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Biology of High Risk CLL and Acquired Resistance

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Acquired Resistance

Incidence and risk factors of acquired resistance

- 50-70% among patients who discontinue BTKi due to progression
- 50% among patients who discontinue venetoclax due to progression
- Risk factors
  - Continuous treatment
  - Relapsed disease
  - *TP53* abnormalities? Complex karyotype?

Primary resistance is rare (ORR >90%)
Discontionuation for intolerance must not be confused with acquired resistance

Mutations of resistance

***BTK* C481 mutations (I, A, Z)**
- preclude irreversible binding of covalent BTKi to BTK
- result in a greatly reduced drug potency

***BTK* T474 gatekeeper (P)**
- interfere with BTKi (both covalent and noncovalent) binding to BTK
- allow for normal B-cell signaling

***BTK* L528W kinase-dead (Z, P)**
- hinder BTK catalytic activity
- B-cell signaling is thought to continue via a BTK scaffolding

***PLCG2* M (I, A, Z, P)**
- co-occur with BTK M
- autonomous signaling

***BCL2* M (V)**
- preclude binding of vnetoclax to BCL2

Biology of persister cells

Pre-treatment CLL

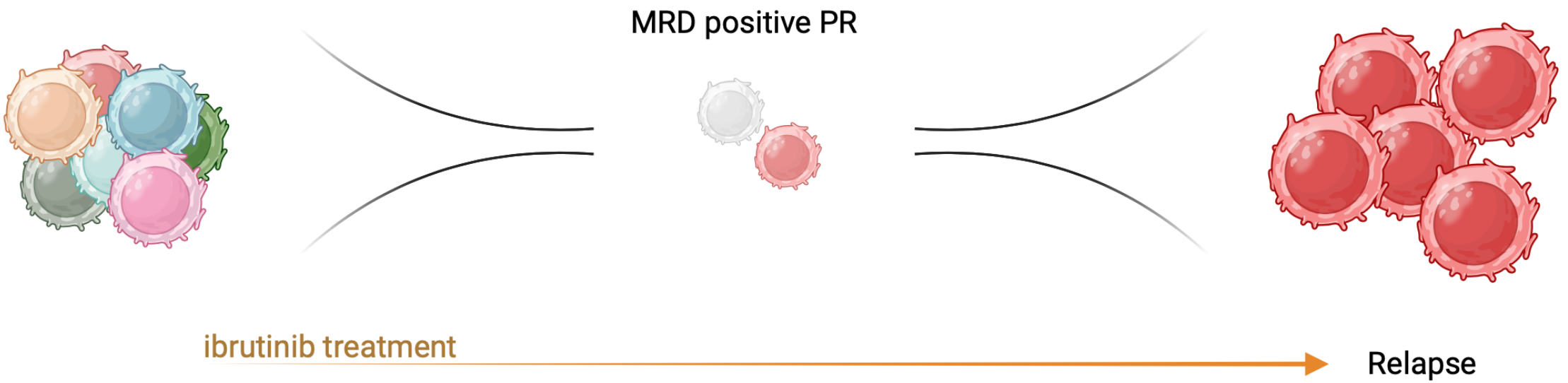
Constitutive activation of BCR
Proliferating state
BTK dependent signaling

MRD positive PR

MRD is rarely negative
BTK Resting/active cells?
Signaling inhibited

Progression

Mainly driven by resistance mutations in *BTK* or *PLCG2*



Epigenetic adaptation?
By-pass signalling?

Richter’s Transformation

Richter's Transformation (RT)

