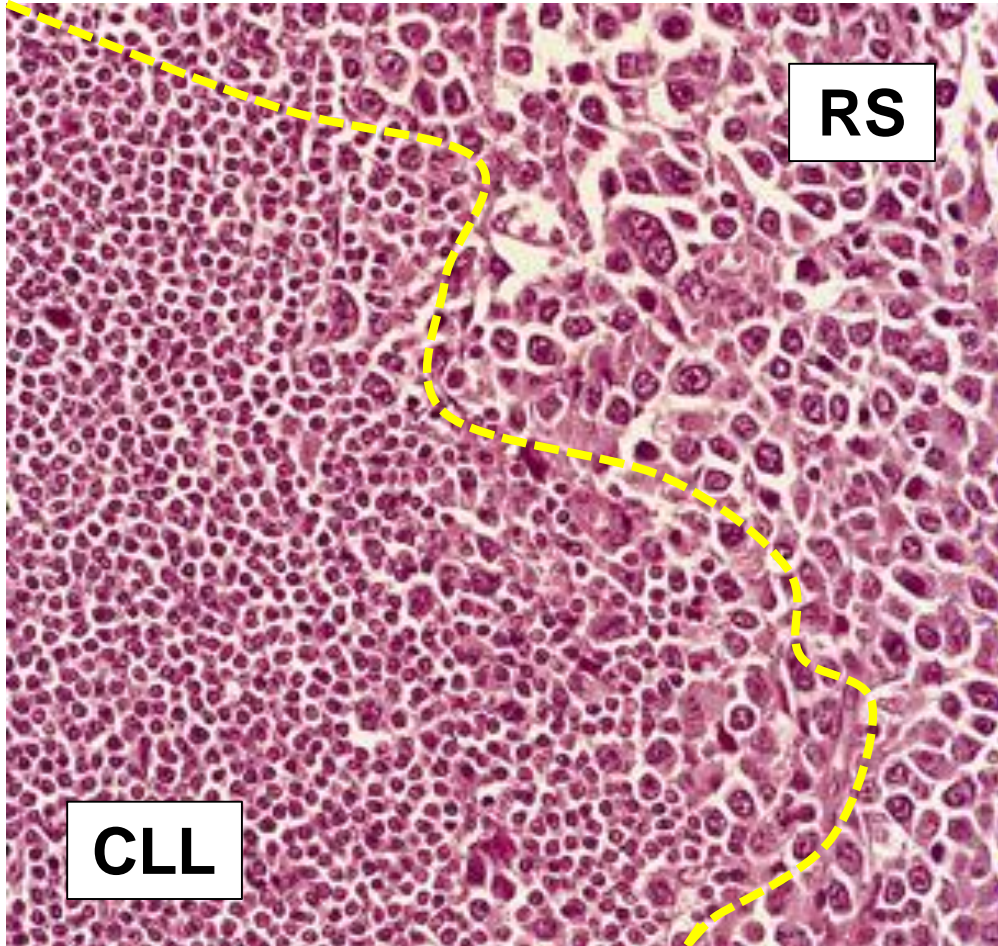
Transformation

Indolent B cell malignancy

Aggressive lymphoma

Chronic lymphocytic leukemia (CLL) ⟶ Richter syndrome (RS)

Richter Syndrome: Unmet need

- Occurs in 5 to 10% of CLL patients

- DLBCL histology (90%)

- Majority clonally related to underlying CLL

- Until recently, little known about molecular basis

- RS is often refractory to existing therapies

Image: Warnke RA et al,
Atlas of Tumor Pathology, 1995

Parikh et al, *Blood,* 2014

Richter syndrome:
Diagnostic and clinical dilemmas in 2023

- Diagnosis

  - Limitations of tissue sampling

  - Morphologic diagnosis – lack of markers, genetics

- Biology

  - Lack of knowledge of unique vulnerabilities/targets
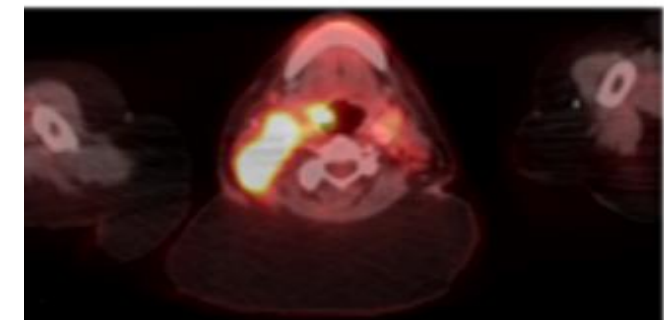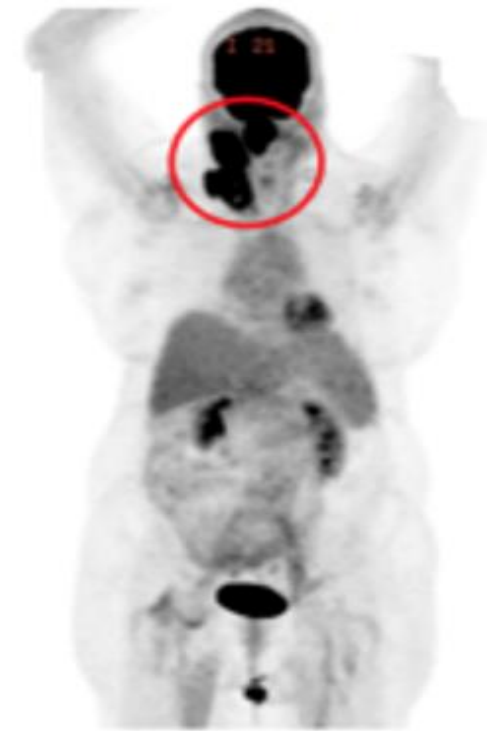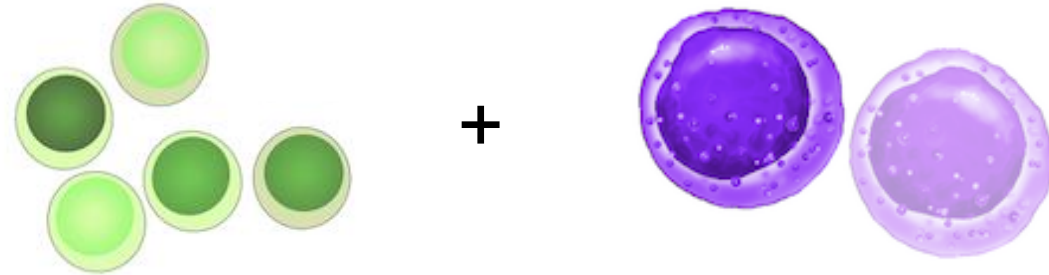
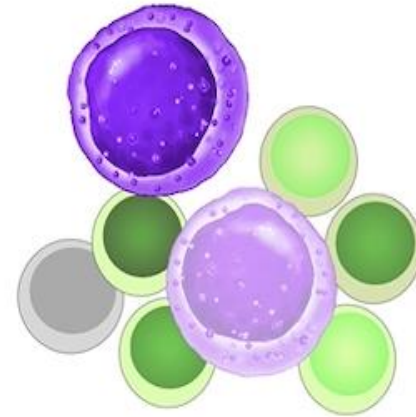 - Molecular changes?

  - Risk subgroups?

Image: N. Jain, *ASH* 2018

Why do we know so little about Richter Syndrome?

Challenges:

1. Sample acquisition

+

2. Sample admixture

Biology of RS: Recent advances

Rossi et al., Blood, 2011
Fabbri et al., JEM, 2013
Klintman et al., Blood 2021
Nadeu et al., Nat Med 2022
Parry et al., Nat Med 2023
Broseus et al., Nat Commun 2023

GEMMs
Knittel et al., Nat Commun 2017
Kohlhaas et al., Blood 2021
Chakraborty et al., Blood 2021
Ten Hacken et al.,
  Blood Cancer Discovery 2022
Hing et al., Nat Commun 2023

PDXs
Vaisitti et al., Cancer Res 2018
Fiskus et al., Leukemia 2021
Playa-Albinyana et al., ASH 2022

Genetics

Epigenetics

Immune microenvironment

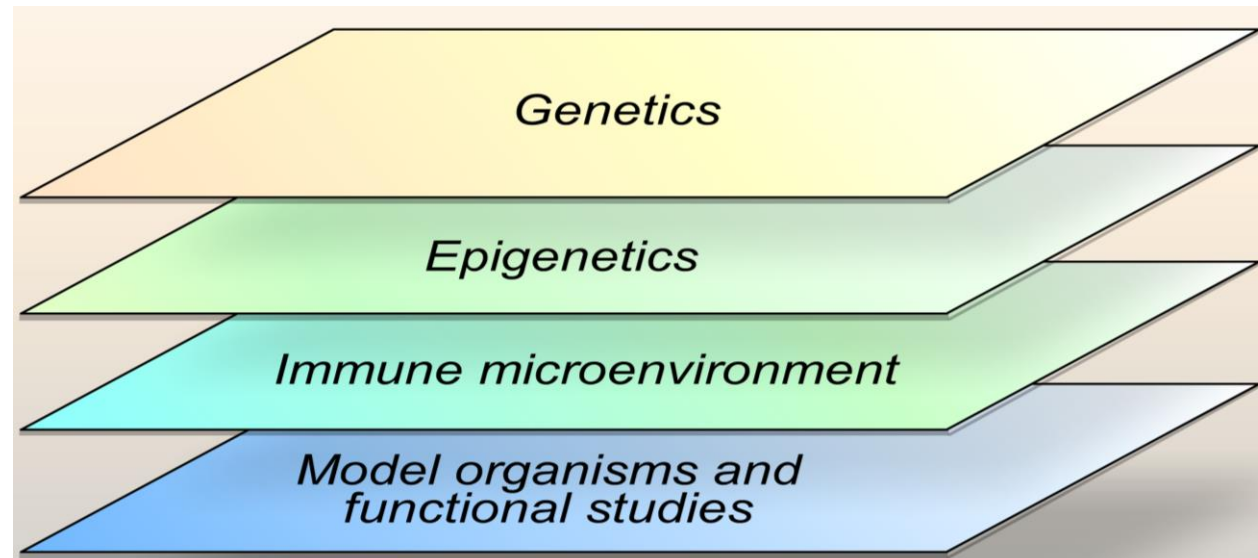Model organisms and
functional studies

Image from:
Parry, Ten Hacken and Wu,
Blood, 2023

Talk Outline

- I: Molecular characterization of transformation

- II: Understanding determinants of response to immunotherapy in transformation

- III: Summary and Future directions

I. Evolutionary history of transformation from chronic lymphocytic leukemia to Richter syndrome
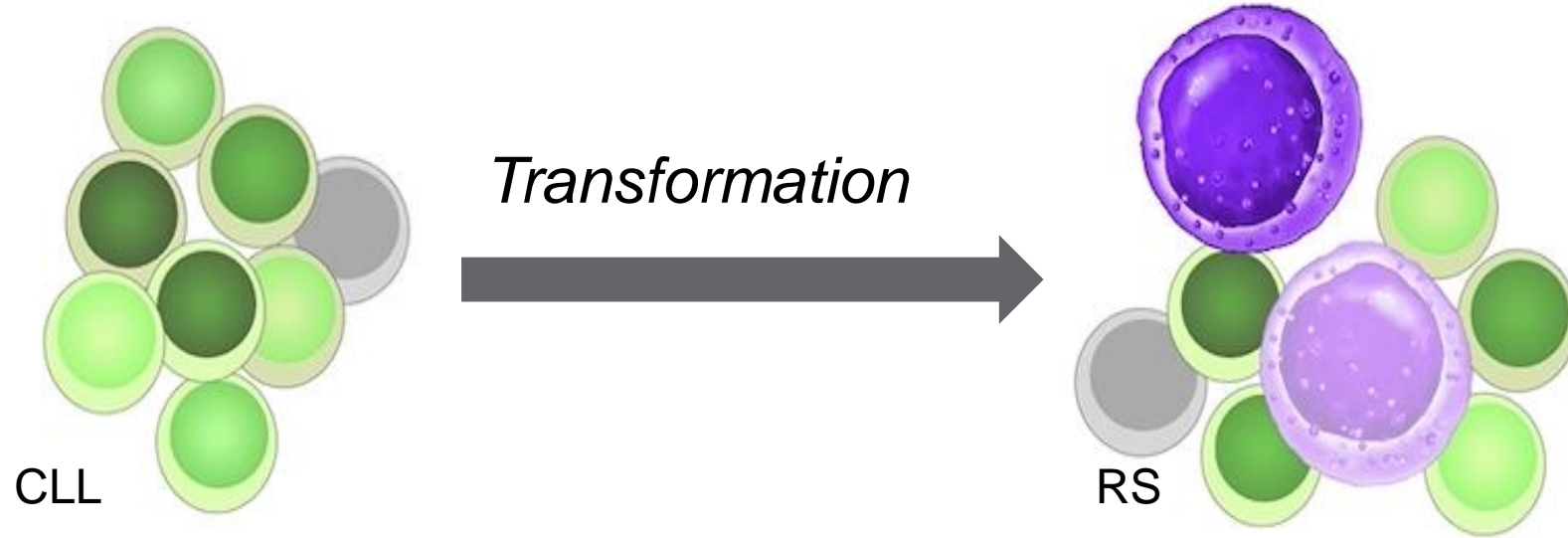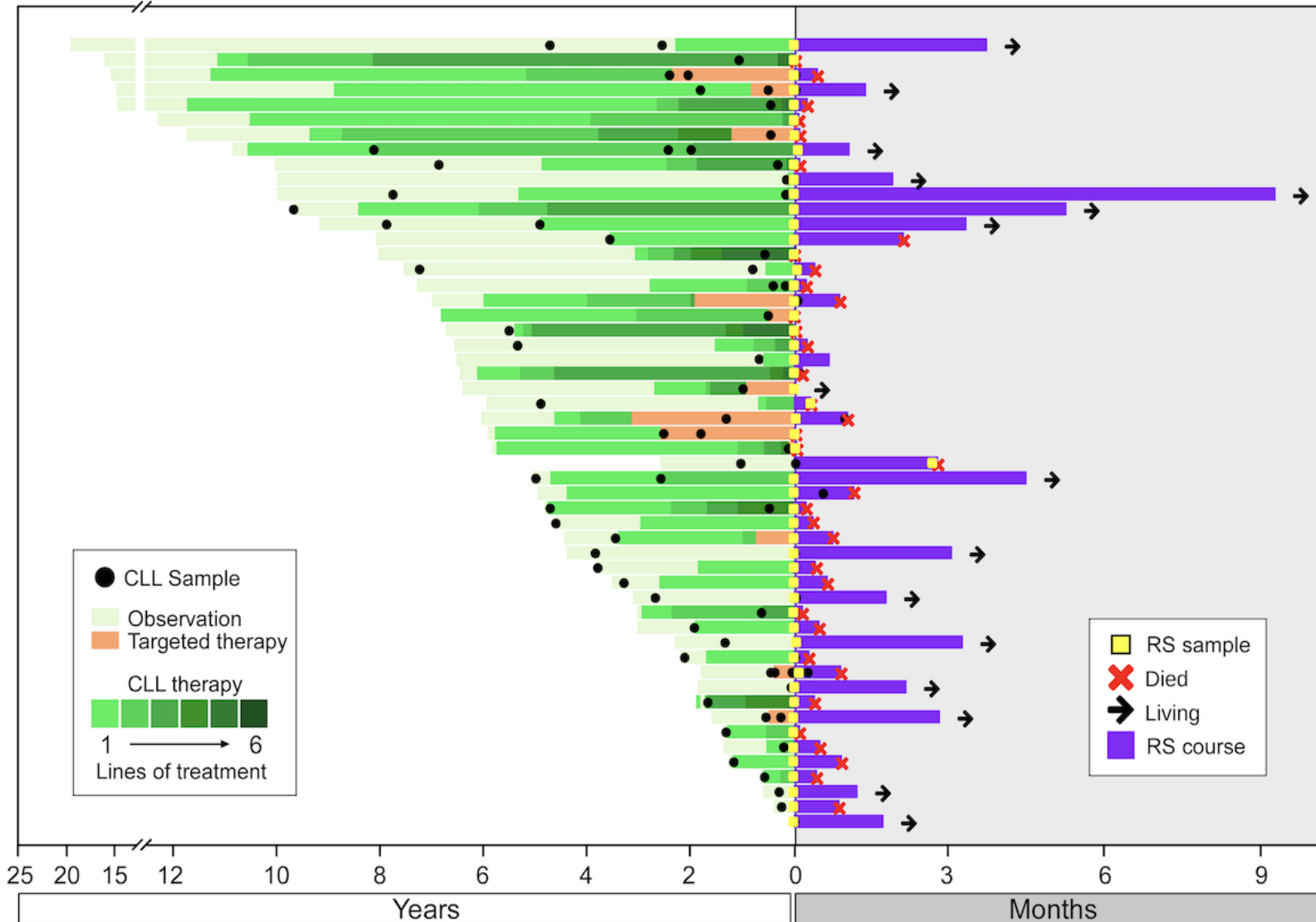

Romain Guieze

Ignat Leshchiner

The biology and genetics of Richter syndrome (RS) remain incompletely understood

CLL

Transformation

RS

- *Clonal relationships?*
- *Genetic drivers?*
- *Pathways of transformation?*
- *Molecular subtypes?*
- *Early or Improved detection?*

I: Assembling RS cohort through collaboration

Discovery Cohort **N=53** (WES)
Ulm, Germany
Dana-Farber Cancer Institute
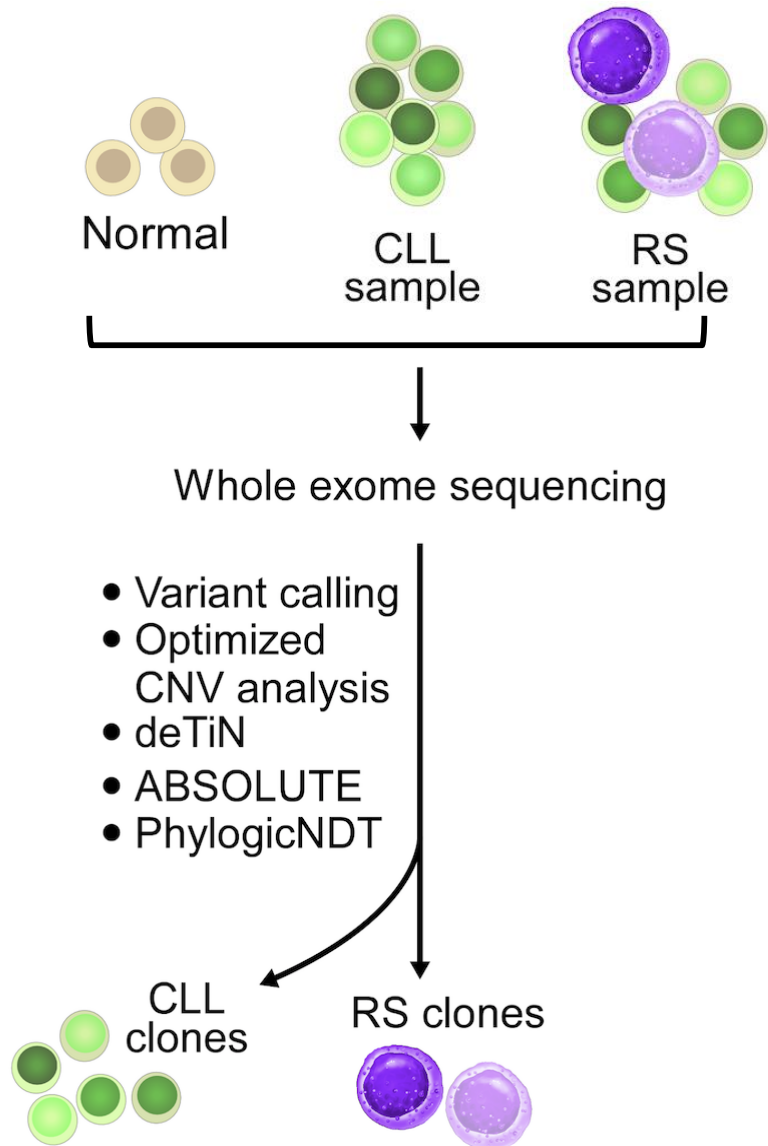Mayo Clinic
FILO
UCSD
MDACC