History of CLL

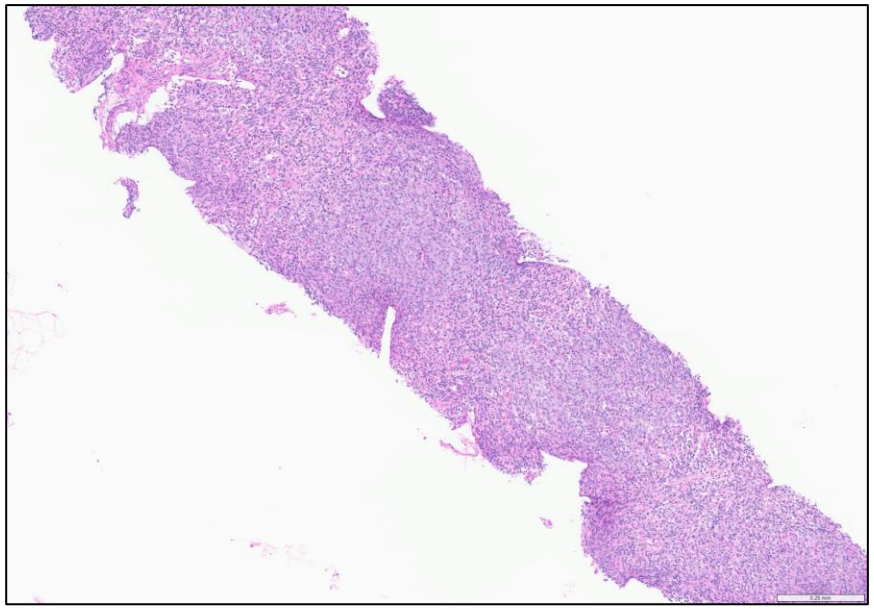
- Previously treated **70%**
- IGVH unmutated **80%**
- *TP53* abn **50%**
- Complex karyotype **40%**

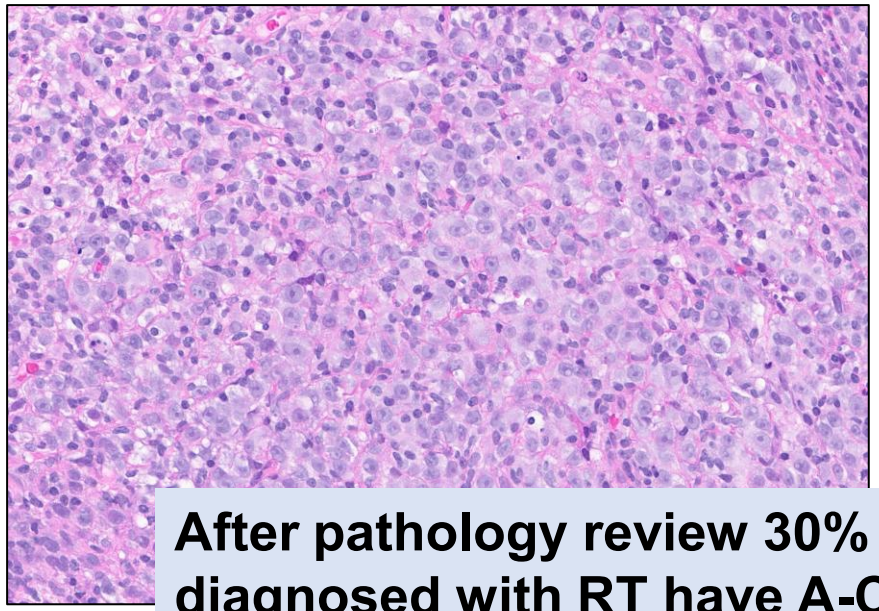
Clinical syndrome

- B symptoms **50%**
- High LDH **70%**
- Fast growing bulky **60%**
- Unusual extranodal sites **10%**
- Hypercalcemia **10%**
- SUVmax >10 **80%**

Pathology aspects

- Sheets of large cells **100%**
- Centroblasts **90%**
- Non-GCB **90%**
- Loss of CD23 **80%**
- Loss of CD5 **70%**
- EBV + **5%**
- Clonally related: **100%**