Validation Cohort **N=45** (WES/RNA)
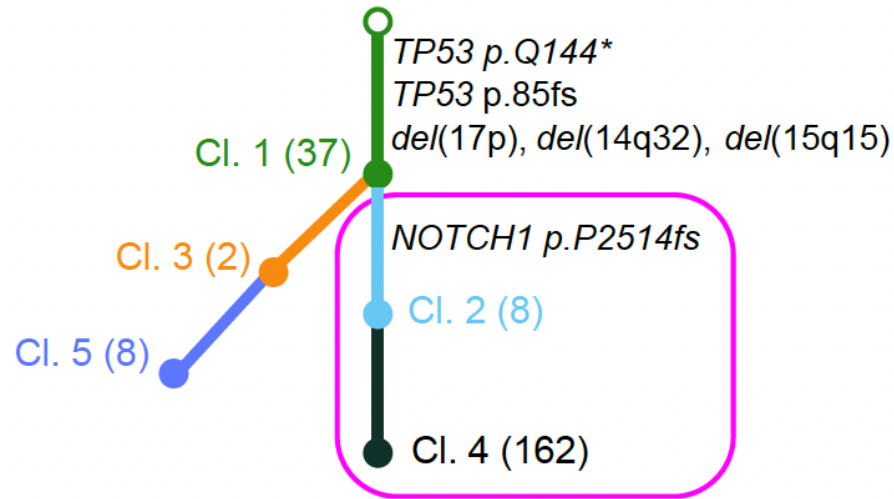FILO

Validation Cohort **N=14** (WGS)
Klintman et al, *Blood* 2021

Legend:
- ● CLL Sample
- Observation
- Targeted therapy
- CLL therapy 1 → 6 Lines of treatment
- ☐ RS sample
- ✗ Died
- → Living
- RS course

Years | Months

25 20 15 12 10 8 6 4 2 0 | 0 3 6 9

II: Computational deconvolution of CLL and RS

WES: clonal related and unrelated RS

Clonal related RS
(86%)

Clonal unrelated RS
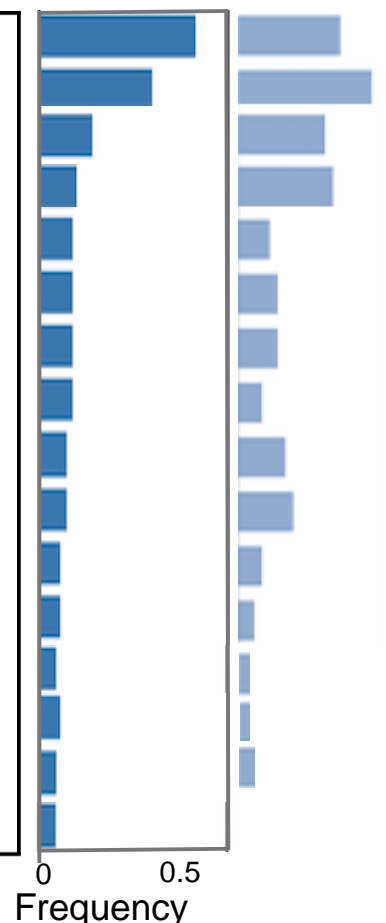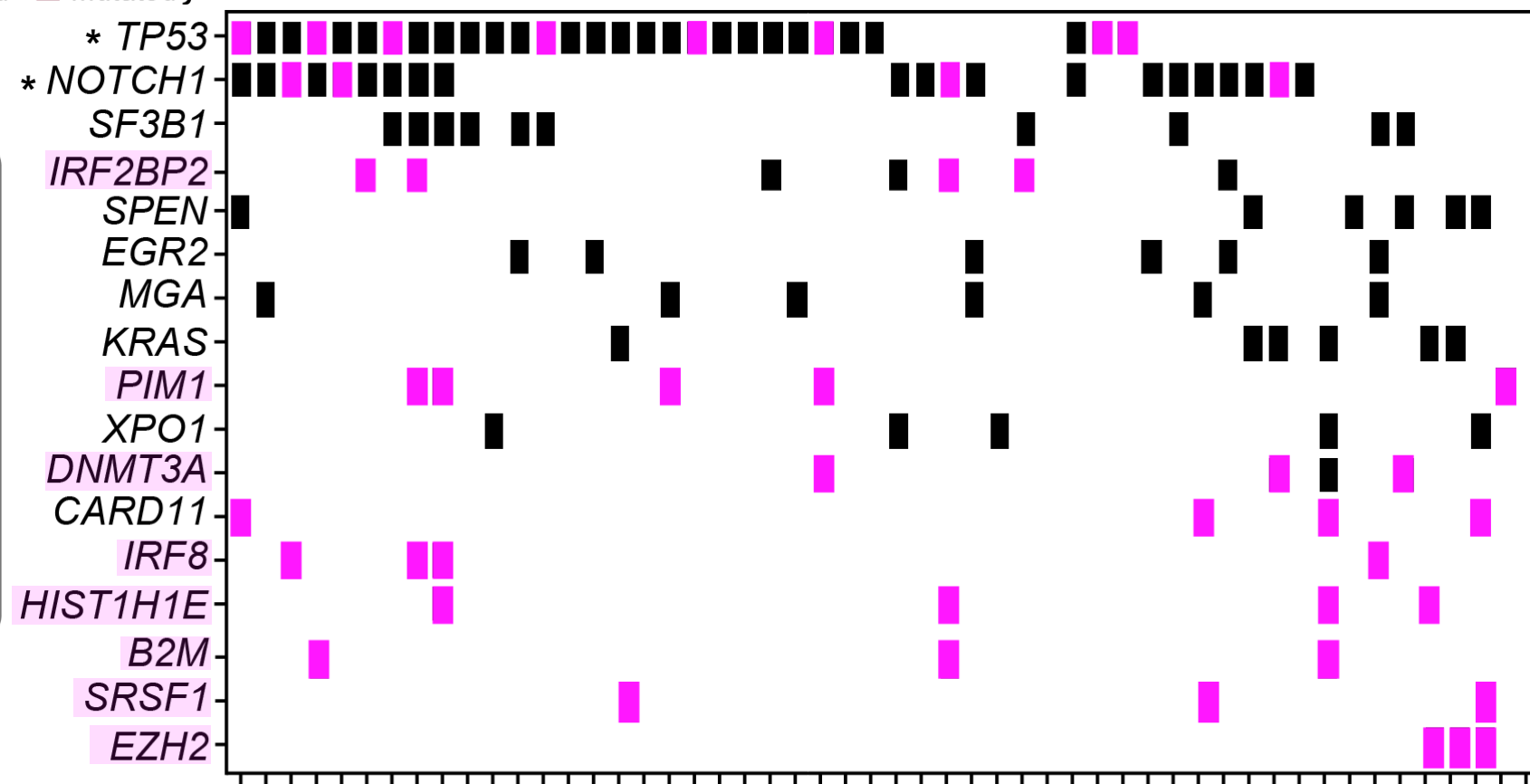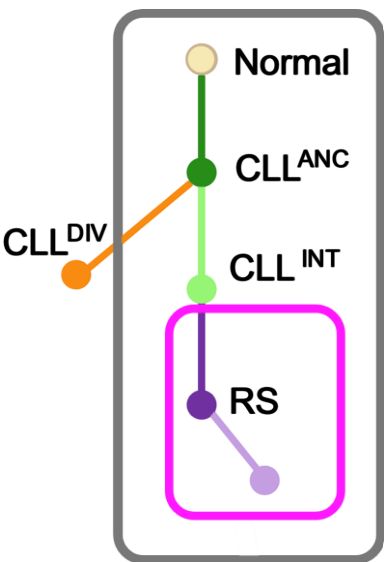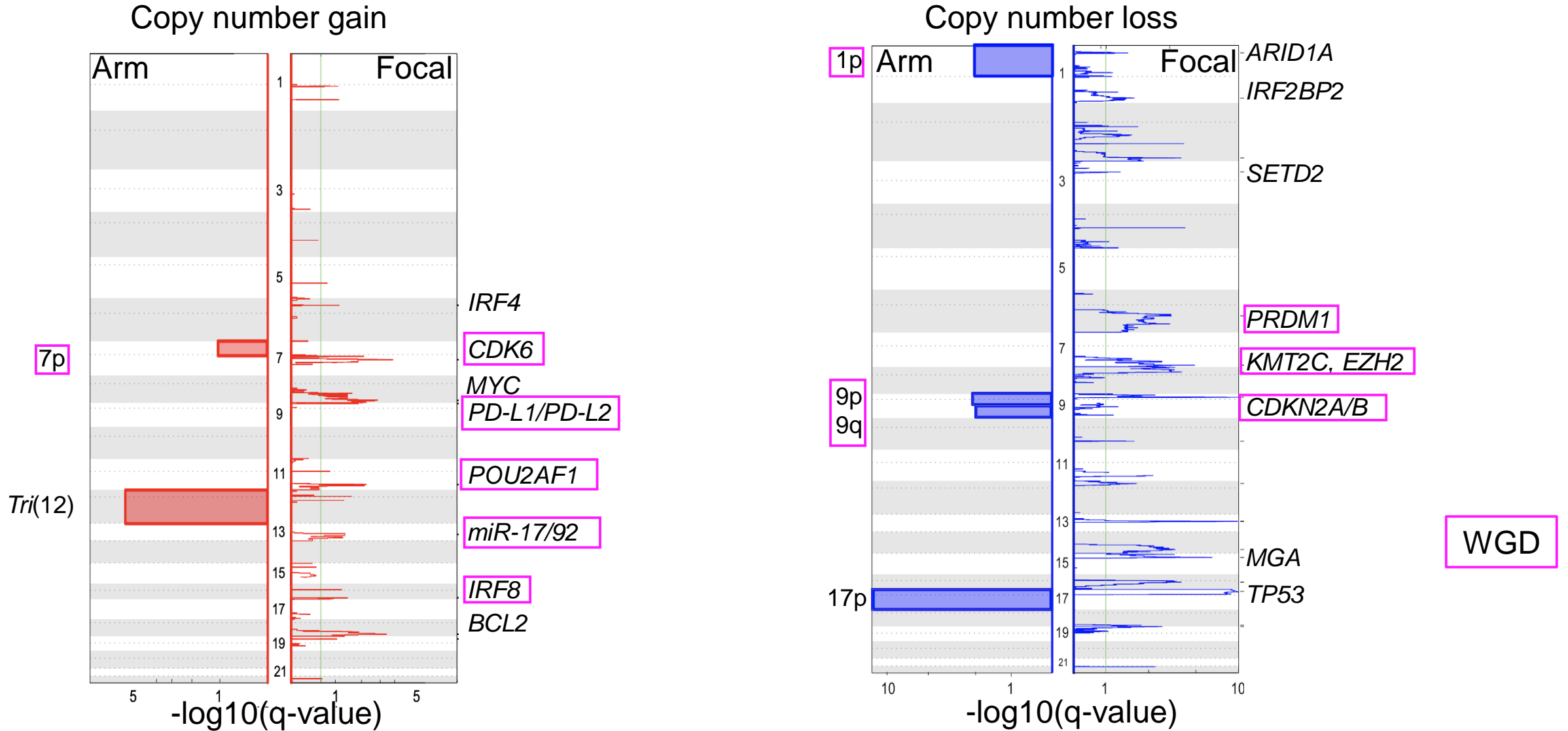(14%)
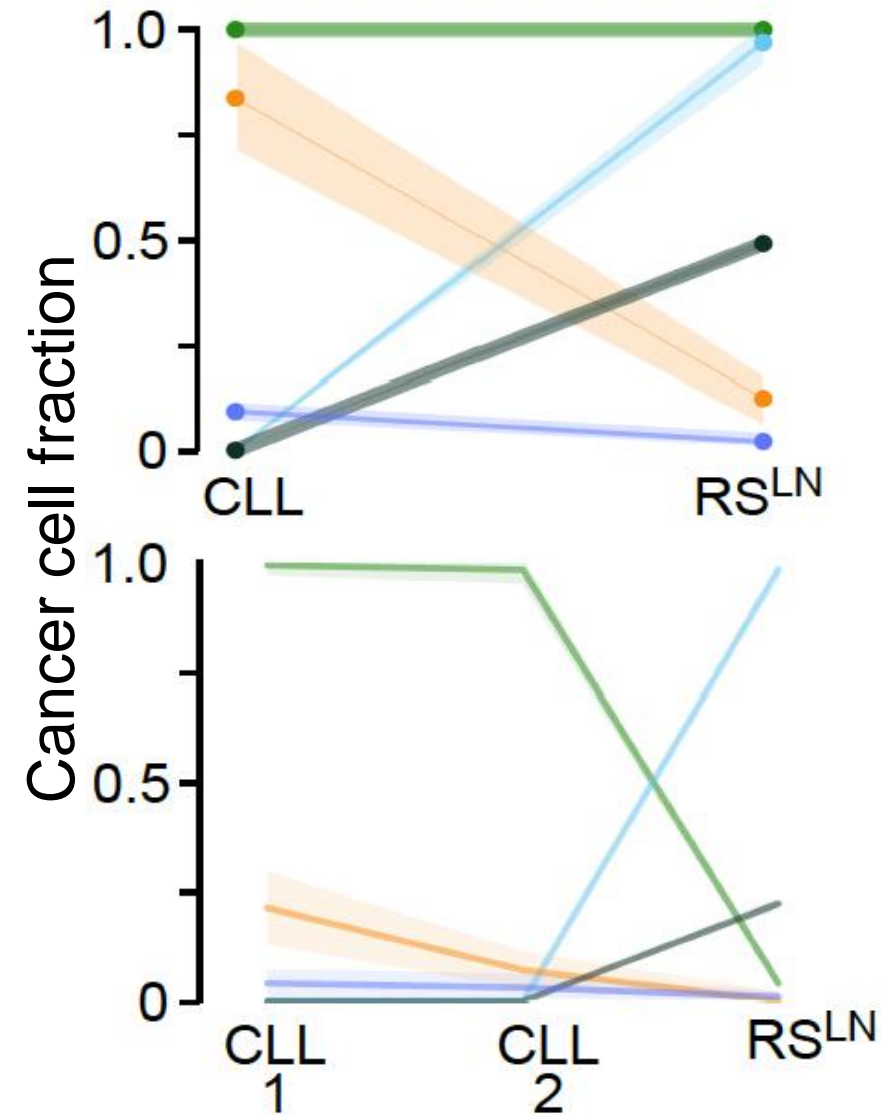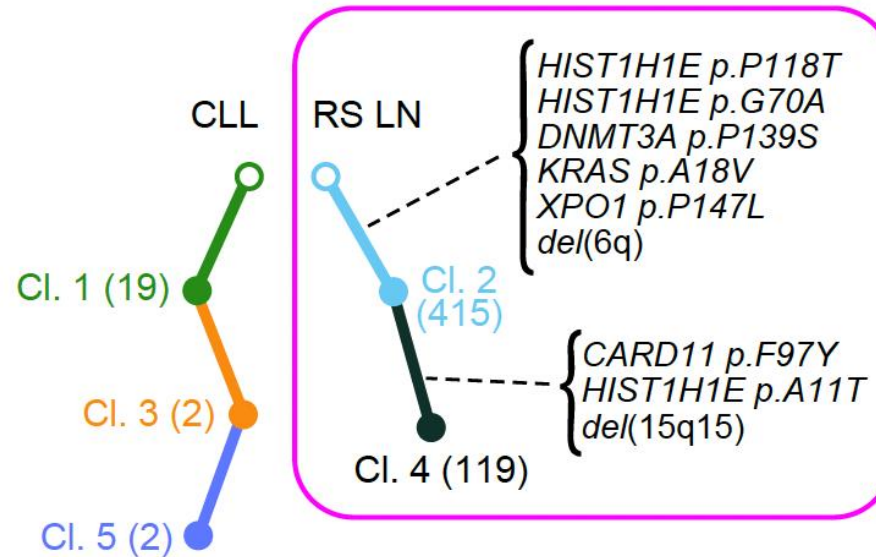
Recurrent driver alterations underlie RS

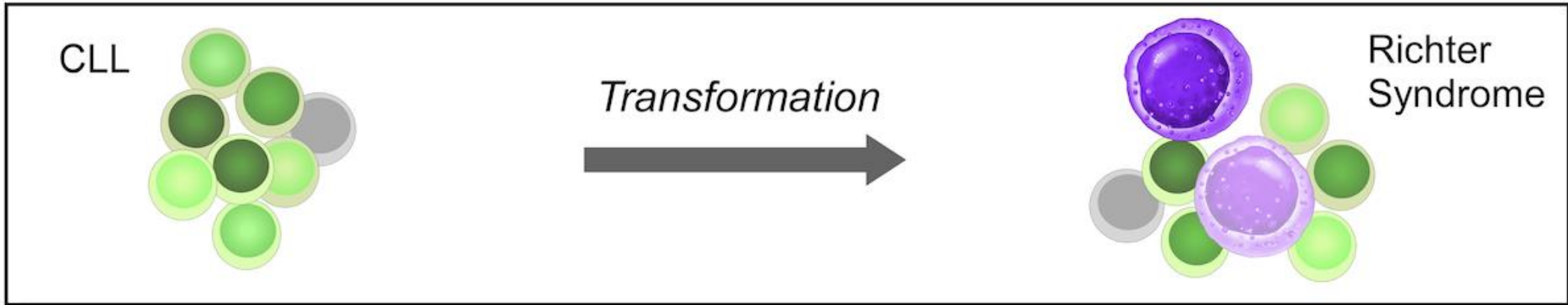Putative novel RS drivers:
IRF2BP2, DNMT3A, B2M, IRF8, SRSF1, EZH2, PIM1

With validation cohort:
BRAF, CCND3, TET2

* P<0.05 vs. CLL 1100 :Knisbacher et al, Nature Genetics 2022
MutsigCV2.0: Lawrence et al, Nature 2013

RS: Recurrent sCNAs

Copy number gain

Copy number loss

Pathways of transformation

Image from:
Parry, Ten Hacken and Wu, *Blood*, 2023

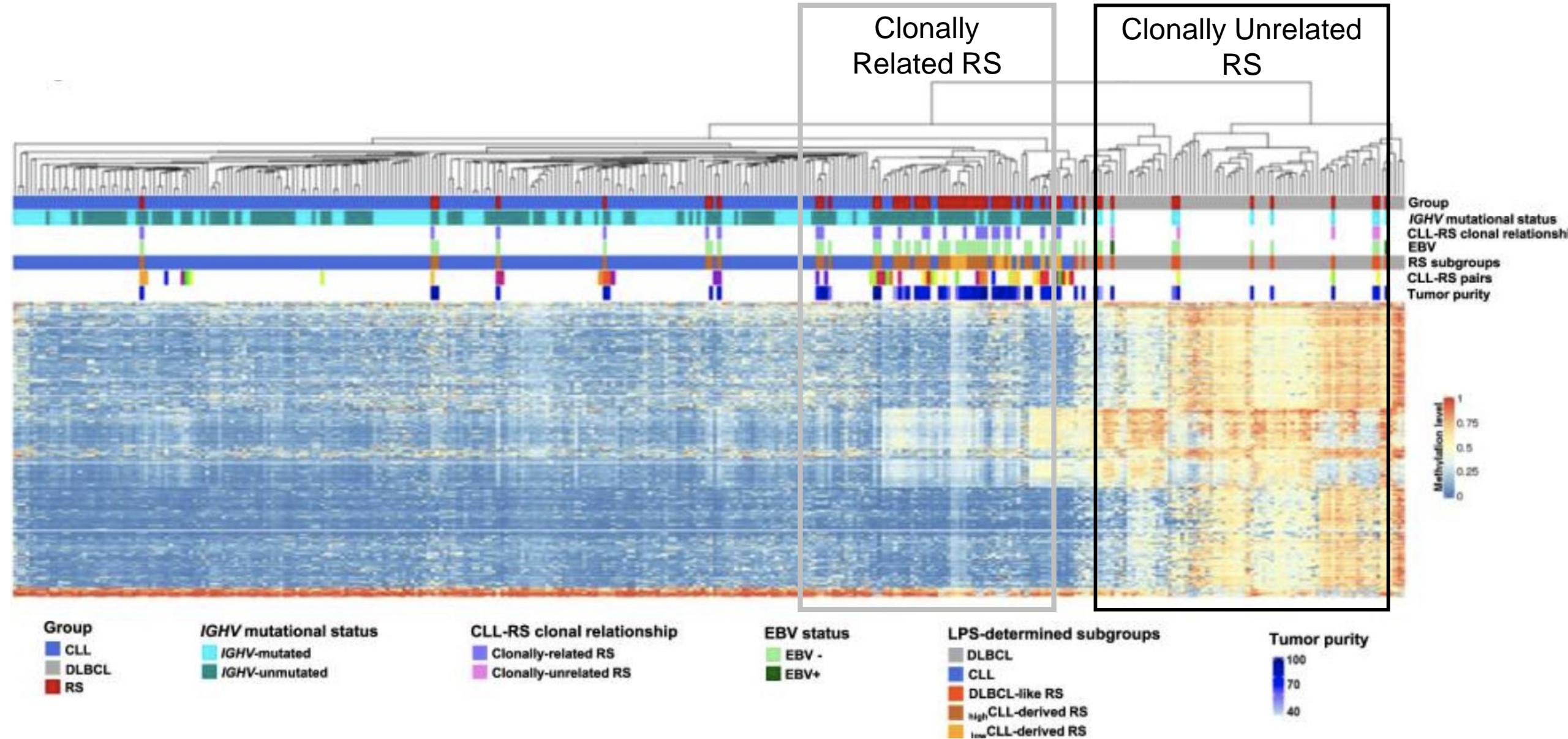# RS genetics: distinct from *de novo* DLBCL
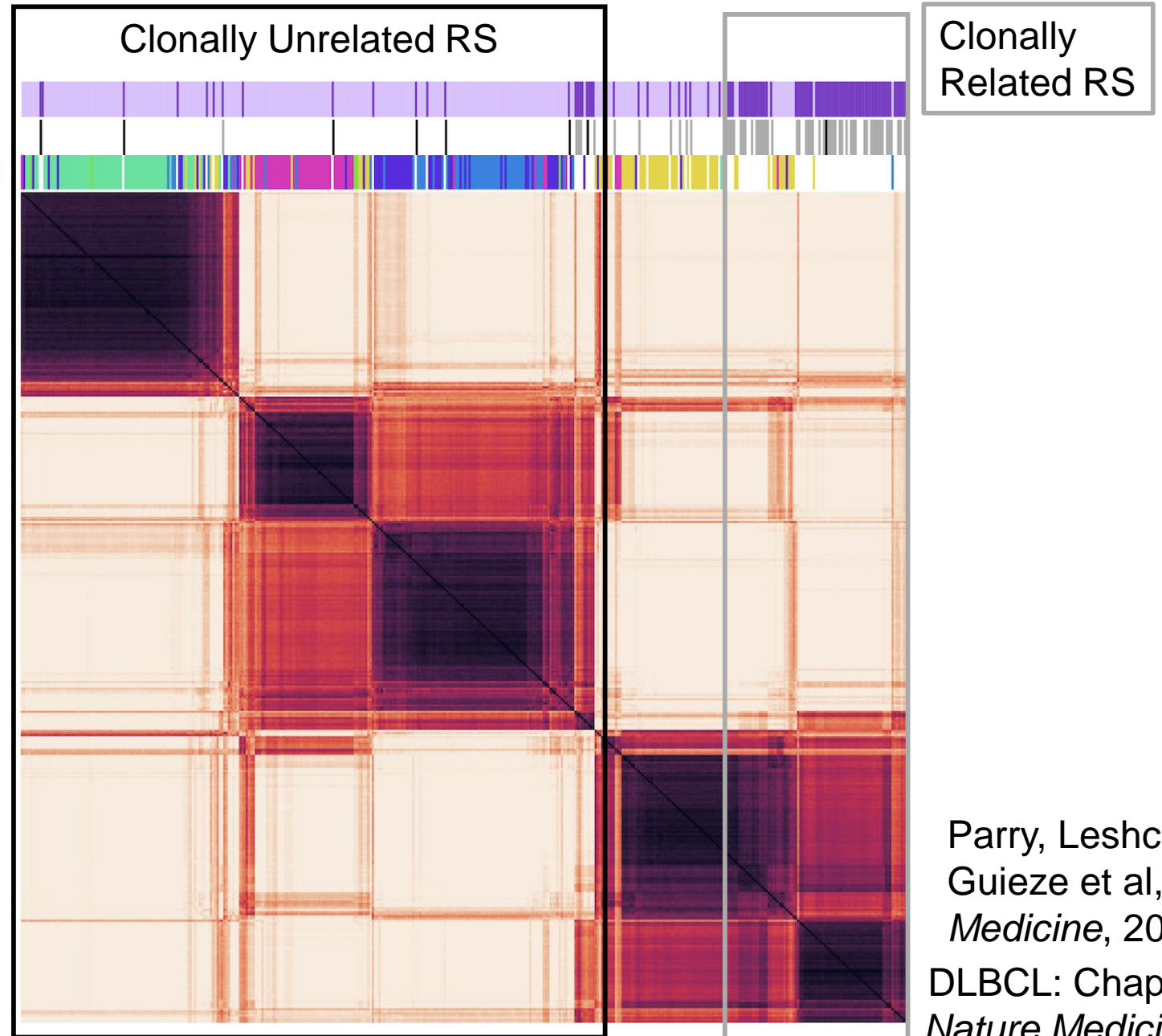
- NMF clustering of RS (N=97) and DLBCL (N=304) alterations

- Clonally <u>un</u>related RS clusters with DLBCL

- Clonally related RS
  - Clusters separate from DLBCL
  - Most similar to C2 DLBCL (Biallelic *TP53* loss)

Parry, Leshchiner, Guieze et al, *Nature Medicine*, 2023
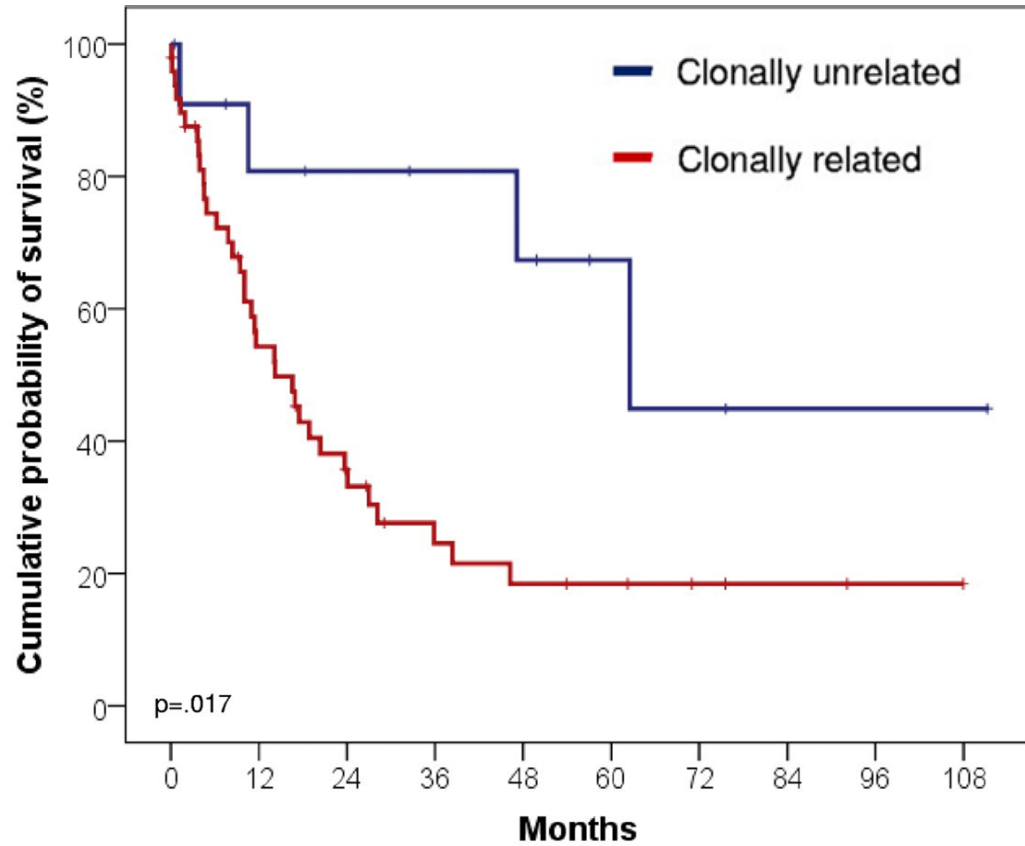
DLBCL: Chapuy et al, *Nature Medicine* 2018

# RS Methylation : distinct from *de novo* DLBCL

Clonally Related RS

Clonally Unrelated RS

Group
- CLL
- DLBCL
- RS

IGHV mutational status
- IGHV-mutated
- IGHV-unmutated

CLL-RS clonal relationship
- Clonally-related RS
- Clonally-unrelated RS

EBV status
- EBV -
- EBV+

LPS-determined subgroups
- DLBCL
- CLL
- DLBCL-like RS
- high CLL-derived RS
- low CLL-derived RS

Tumor purity
- 100
- 70
- 40

Transcriptomic differences present as well

Broséus et al, *Nature Comm* 2023

Clonal and unrelated RS: different biology
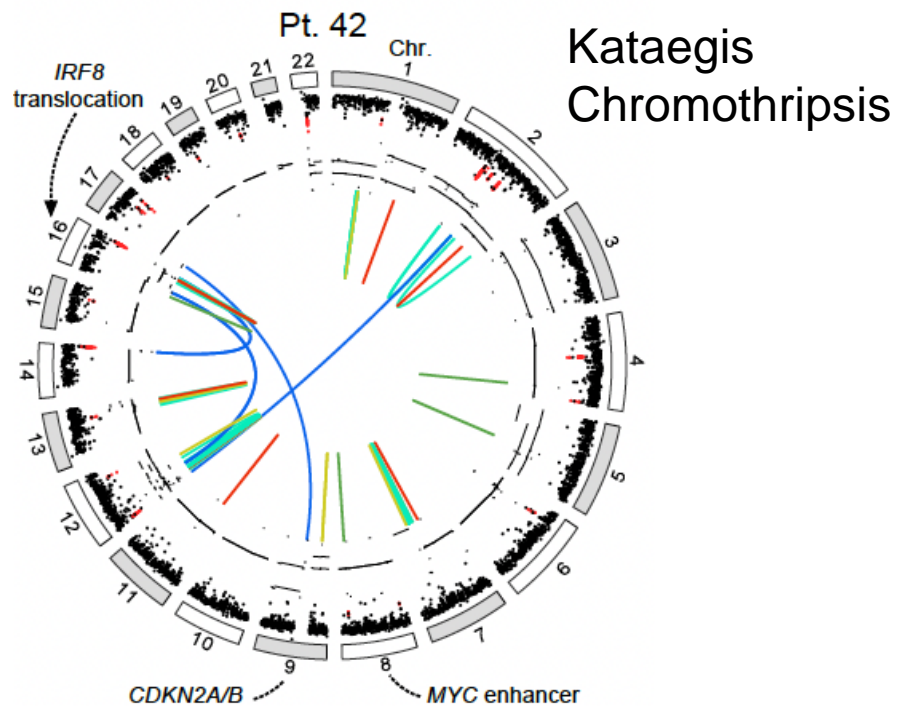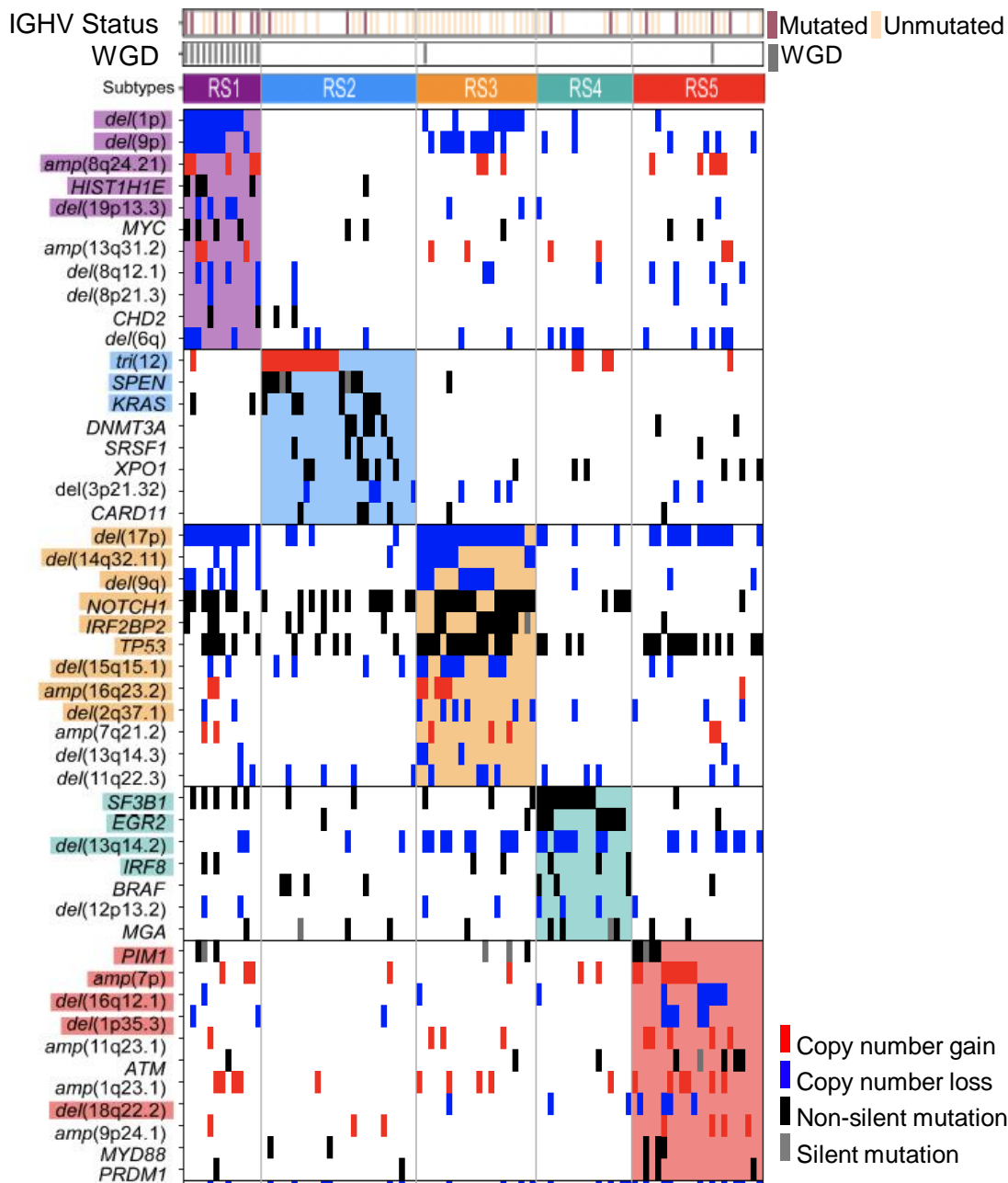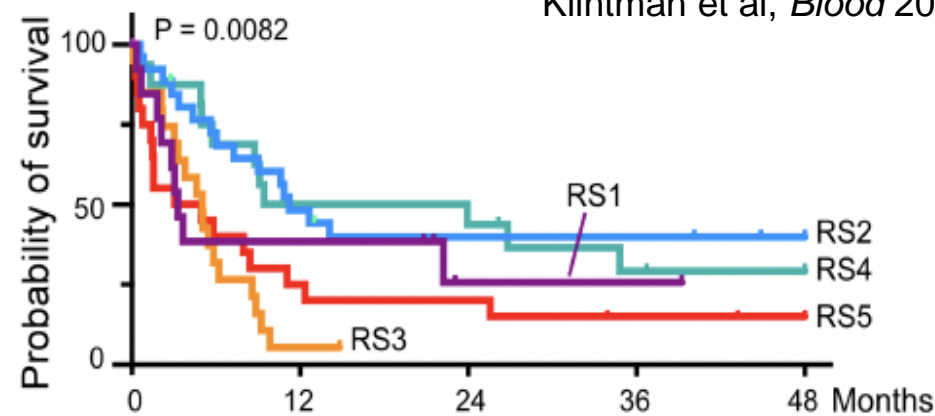

Rossi et al, *Blood* 2011
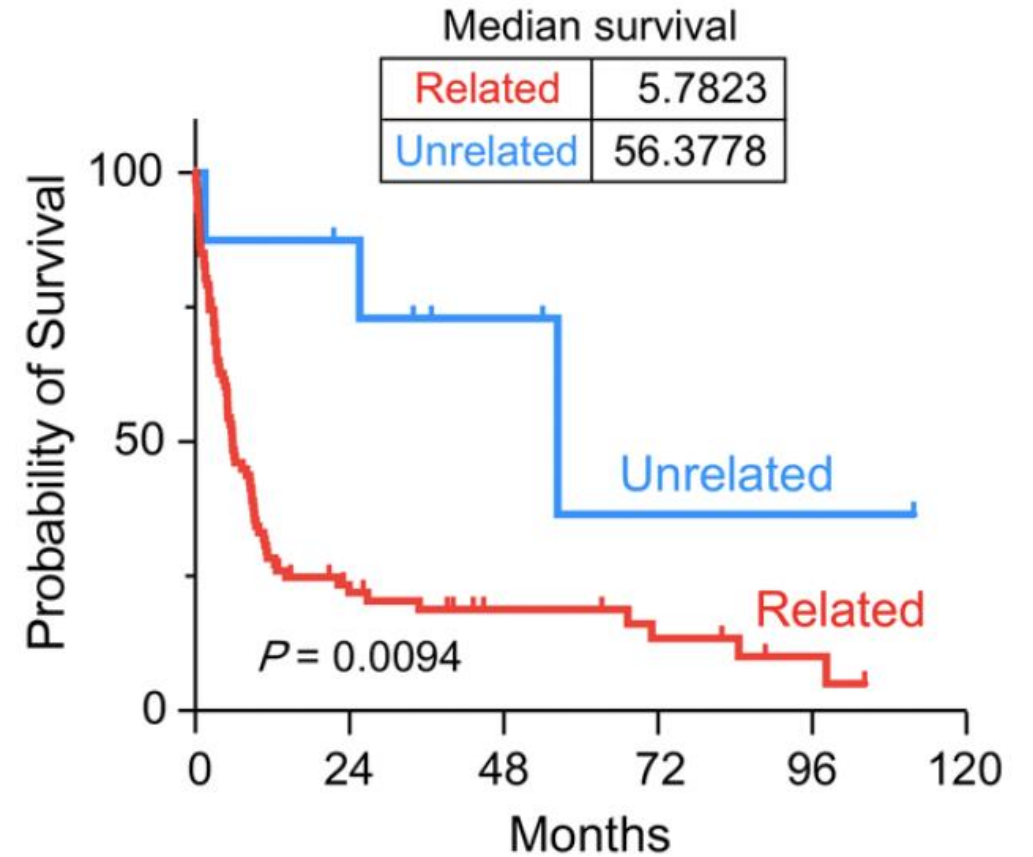
Parry, Leshchiner, Guieze et al, *Nature Medicine*, 2023