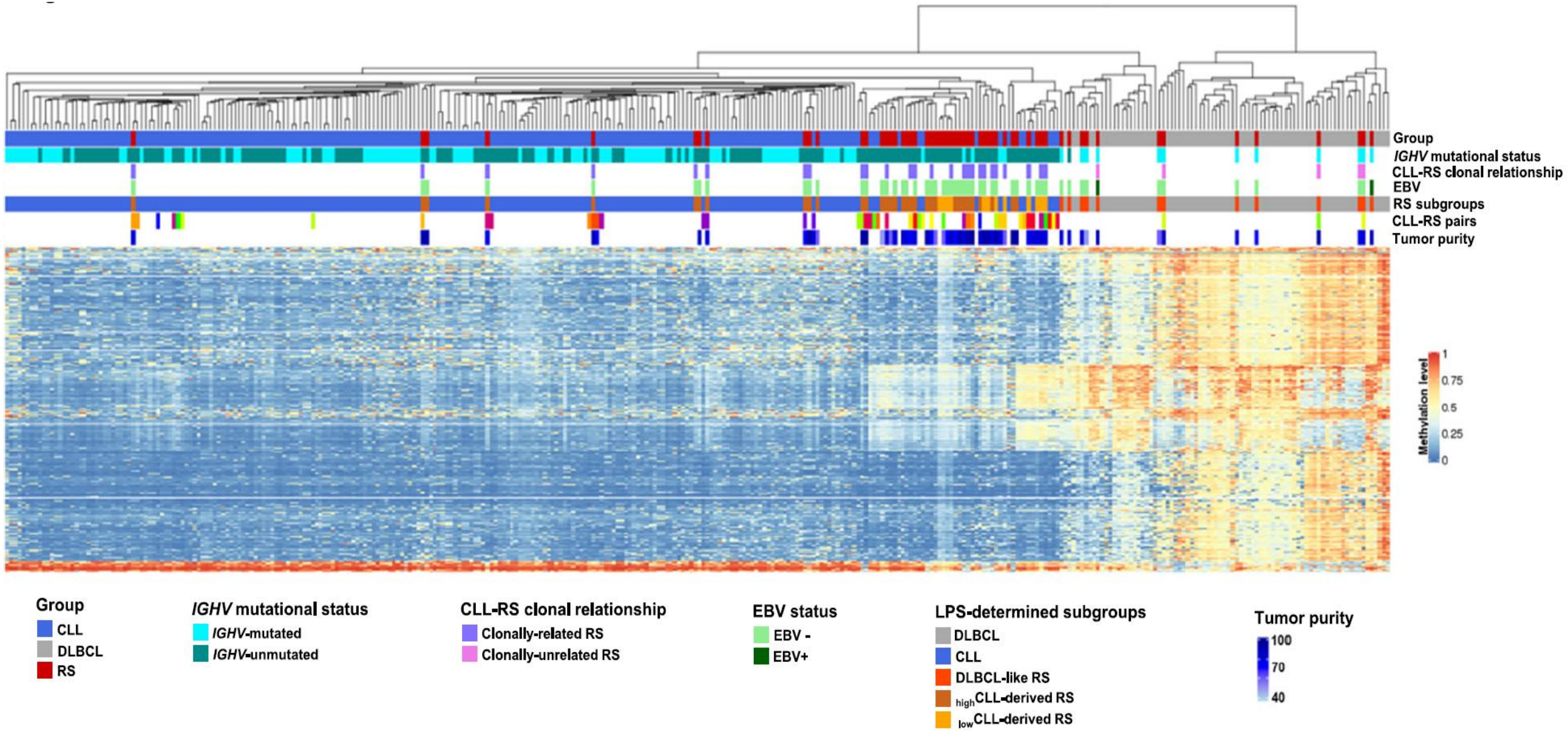




After pathology review 30% of cases diagnosed with RT have A-CLL or cHL

WHOHAEM5, Mao Z et al. Am J Surg Pathol 2007, Rossi D, et al. Blood 2011, Kittai AS, et al. Blood Cancer J. 2025, Miller CR, et al. Am J Hematol. 2025, Kittai AS, et al. Am J Hematol. 2023, Hampel PJ, et al. Leukemia. 2025

Clonally unrelated RT differs from RT and is similar to LBCL



Broseus et al, Nat Comm 2023

Clonally unrelated RT

	Number of patients (% clonally unrelated)	Median OS in clonally related	Median OS in clonally unrelated	P
Mao et al,[-]2007	23 (22%)	NA	NA	NA
Rossi et al,[-]2011	63 (21%)	14.2 mo	62.5 mo	.017
Abrisqueta et al, 2020	35 (15%)	5.4 mo	74.8 mo	.05
Broséus et al, 2023	58 (25%)	8 mo	35.5 mo	.018
Parry et al, 2023	52 (14%)	5.8 mo	56.4 mo	.0094

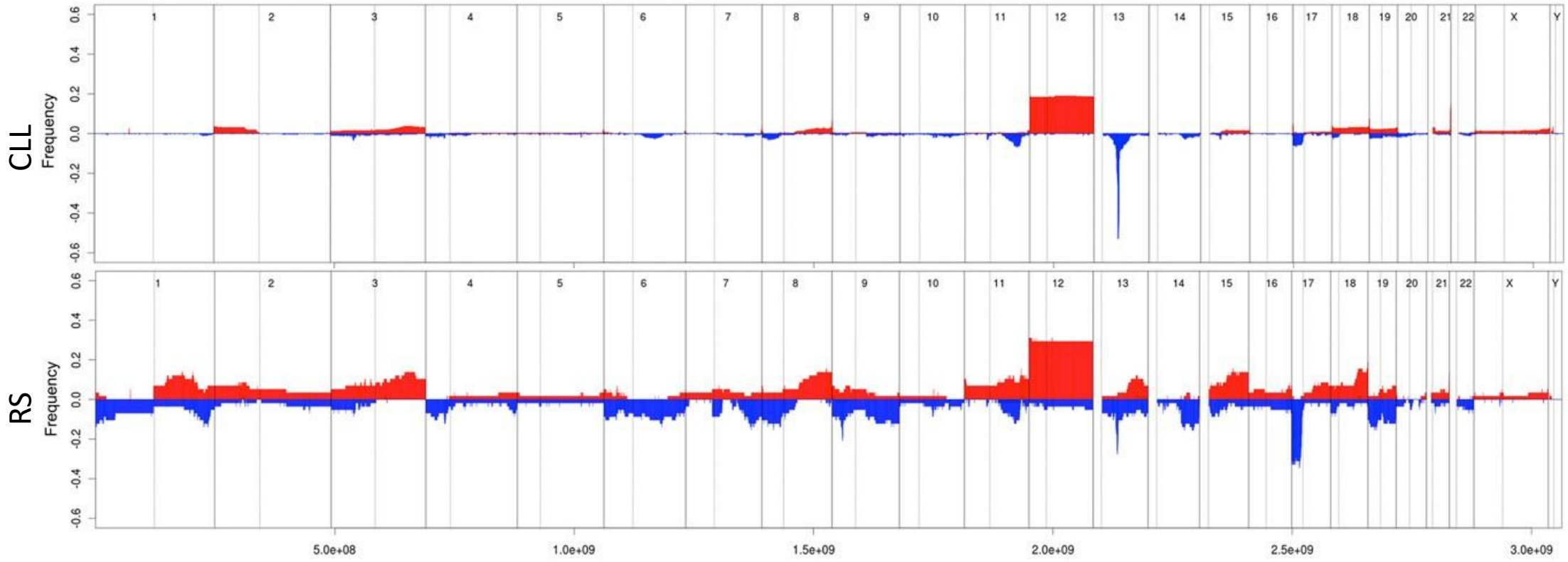
Clonal relationship between CLL and LBCL

- IG rearrangement PCR comparison of peak sizes
- IG gene sequencing
- NGS or FISH to confirm shared mutations and cytogenetic abnormalities
- Tissue biopsy as source of LBCL
- Blood and/or marrow are source of CLL