NMF clustering identifies 5 RS subtypes

-17p/*TP53*
WGD

Tri(12)
KRAS
SPEN/NOTCH1

-17p/*TP53*
NOTCH1
IRF2BP2
del(15q)*(MGA)*

SF3B1
EGR2

-17p/*TP53*
WT *NOTCH1*
sCNAs

IGHV Status — Mutated — Unmutated
WGD — WGD
Subtypes: RS1 RS2 RS3 RS4 RS5

Copy number gain
Copy number loss
Non-silent mutation
Silent mutation

Kataegis
Chromothripsis

Pt. 42

IRF8 translocation

CDKN2A/B   *MYC* enhancer

Nadeu et al, *Nature Medicine* 2022
Klintman et al, *Blood* 2021

P = 0.0082

Parry et al, *Nature Medicine*, 2023
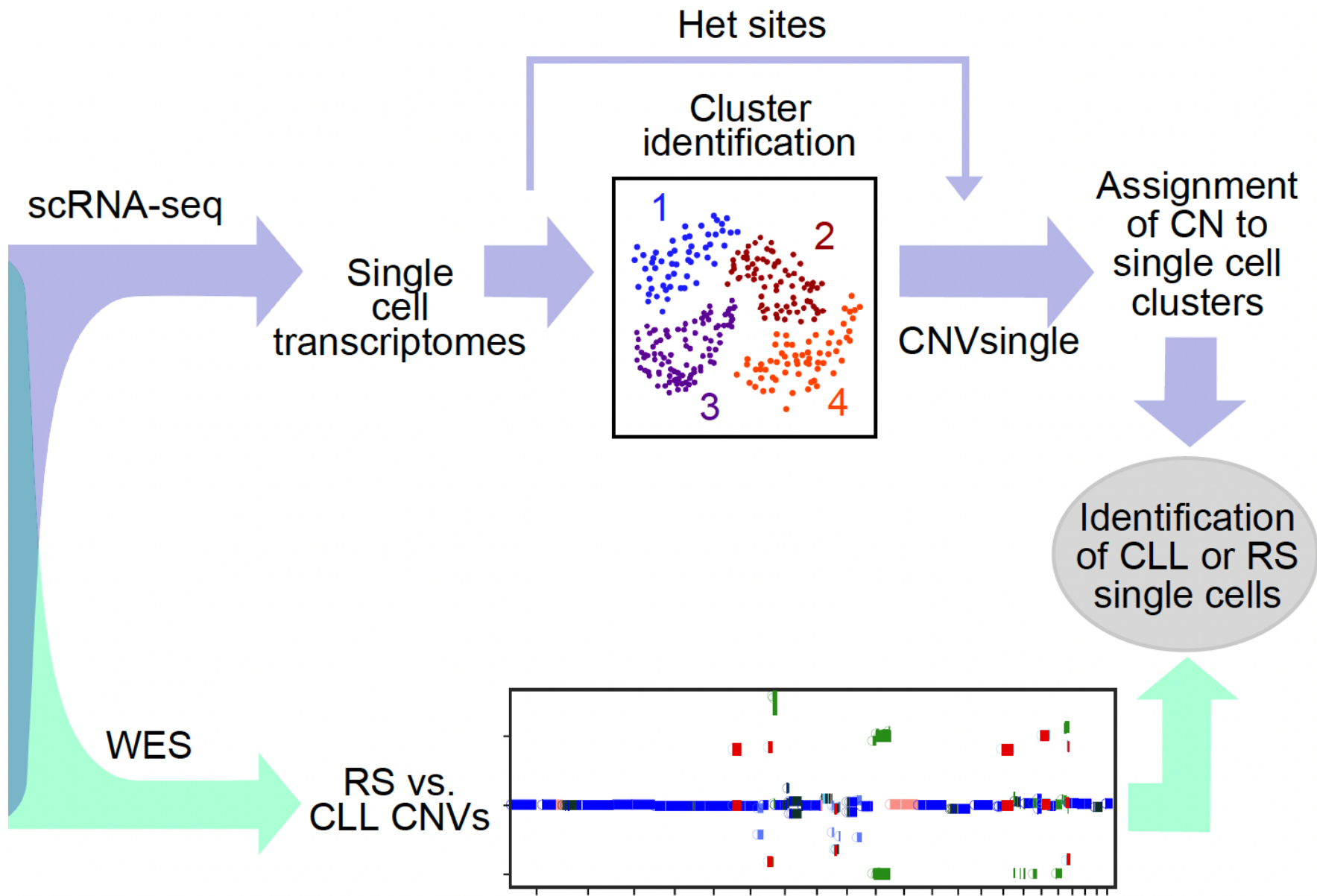
RS upregulates pathways of cell growth
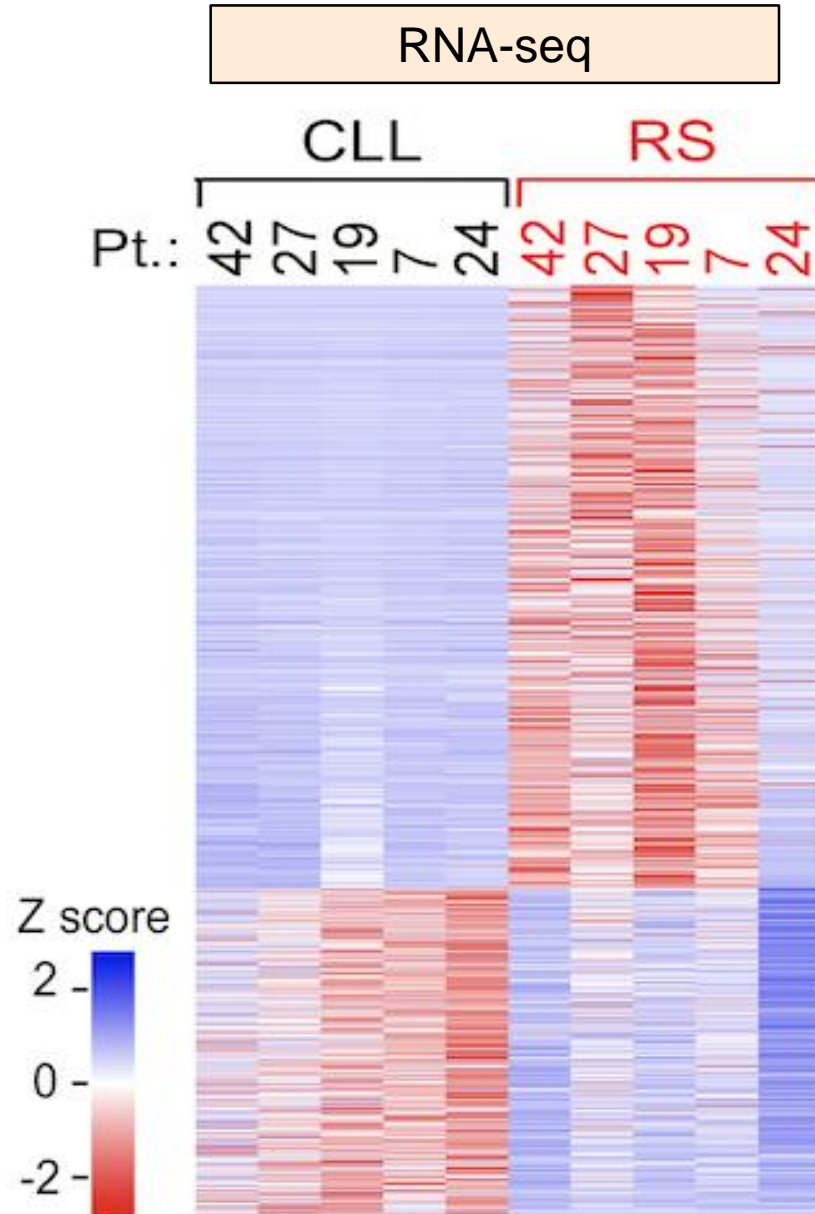
RNA-seq

Upregulated pathways

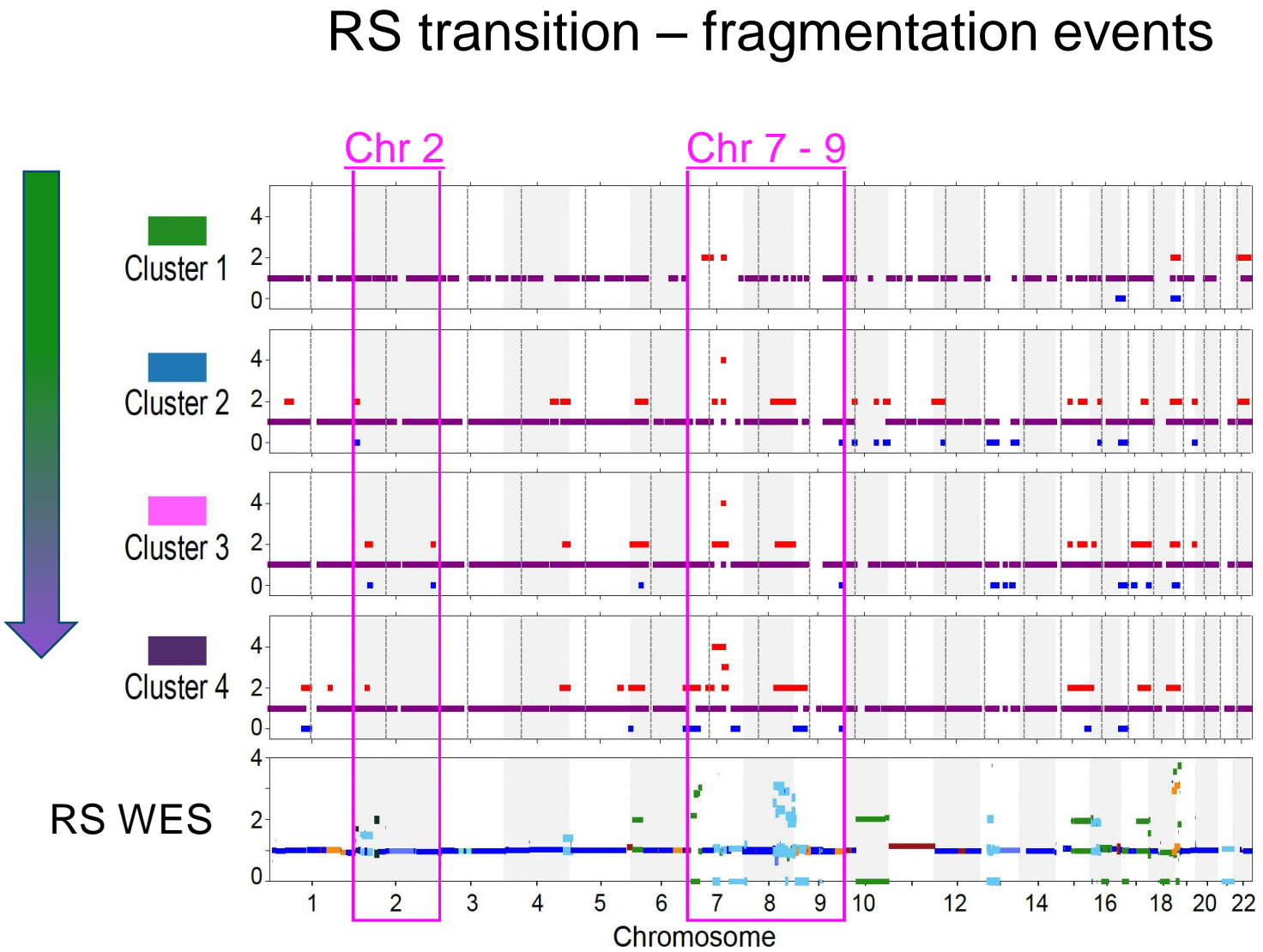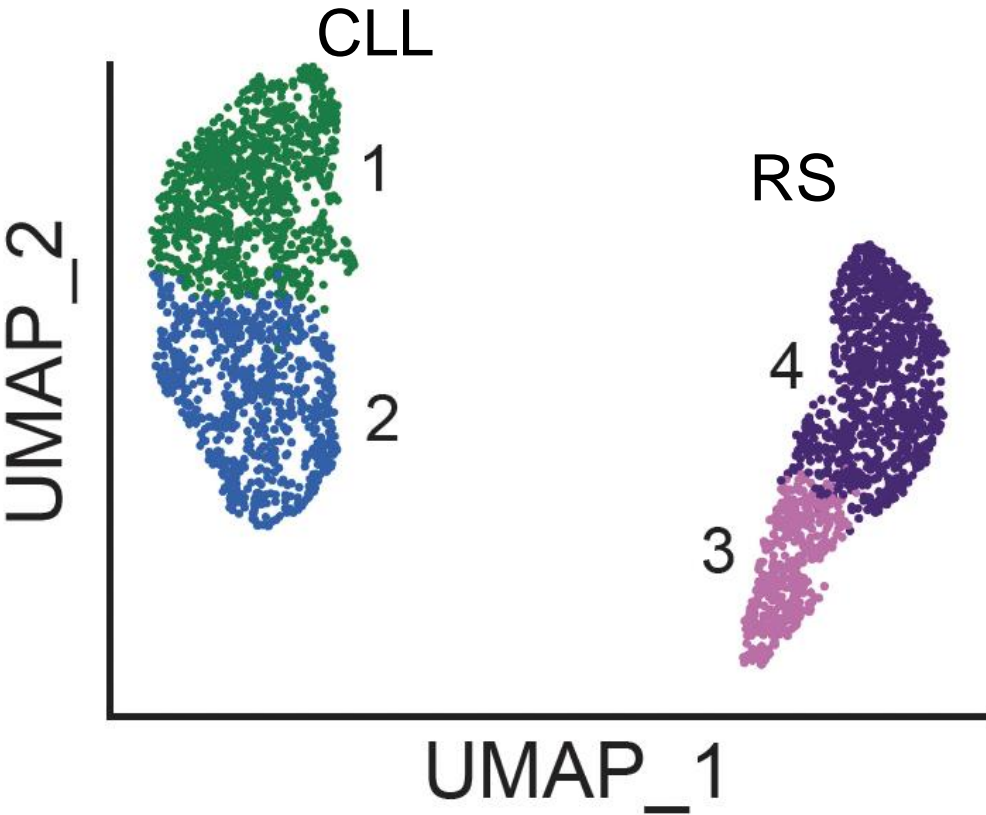- Cell cycle
- MYC targets
- MTORC1 signaling
- Mitotic spindle

Downregulated pathways

- BCR signaling

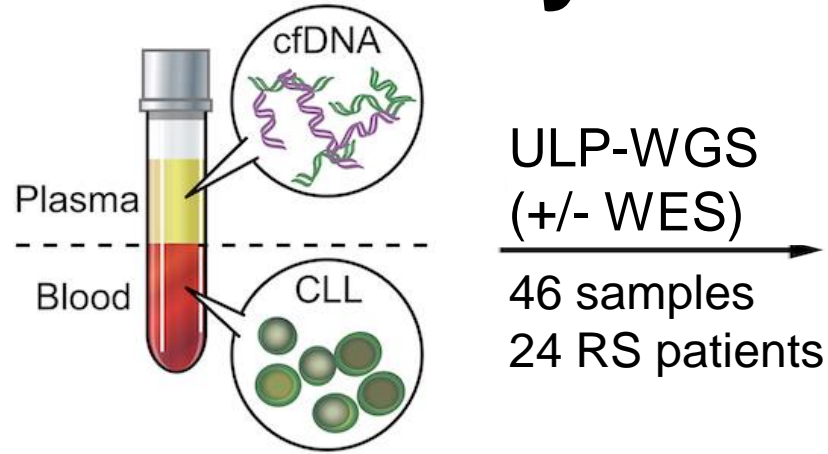Similar findings: Nadeu et al, *Nature Medicine,* 2022

Linking transcriptome to WES

Single-cell RNA-seq reveals RS in transition

Towards early detection of RS?

cfDNA

Plasma

Blood

CLL

ULP-WGS
(+/- WES)

46 samples
24 RS patients

RS specific events
- WGD
- Recurrent sCNAs
- Chromothripsis
- Driver events

Early diagnosis:

cfDNA

1
0
-1

CLL^{PB} WES

3
1

Day
-162

RS^{LN} WES

3
1

1   Chromosome   22

6   5   4   3   2   1   Dx
of RS
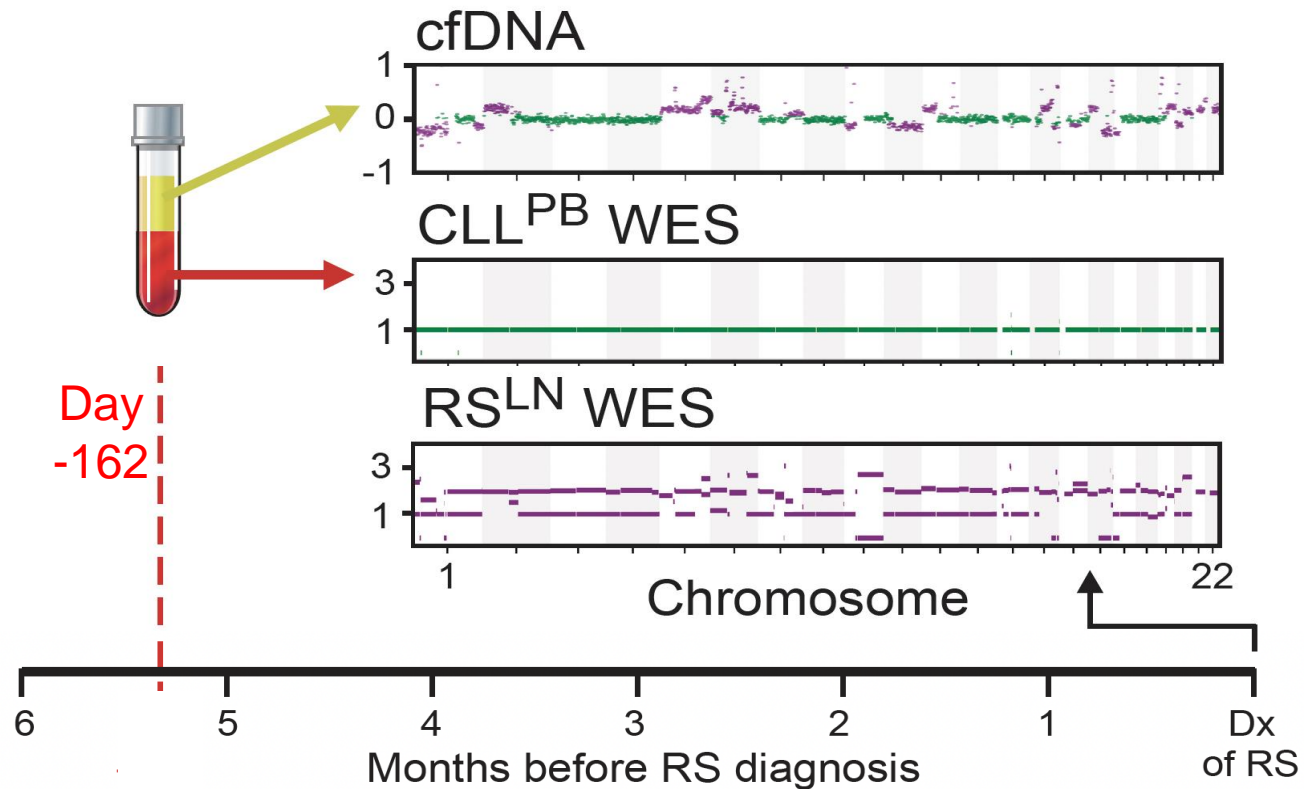
Months before RS diagnosis