Kittai AS, Blood. 2025

Genetics of LBCL-type RT

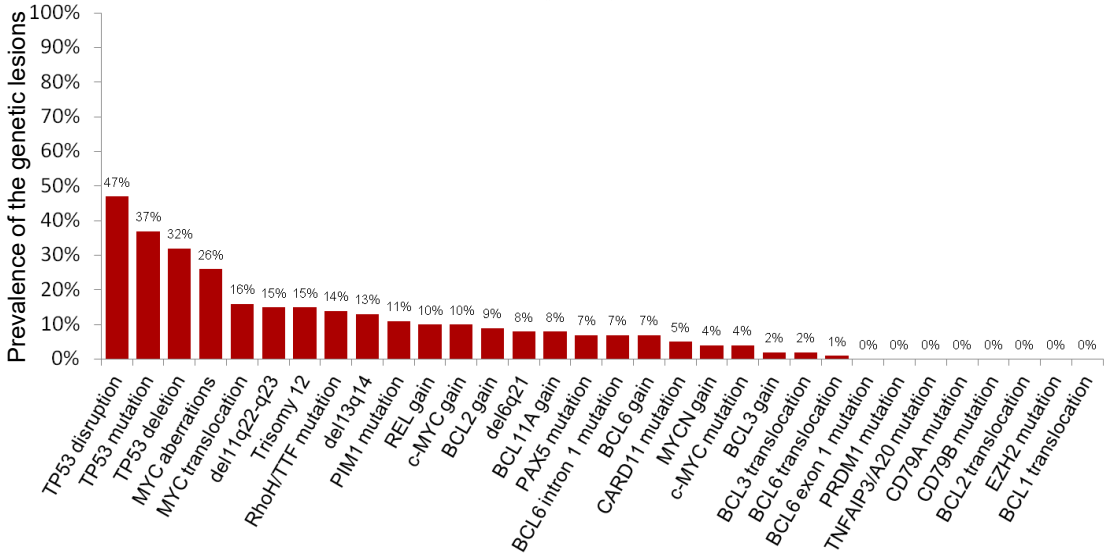
The genomic complexity of RT

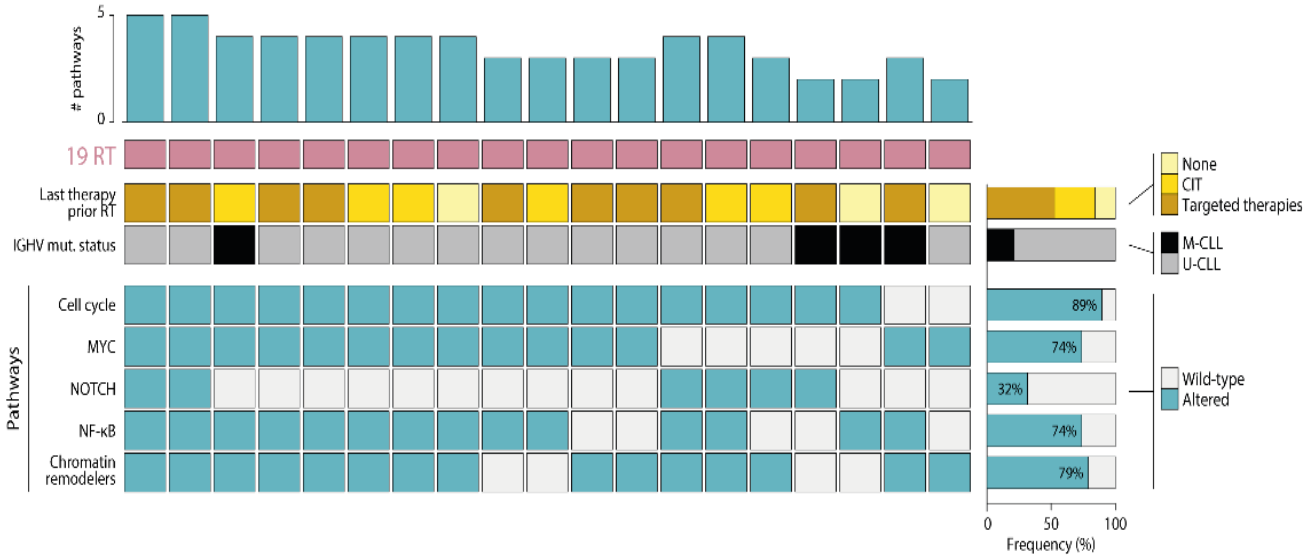


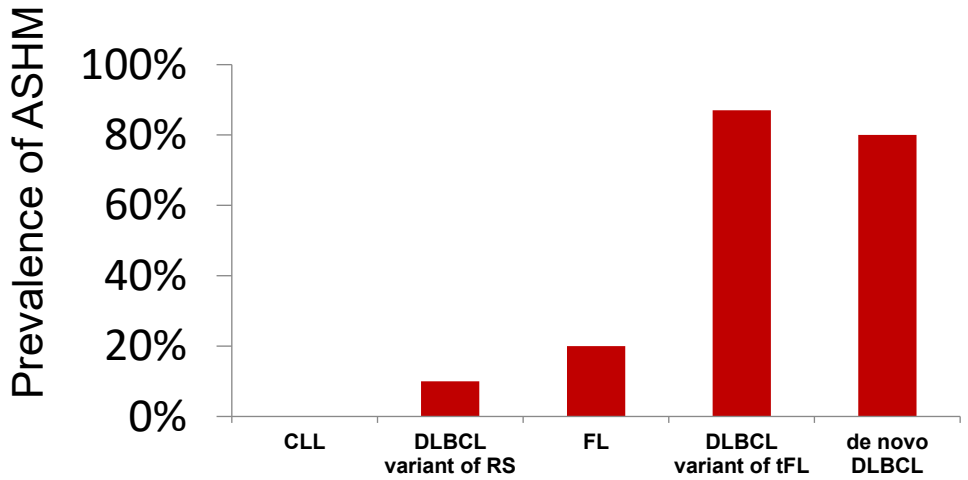
Copy number abnormality load

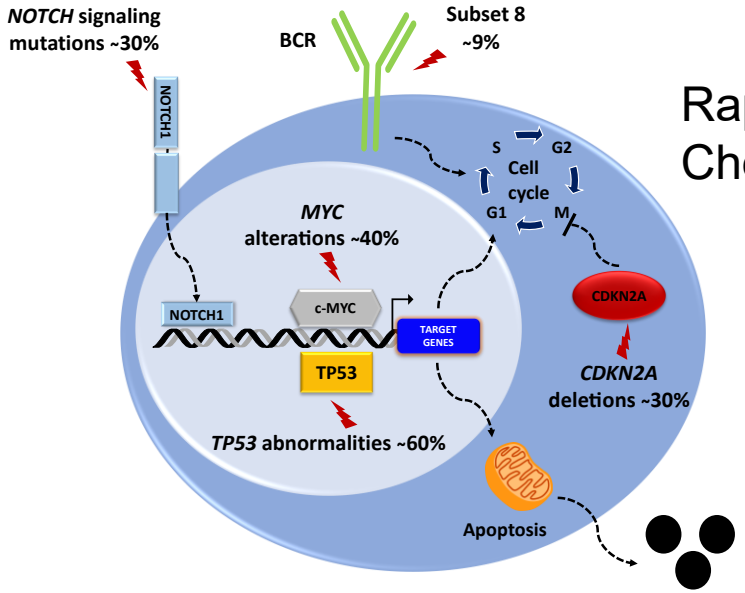
Chigrinova E, et al. Blood. 2013, Fabbri G, et al. J Exp Med. 2013