Towards improved detection of RS?
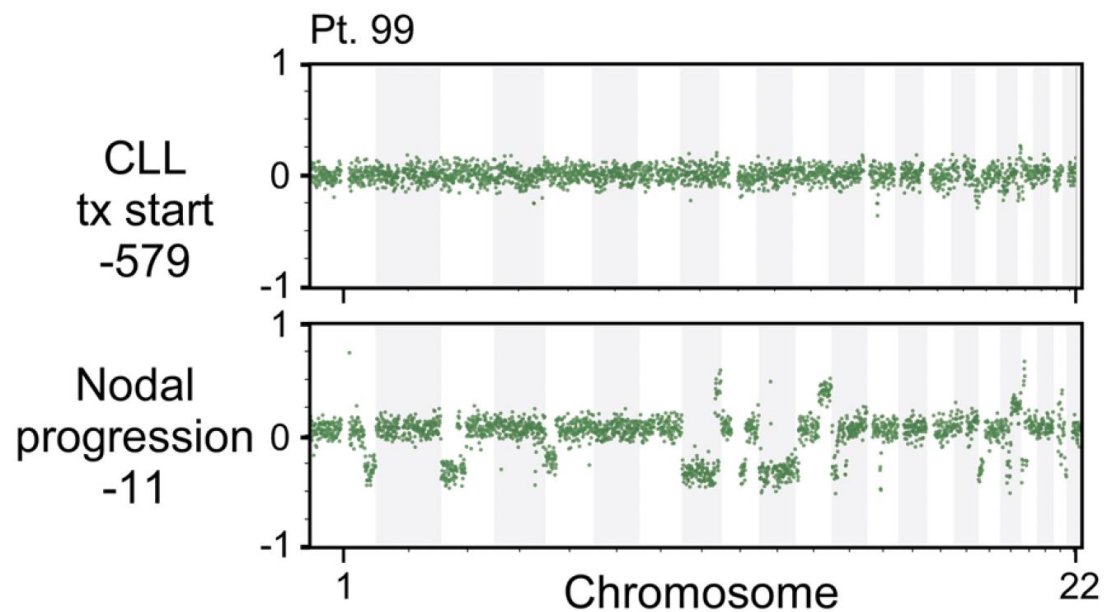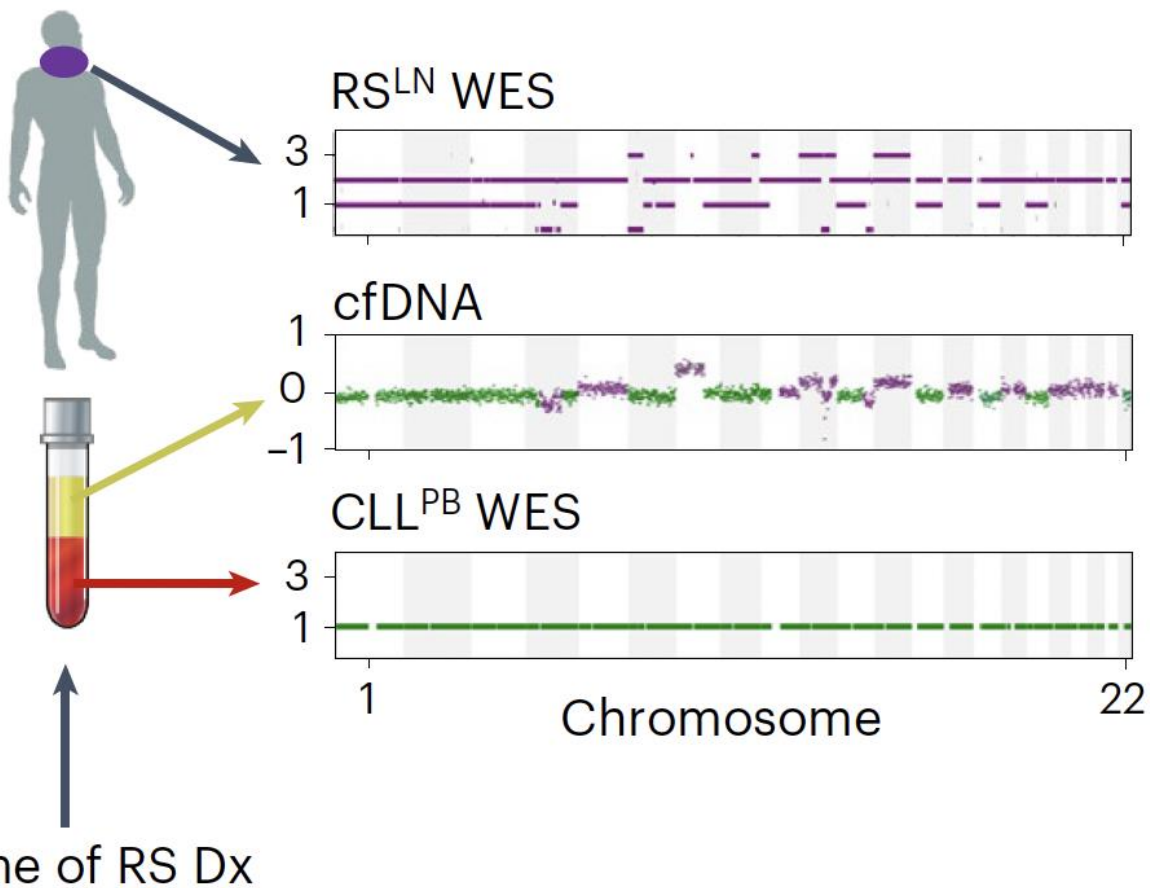
Towards improved detection of RS?
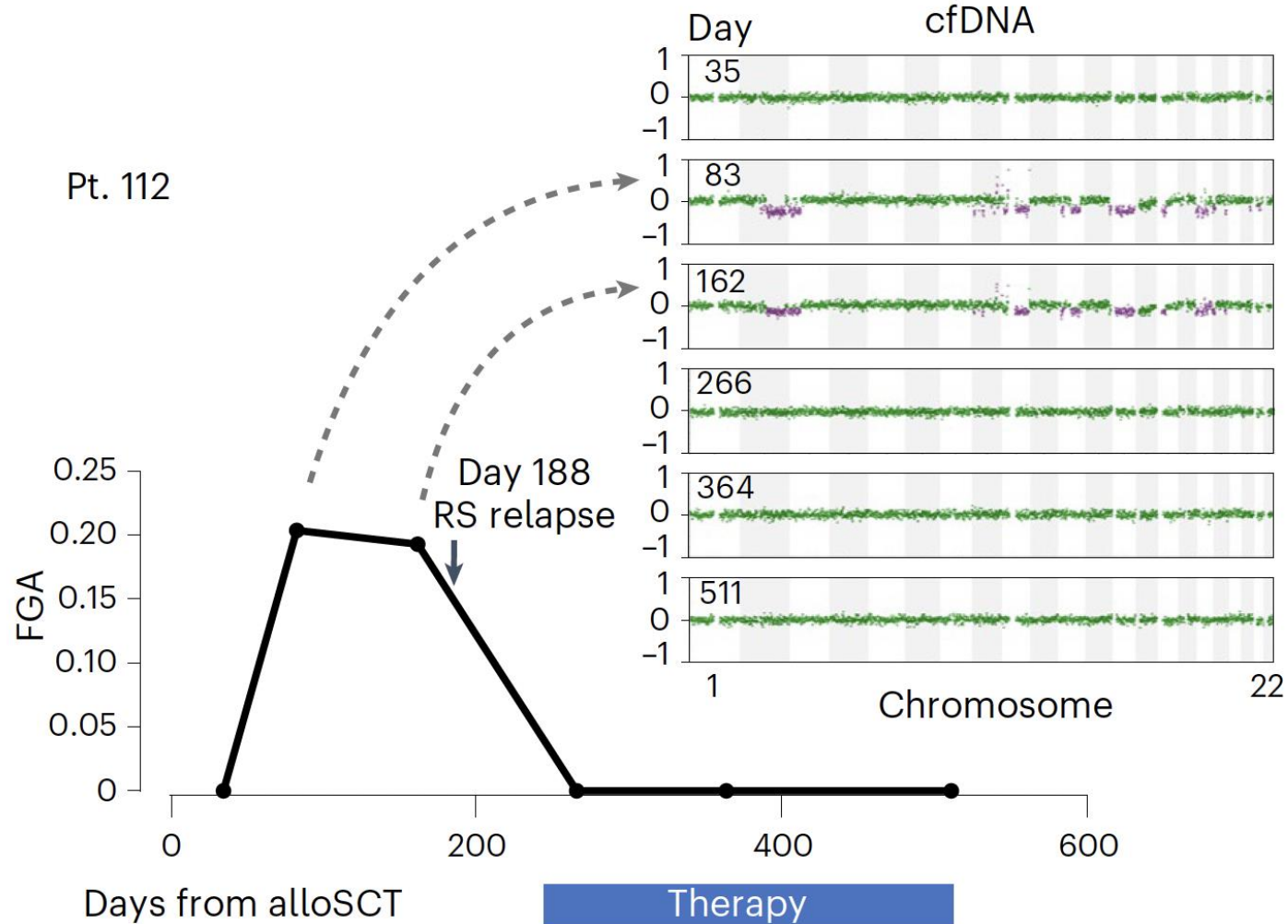
Is RS present prior to diagnosis?
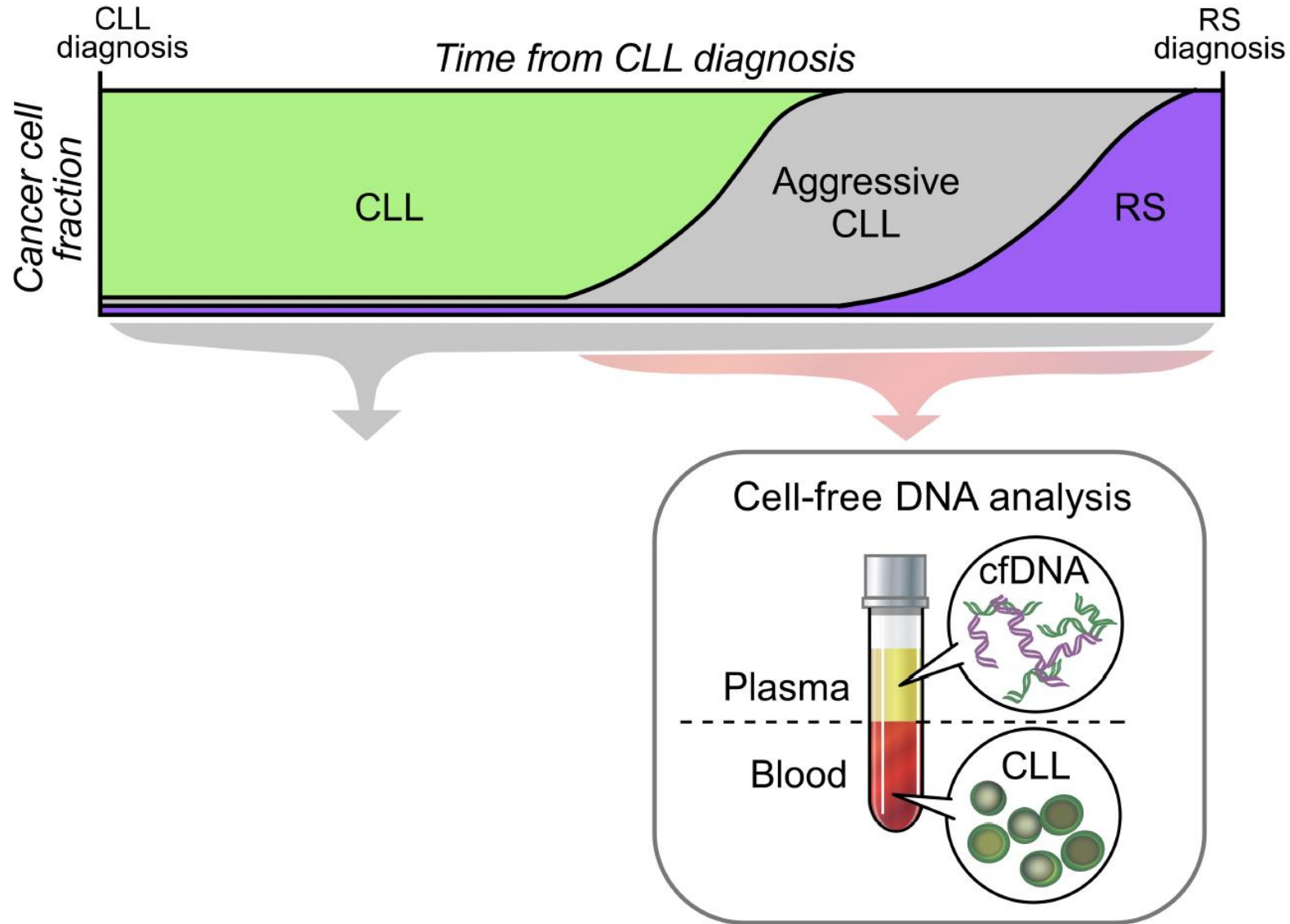
Conclusions I – Molecular basis of RS

- The majority of RS evolves from CLL subclones through acquisition of additional driver events

- Clonally related RS is distinct from *de novo* DLBCL

- Molecular subtypes of RS exist with prognostic significance

- ULP-WGS cfDNA may hold promise for non-invasive and early diagnosis of RS

II. Understanding determinants of response to immune therapy in transformation

Camilla Lemvigh

PD-1 blockade shows promise in Richter Syndrome

- 42-65% response rates to PD-1 blockade in RS

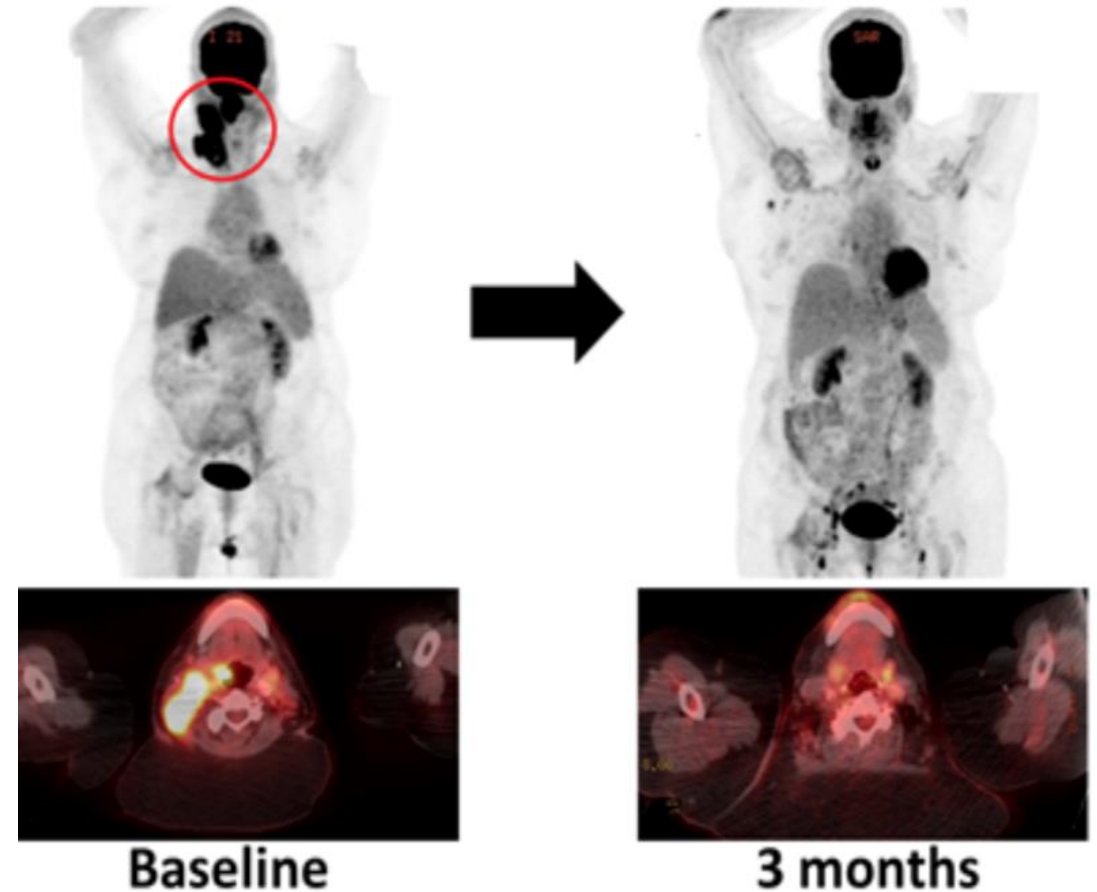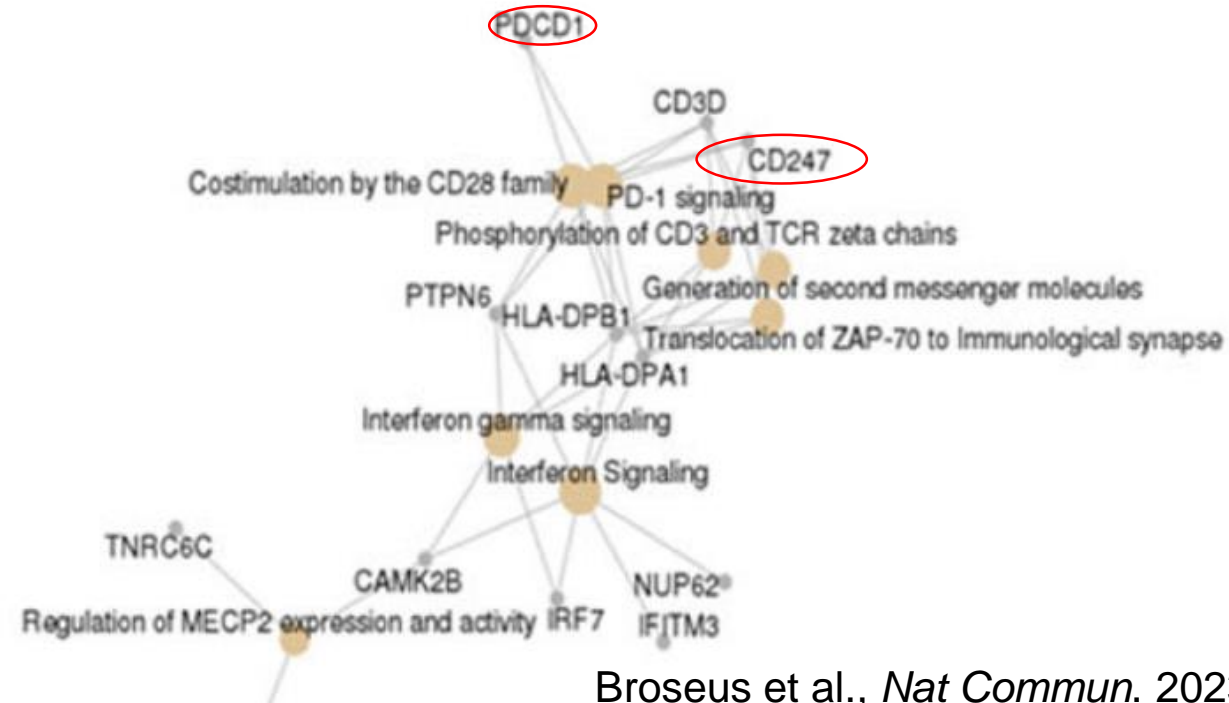- Opportunity: understand immune response to PD-1 CPB in hematologic malignancy

Baseline

3 months

Image courtesy of N. Jain, *ASH* 2018
Ding et al, *Blood* 2017; Jain et al, *Blood Advances 2022*;
Younes et al, *Lancet Haematology* 2019

RS: Pathways of immune evasion

Genetics

- 9p24 gain – 7%
- *B2M* LOF mutation– 4%

Parry, Leshchiner, Guieze et al, Nature Medicine 2023

Transcriptomics

HLA: Class I   Class II

HLA: Class I   Class II

Methylation

Broseus et al., *Nat Commun*. 2023

Protein

PD-1 expression on RS cells

Ten Hacken et al., *Blood Cancer Discov*., 2022
Nadeu et al., *Nature Medicine* 2022,

Wang et al, Blood Cancer J. 2021
He et al., Am J Surg Pathol. 2018
Behdad et al., Br J Haematol. 2019

RS: Neoantigen burden
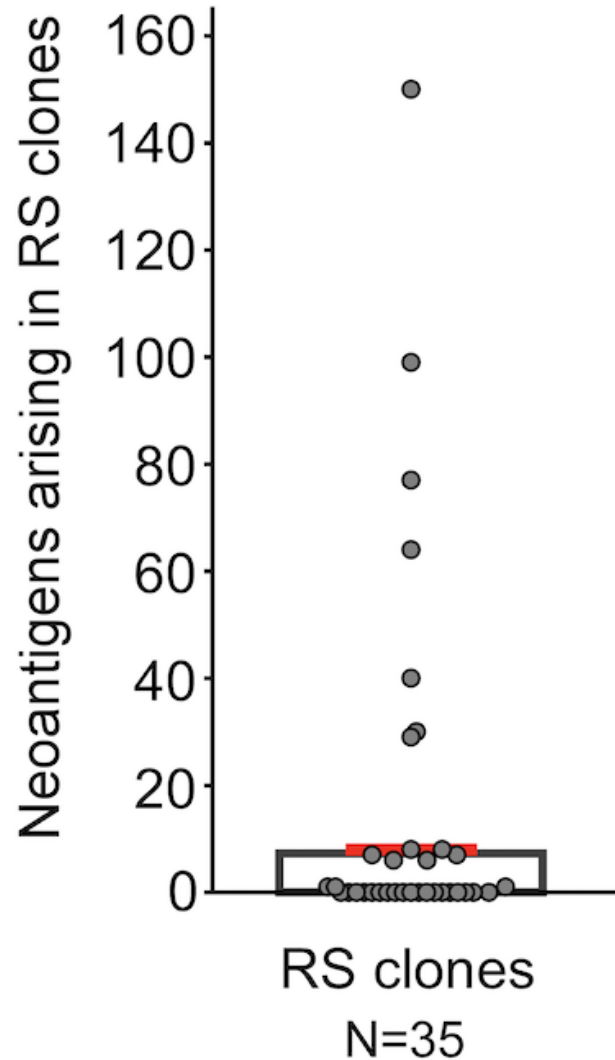
Neoantigen prediction
- HLAthena
- Inferred HLA-type from WES data