The genetics of DLBCL-type RT







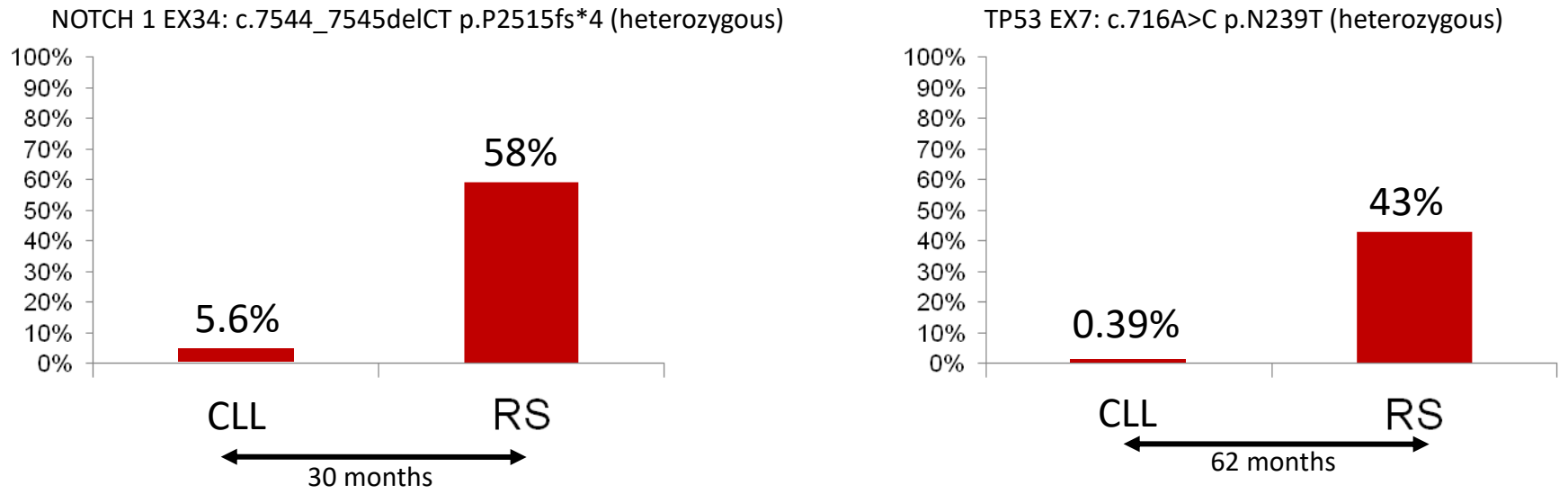


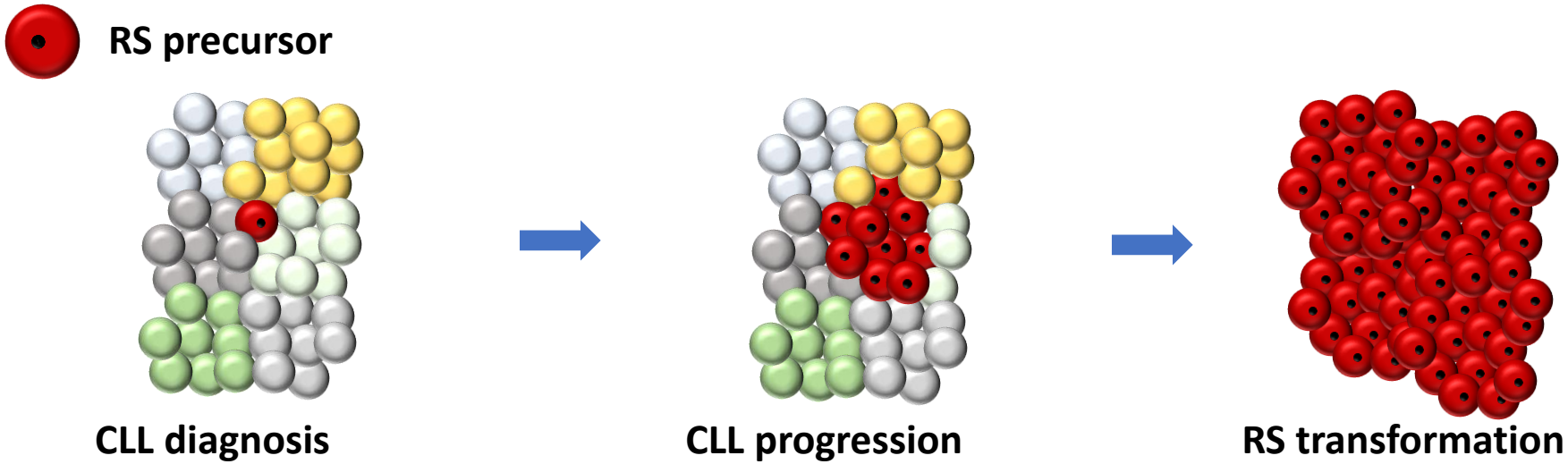
Rapidly progressive kinetics
Chemorefractoriness

Rossi D, et al. Blood 2011, Rossi D, Haematologica 2006, Pasqualucci L, Cell Rep 2014, Fabbriu J, et al, J Exp Med 2013, Klintman J et al, Blood 2020, Nadeu et al, Nat Med 2022, Parry et al, Nat Med 2023