7/36 with >250 novel neoantigens at transformation

High neoantigen cases were in *TP53*-altered RS molecular subtypes

HLAthena: Sarkizova et al., *Nat Biotechnol.*, 2020
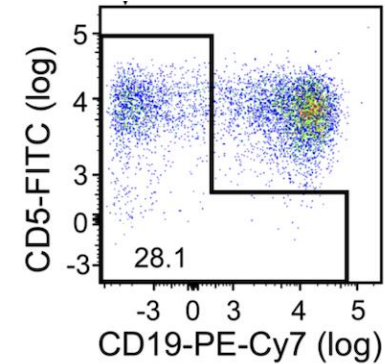*NetMHCpan 4.1*: Brynisson et al, NAR, 2020

Study Questions

- *What are the immune determinants of response and resistance to PD-1 blockade in RS?*

- *How to these determinants impact immune function?*

- *Are these immune determinants relevant in other disease settings?*

Approach: Analysis of RS marrow populations

NCT02420912

14 NR · 10 R

- Bone marrow sampling times

RS response
- ○ Complete (green)
- ○ Partial (yellow)
- ○ Progression (red)
- ○ Stable (gray)

Nivolumab 3 mg/kg every 2 weeks
Ibrutinib 420 mg daily

RS
- Responders: RS-R1, RS-R2, RS-R3, RS-R4
- Non-responders: RS-NR1, RS-NR2

CLL
- CLL-1, CLL-2

Months from treatment initiation
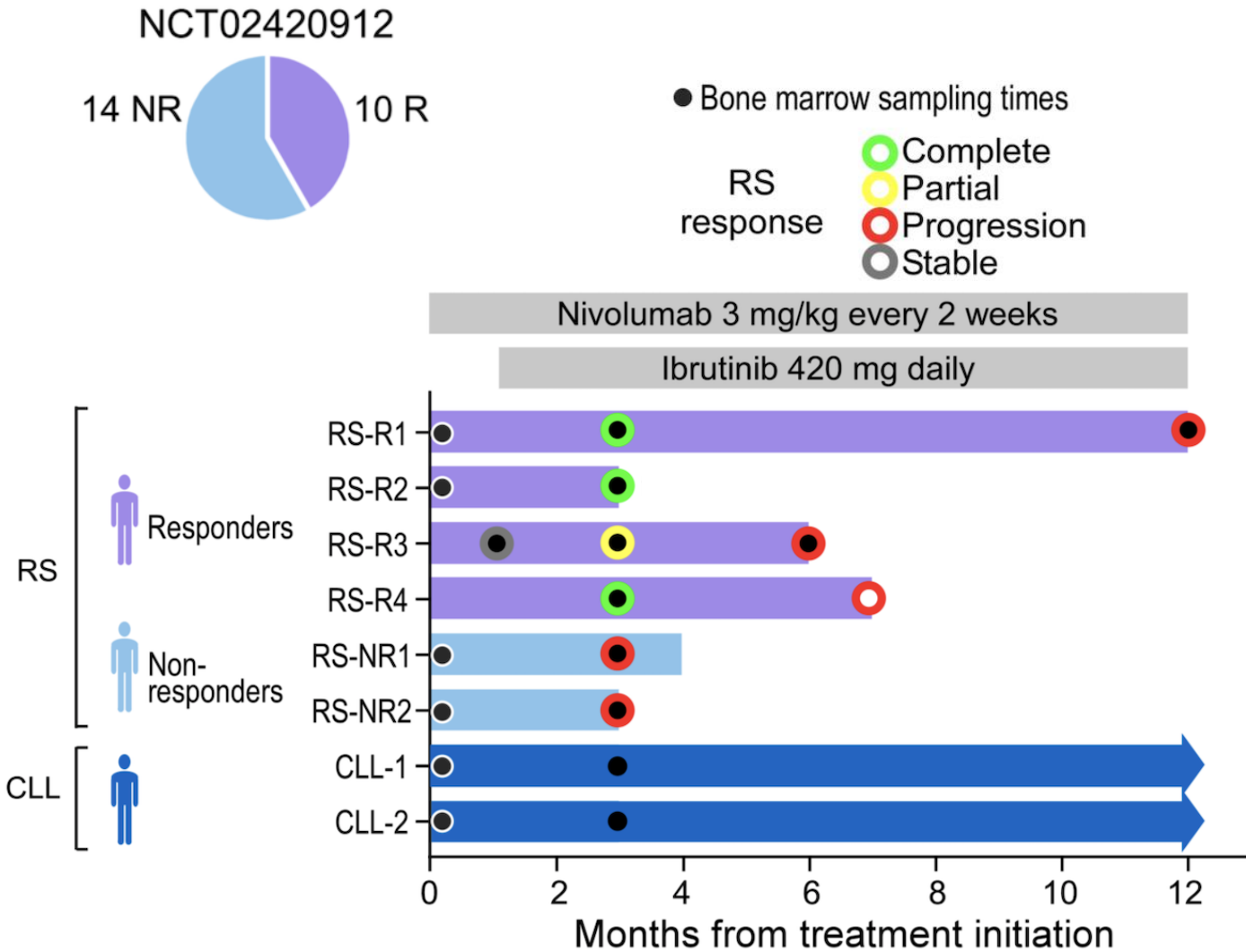
Flow cytometry sorting of bone marrow

CD5-FITC (log) vs CD19-PE-Cy7 (log)
28.1

- Sorted all viable lymphocytes

- Selected non-tumor immune fraction

Analysis

NK cells, T cells, B cells, Erythroid progenitor, CD34+ stem cells, Monocytes/DC
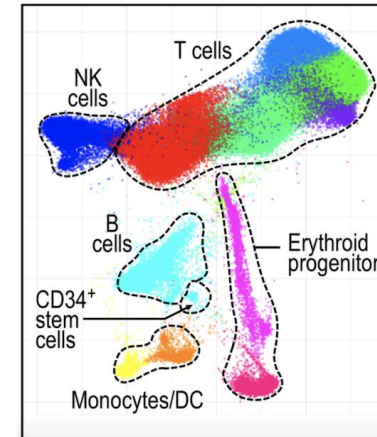
- scRNA-seq (10x)
- Data processing (Pagoda 2)
- Joint clustering (Conos)

Nitin Jain, William Wierda

Jain et al., *Blood Advances* 2022; Parry Lemvigh et al, *Cancer Cell,* 2023

Barkas et al., *Nat Methods* 2019;
Fan et al., *Nat Methods* 2017

RS-R: Enriched in CD8 Effector/Effector Memory

All cells

78,488 cells

Sub-clustering of T/NK cells

CD8 Effector/Effector Memory

60,727 cells

RS-R
RS-NR
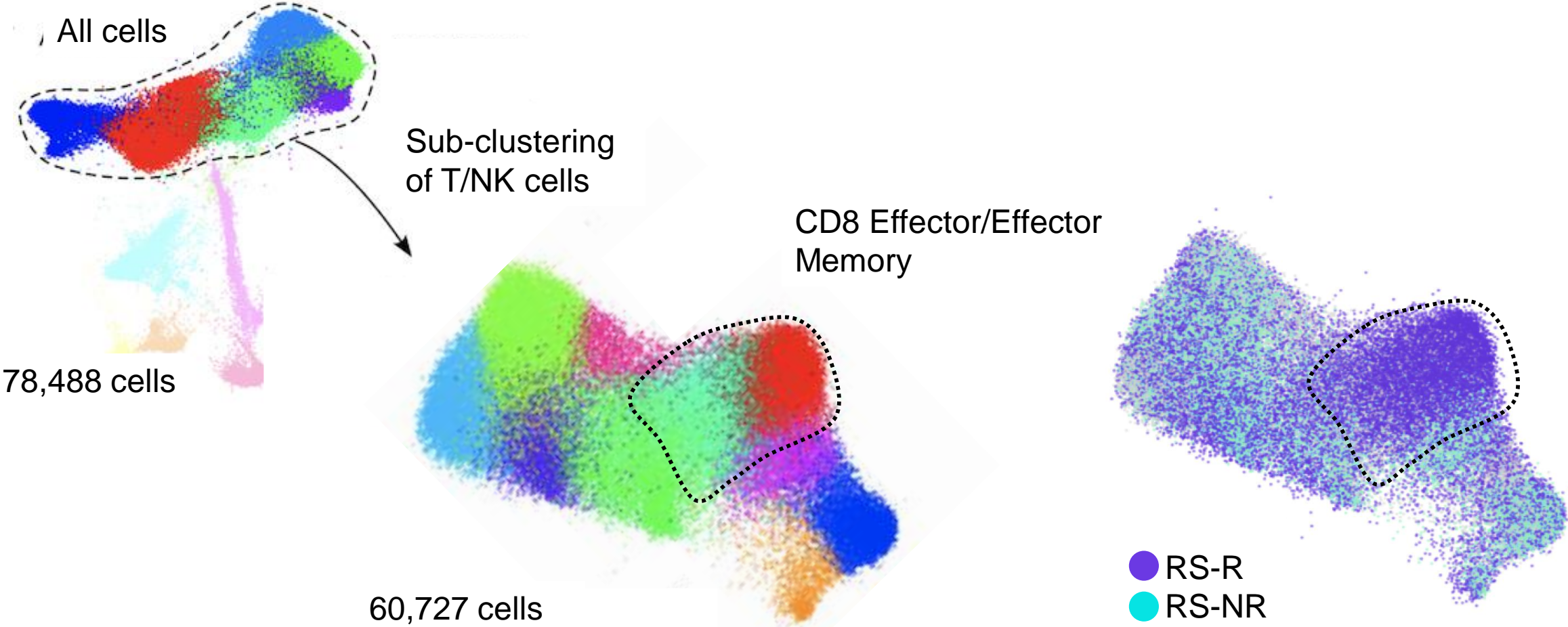
CD8+ E/EM (C1) population is marked by *ZNF683*
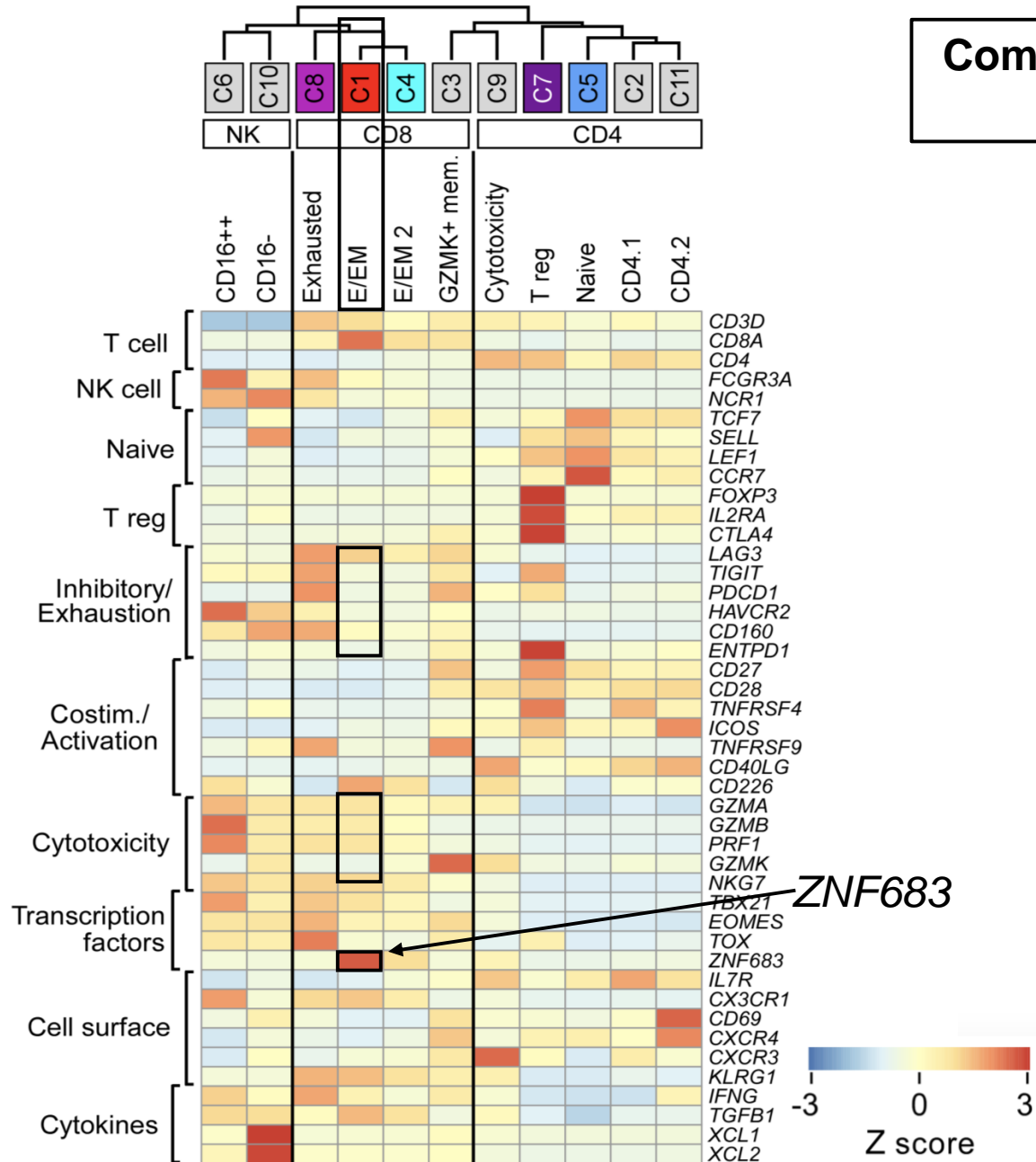
Compared to Normal Marrow (n=32)
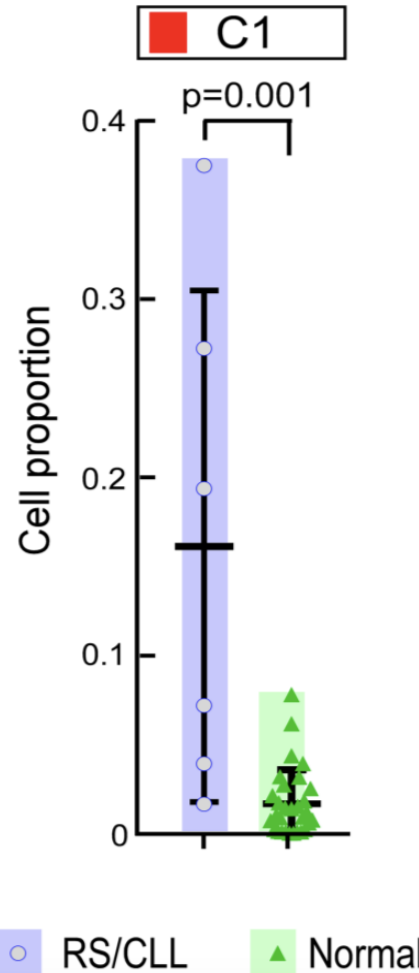
Cluster 1 (C1) CD8+ T cells
- Effector/effector memory (E/EM)
- Preserved cytotoxicity
- *Intermediate* exhaustion
- Rare in normal bone marrow
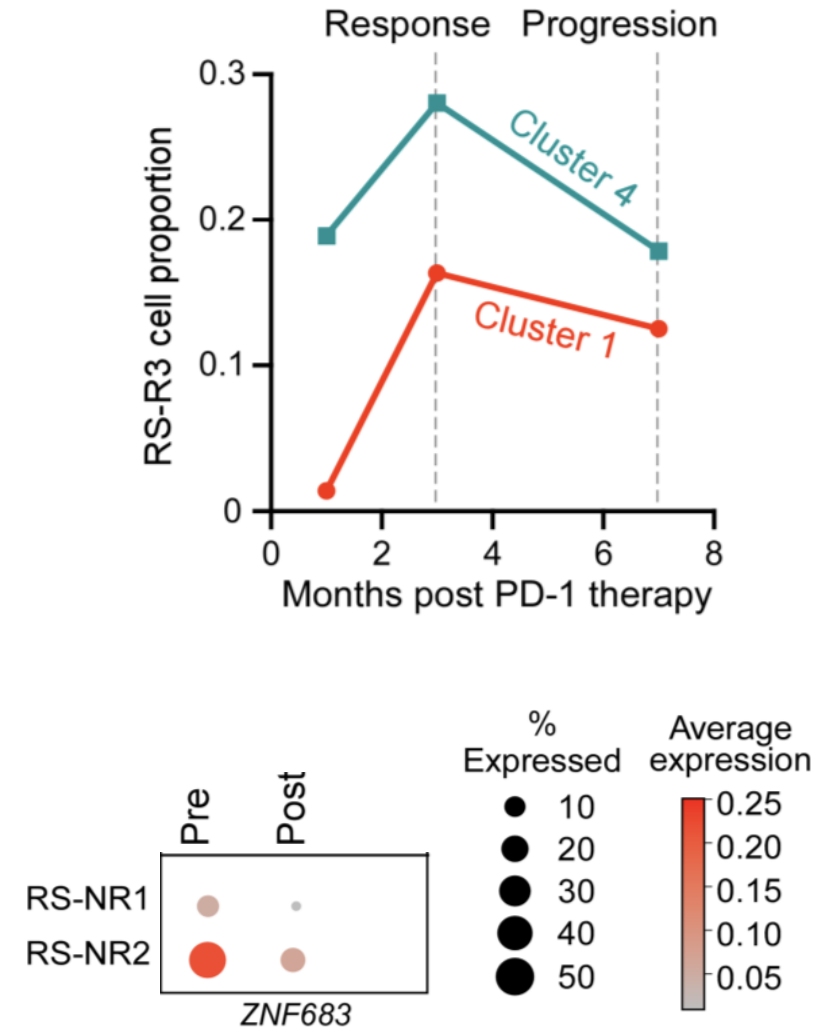- Marked by expression of *ZNF683* (Hobit, Homolog of Blimp1)

RS Responders: *ZNF683* and response
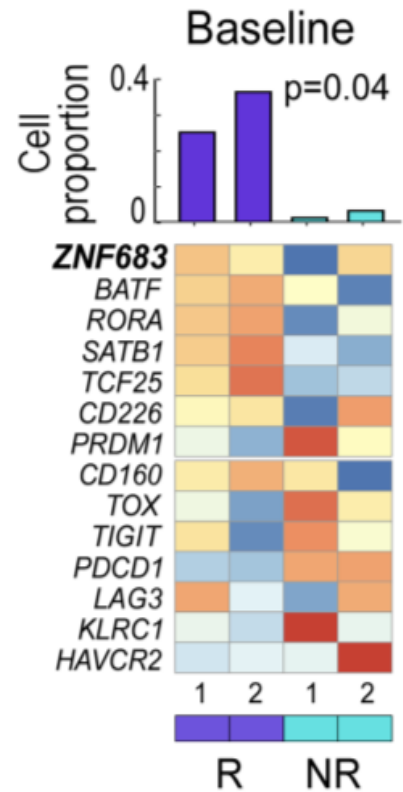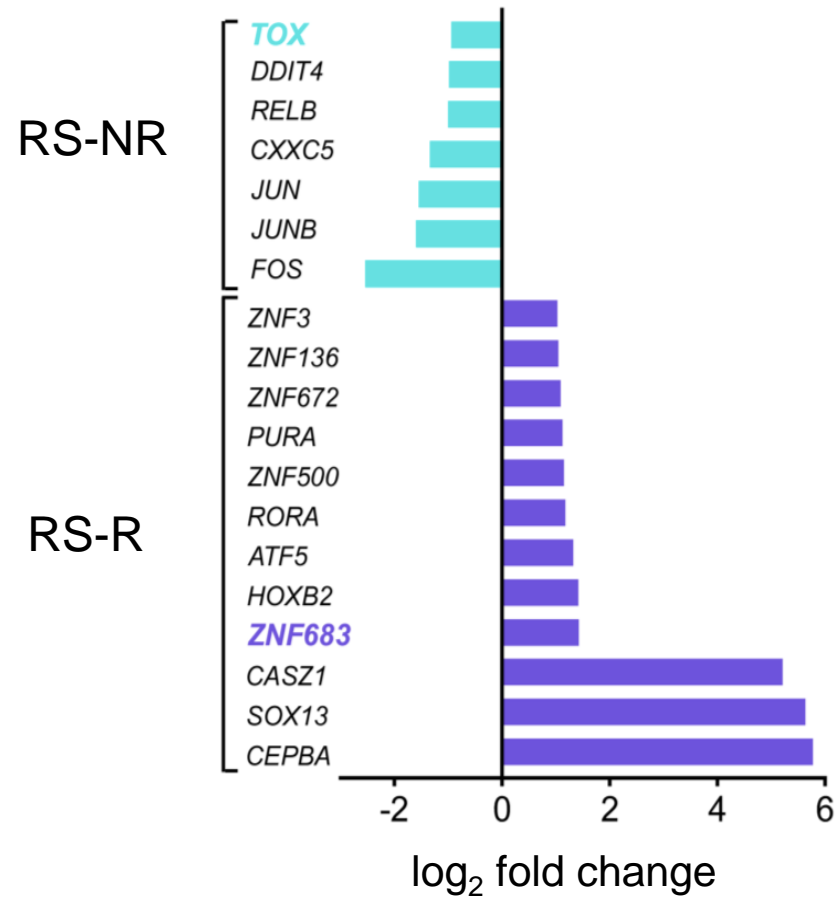
C1 ZNF683^{high}: Clonotype enrichment
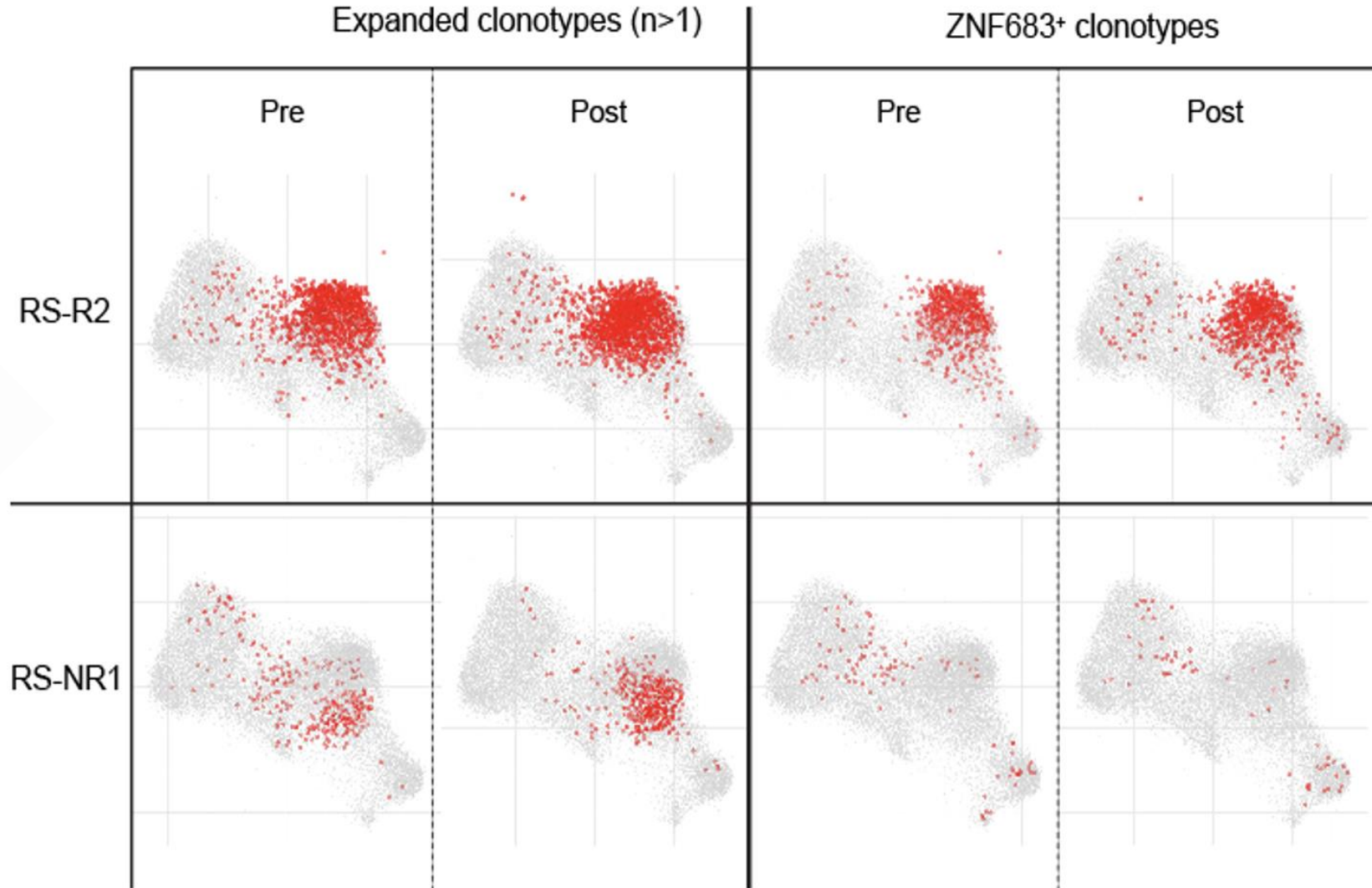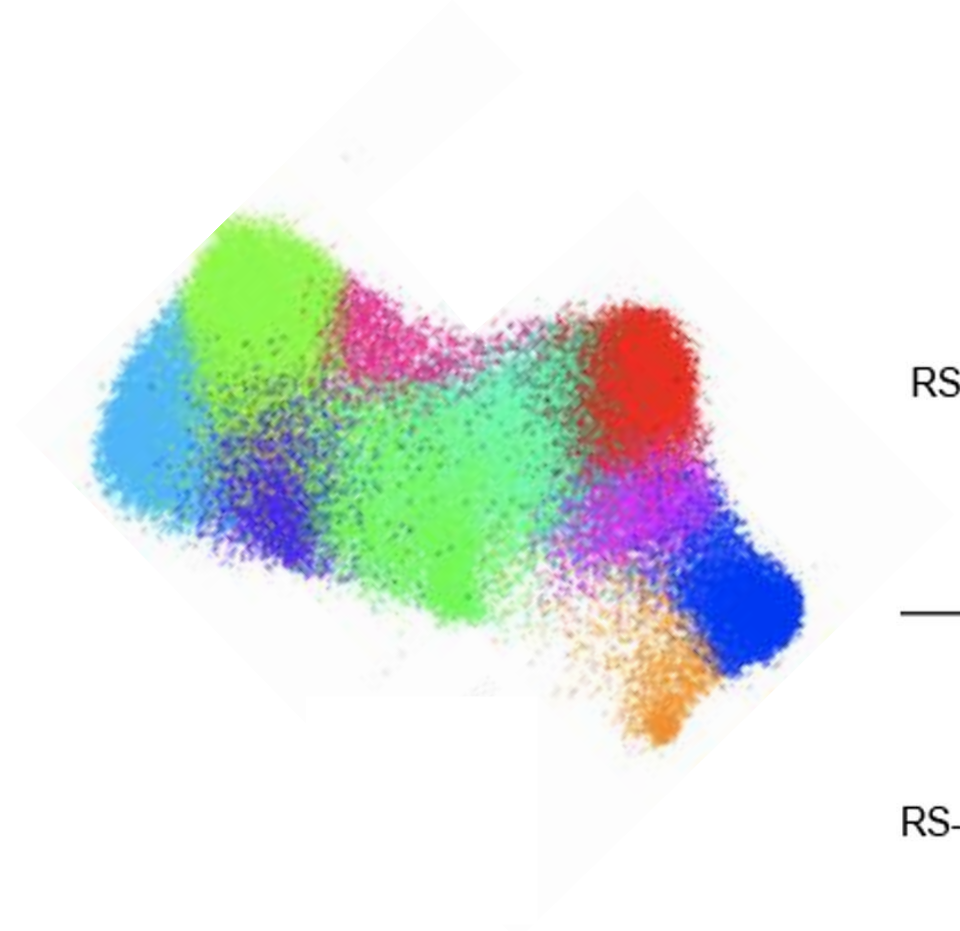
RS-R: Clonotype stability

Bone marrow – scTCRseq
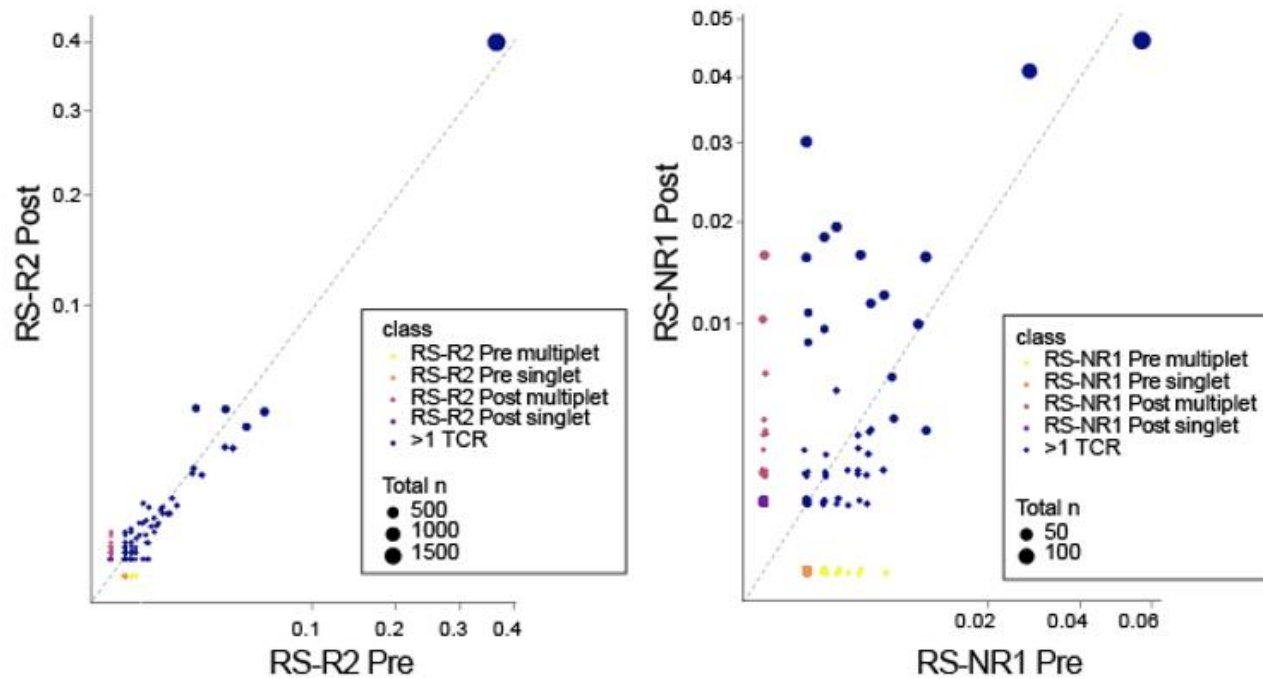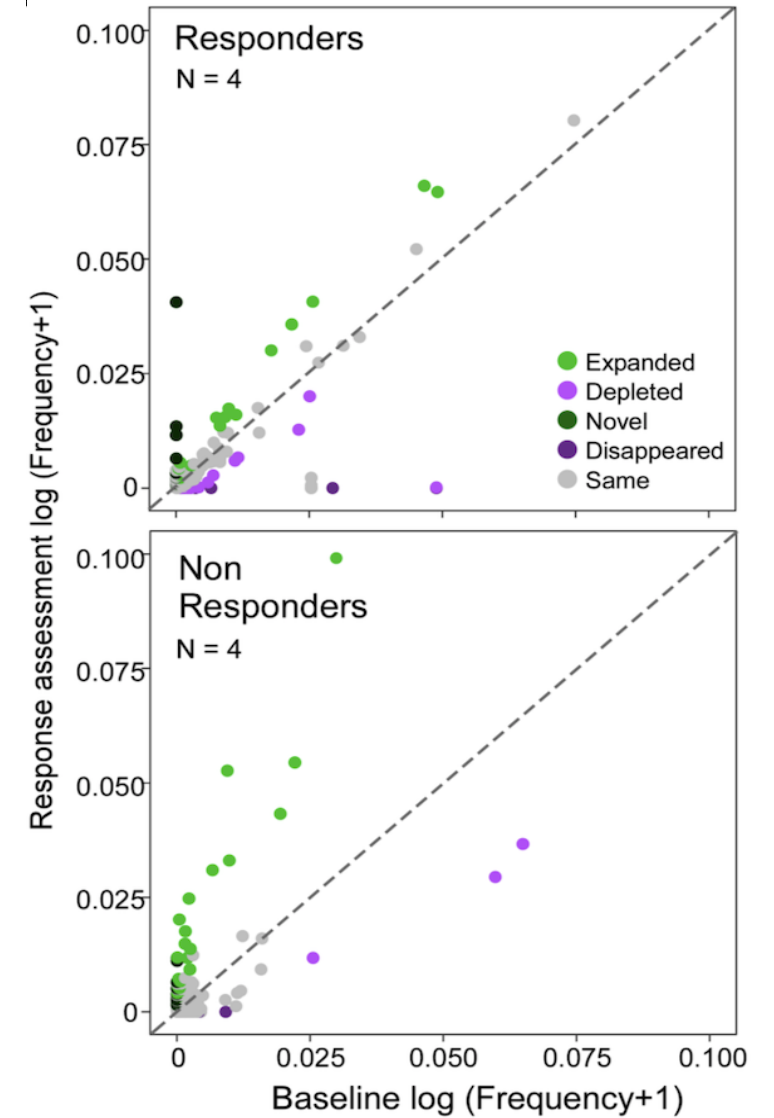
C1 ZNF683^{high}: Divergent from terminal exhaustion
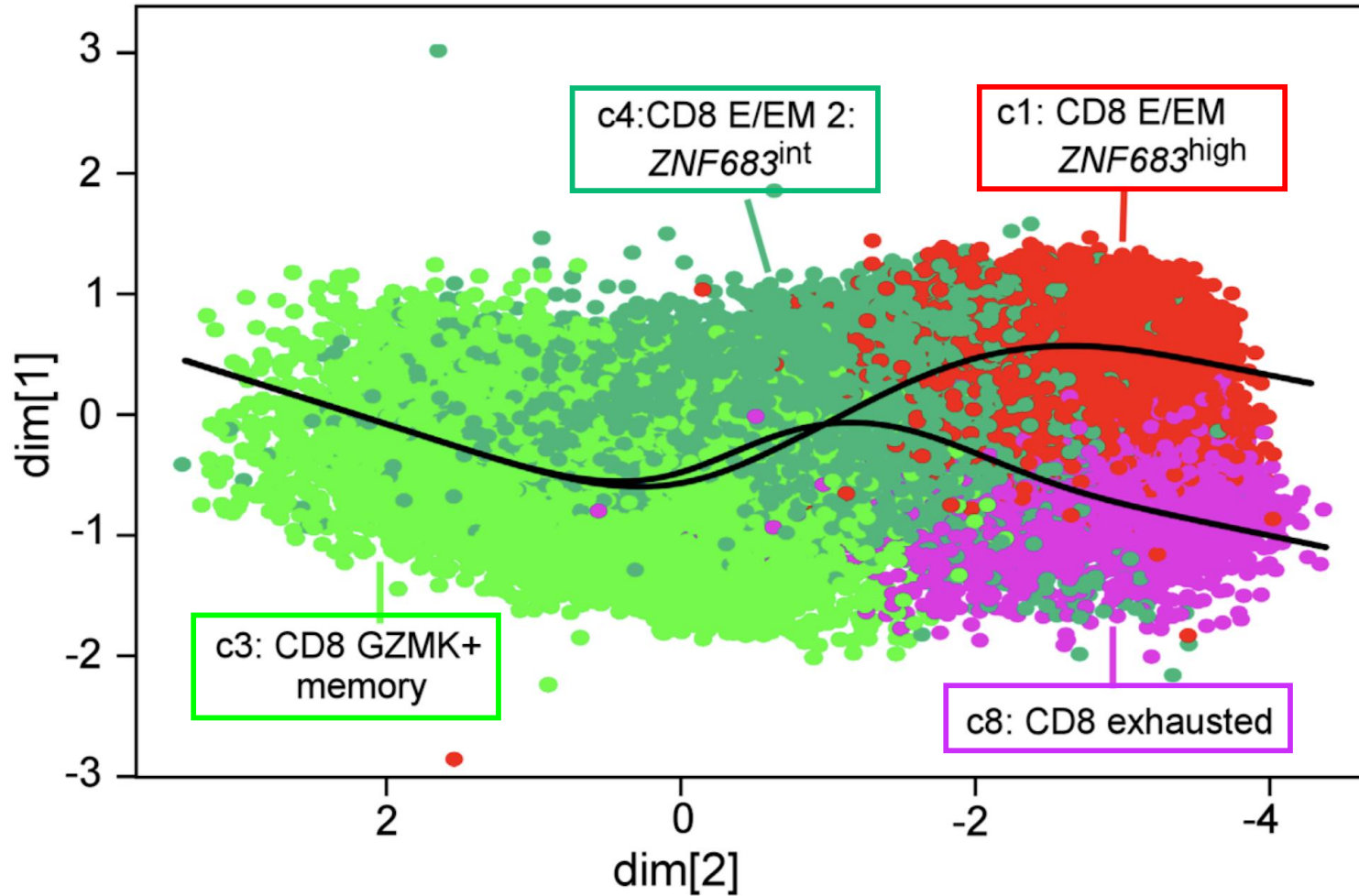
- Displays overlap with recently discovered CD8 populations
  - Exhausted intermediate
  - Exhausted divergent (KLR)

Giles et al, *Immunity*, 2022
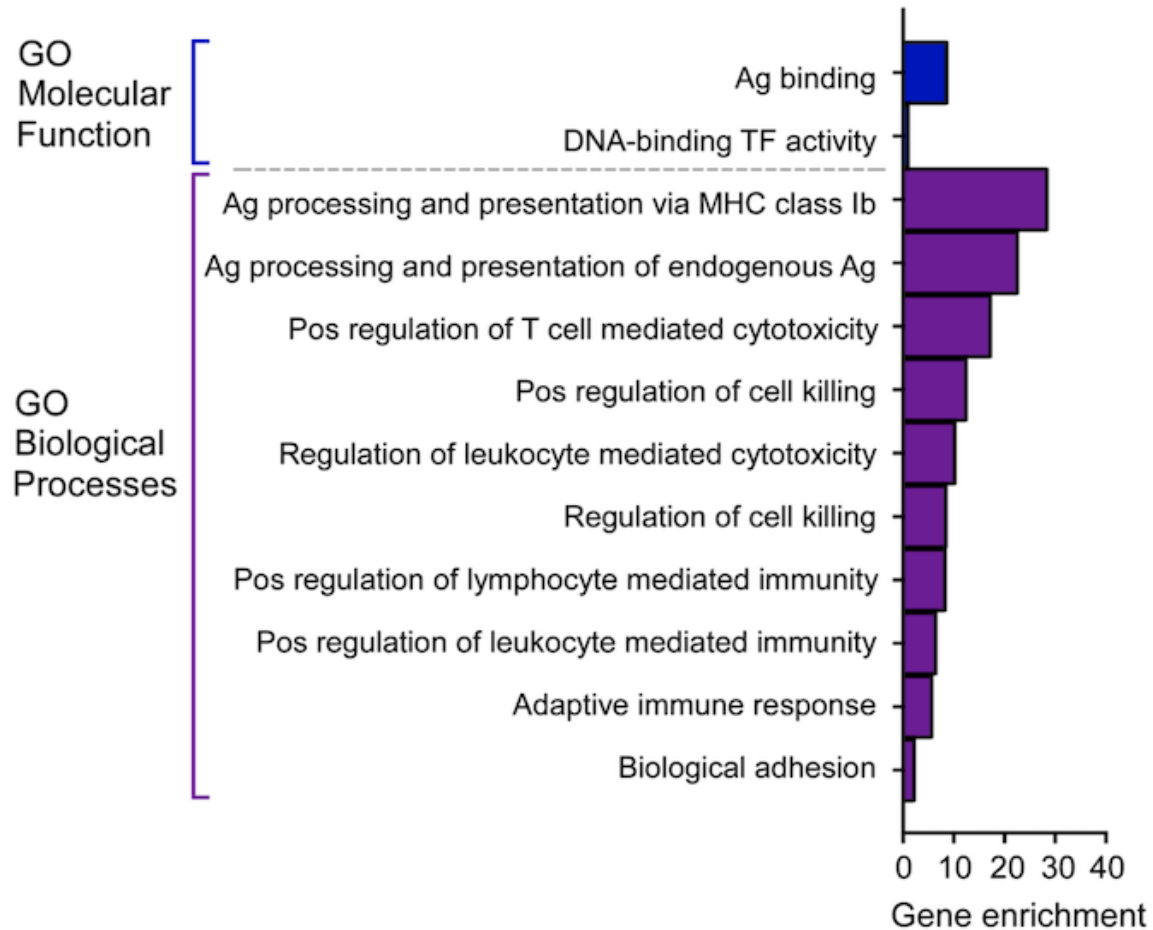Daniels et al, *Nature Immunology*, 2022

ZNF683 function?

Slingshot: Street et al., *BMC Genomics* 2018

ZNF683 regulates key immune pathways

ATAC-seq data: Philip et al., *Nature* 2017

ZNF683 regulates key immune pathways

Cistrome-GO: Li et al, *Nucleic Acids Research*, 2019
Meme suite: Bailey et al, *Nucleic Acids Research*, 2015

*ZNF683*high signature is detected in the peripheral blood

*ZNF683*high signature is detected in the peripheral blood

Melanoma blood

CD8+ T cell
PD-1 response

Cluster1

ZNF683
FGFBP2
176   103   60
GNLY
GZMH

P < 0.001
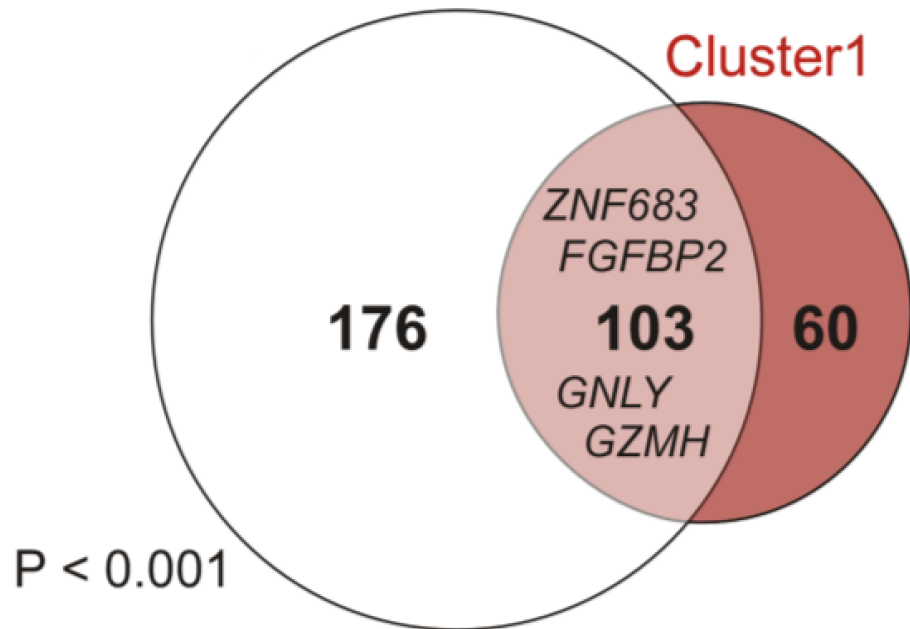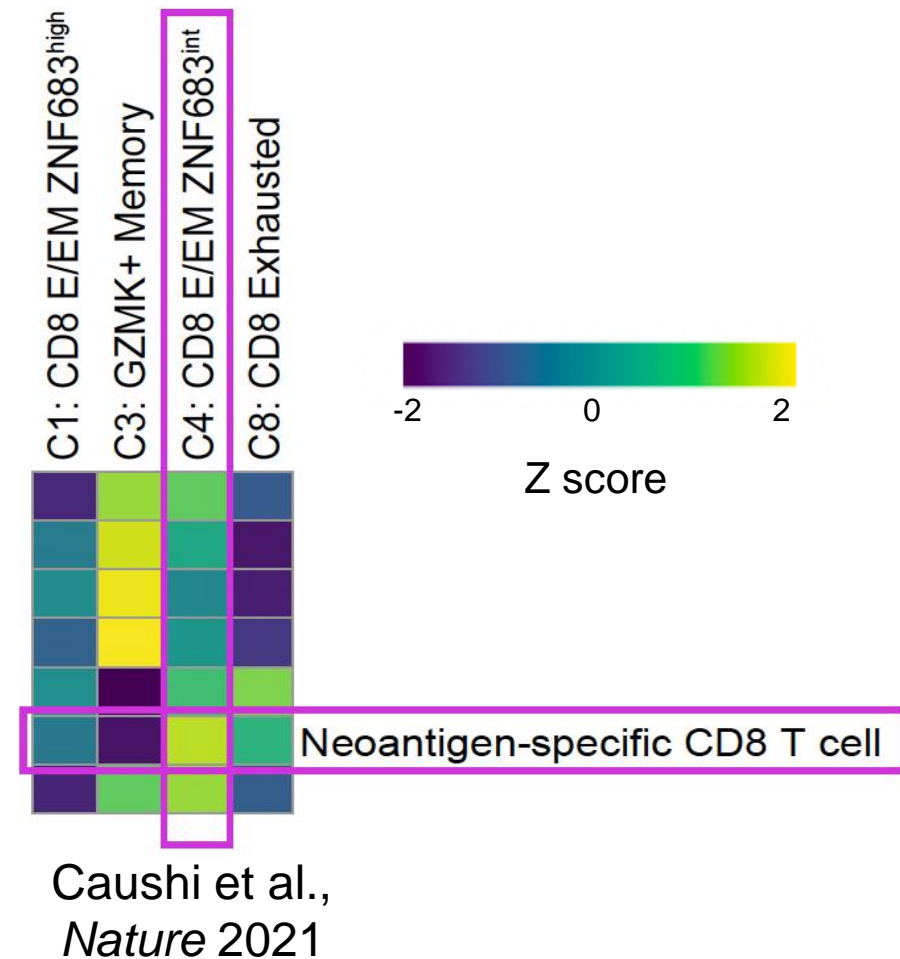
Fairfax et al.,
Nature Medicine 2020

Lung cancer blood

Neoantigen-specific CD8+ T cell
Early in PD-1 treatment

C1: CD8 E/EM ZNF683high
C3: GZMK+ Memory
C4: CD8 E/EM ZNF683int
C8: CD8 Exhausted

Z score
-2   0   2

Neoantigen-specific CD8 T cell

Caushi et al.,
Nature 2021

ZNF683^{high} signatures across cancer
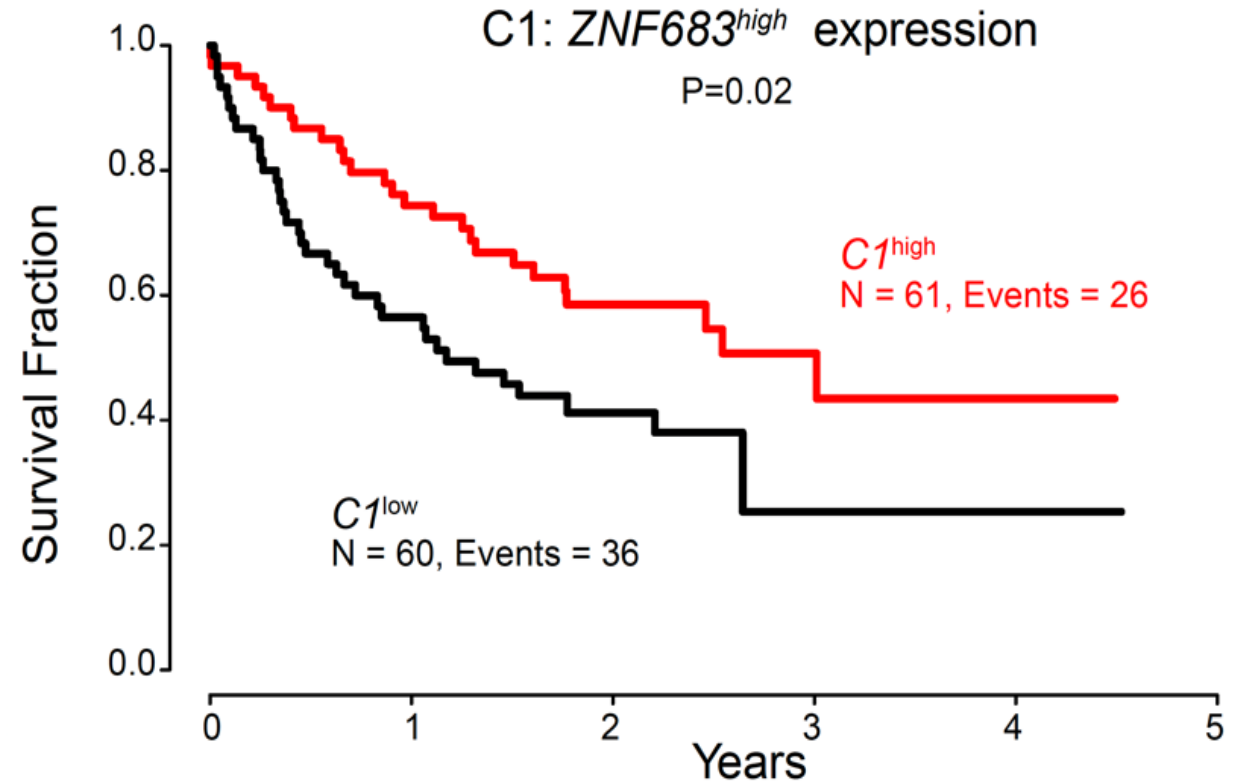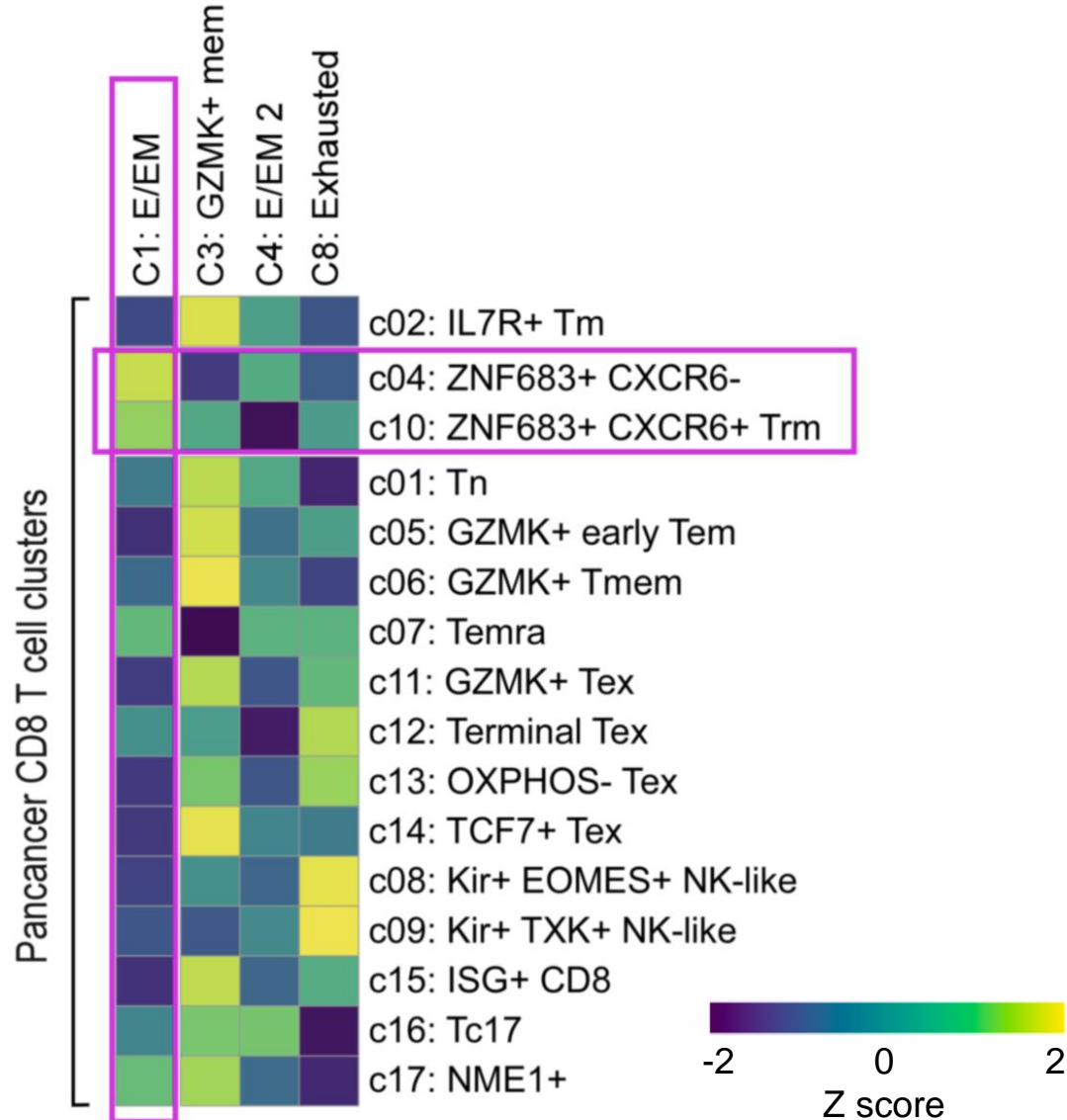

Pan-cancer: Zheng et al., *Science*, 2021
Melanoma: Liu et al., *Nature Medicine*, 2018

Conclusions II: *ZNF683*

- RS-R show increased marrow *ZNF683*high CD8 E/EM T cells compared to RS-NR

- ZNF683 regulates pathways of T cell differentiation, activation and cytotoxicity

- The *ZNF683*high signature is detectable in the peripheral blood and associates with checkpoint blockade response

- Ongoing studies are focusing on investigating
  - The predictive potential of this population
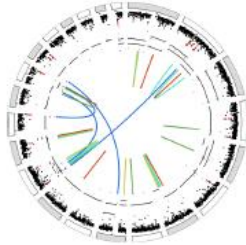 - Dissecting the function of ZNF683 *in vivo*
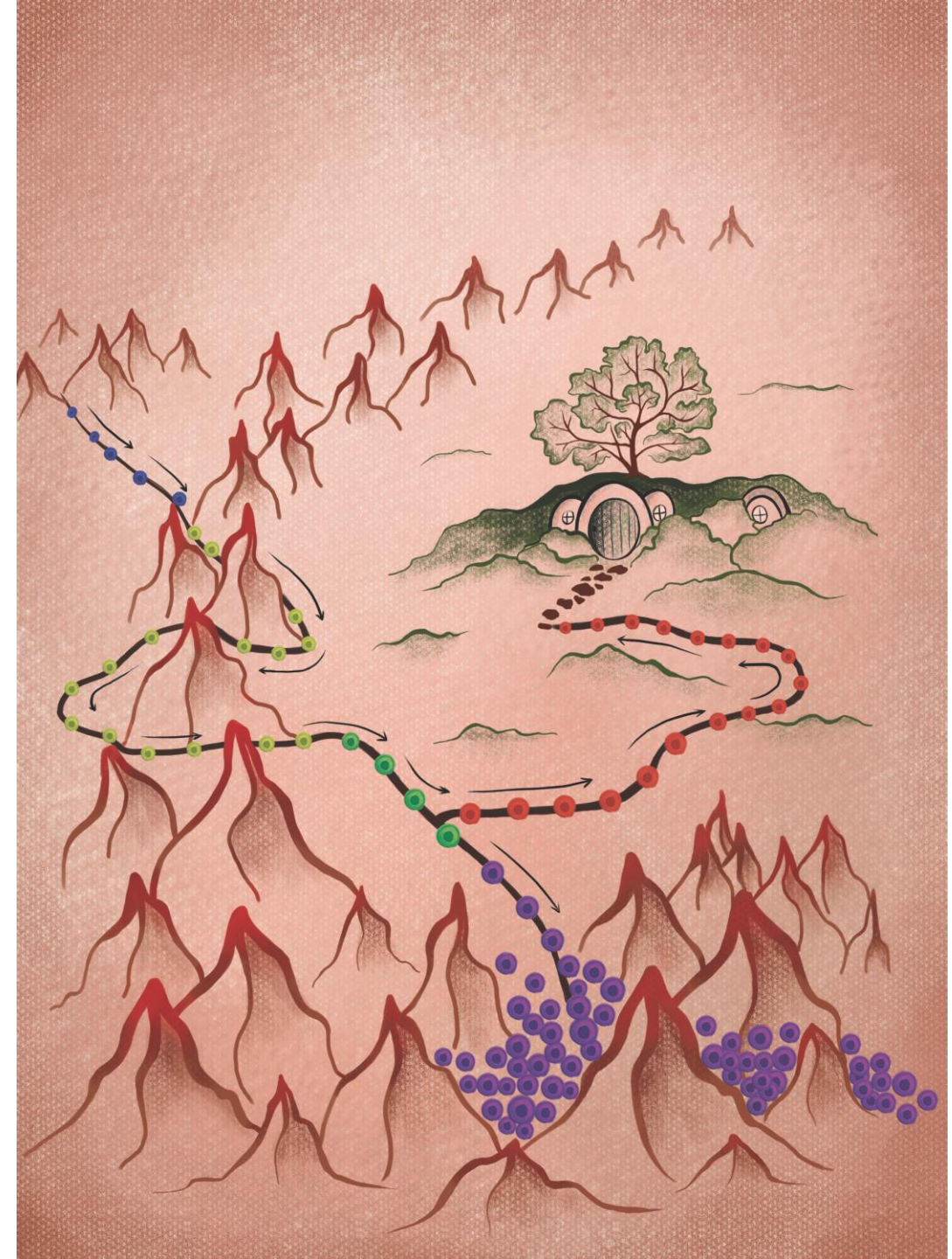
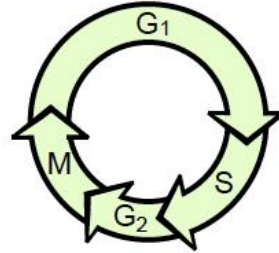# Summary: Current and future insights into transformation

Summary insights

Genetics
- ↑ Mutations
- ↑ sCNAs
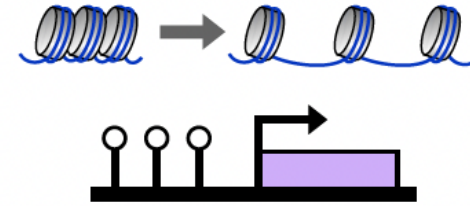- Chromothripsis, kataegis
- Novel recurrent drivers
- Molecular subtypes

Transcriptomics
- ↑ Cell cycle, MYC activation, oxidative phosphorylation
- ↓ BCR

Epigenetics
- ↑ Chromatin accessibility
- Global hypomethylation

- Clonal related and unrelated RS are separate biological entities
- Related RS is distinct from DLBCL
- Altered core pathways of transformation have been identified
  (including MYC activation, cell cycle, IRF signaling, NOTCH signaling, immune evasion, oxidative phosphorylation)

?Immune microenvironment

Image from:
Parry, Ten Hacken and Wu, *Blood*, 2023

Dana-Farber
Cancer Institute

Merci