Early seeding

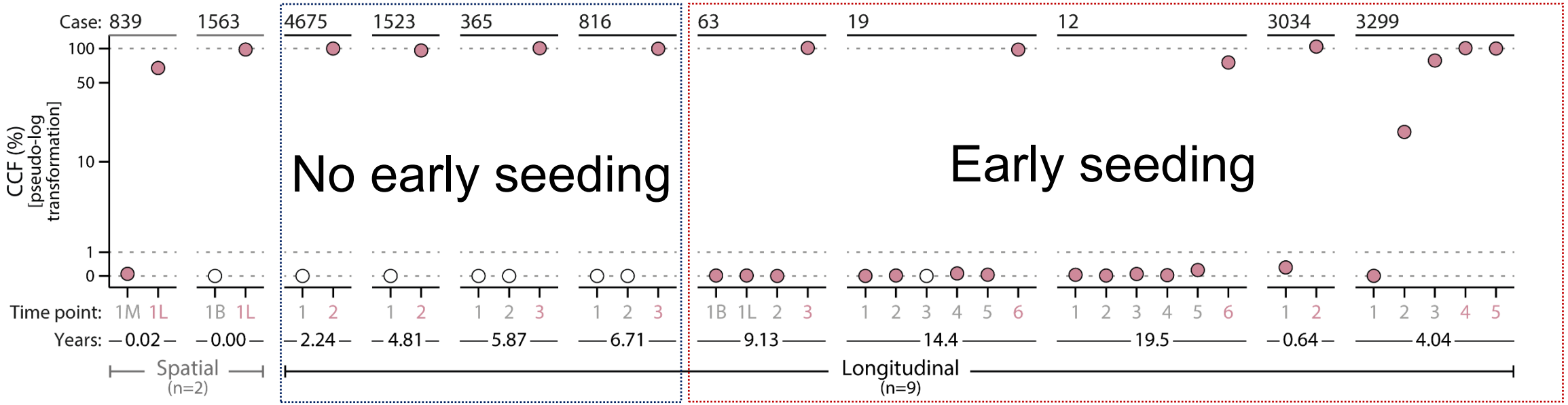
Early seeding of the RT clones



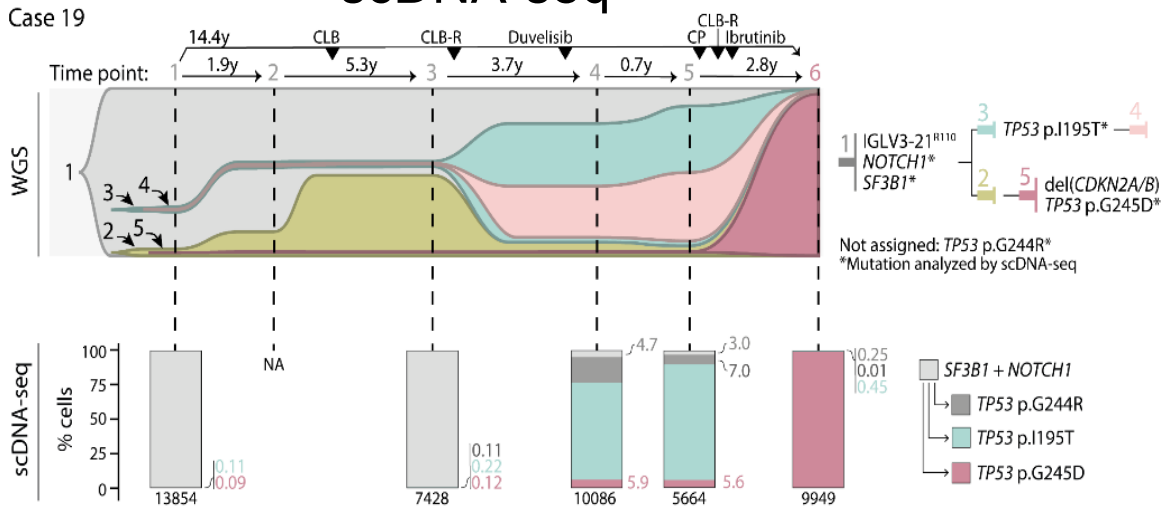


Fabbri G, et al, J Exp Med 2011

Early seeding of the RT clone is frequent (ca. 50%)



scDNA-seq



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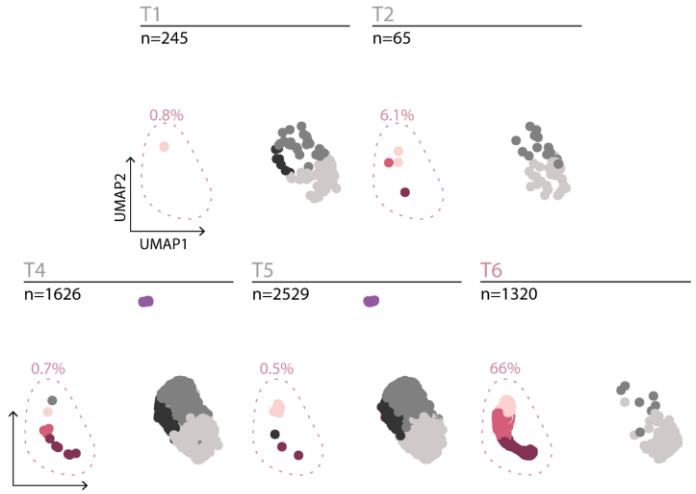
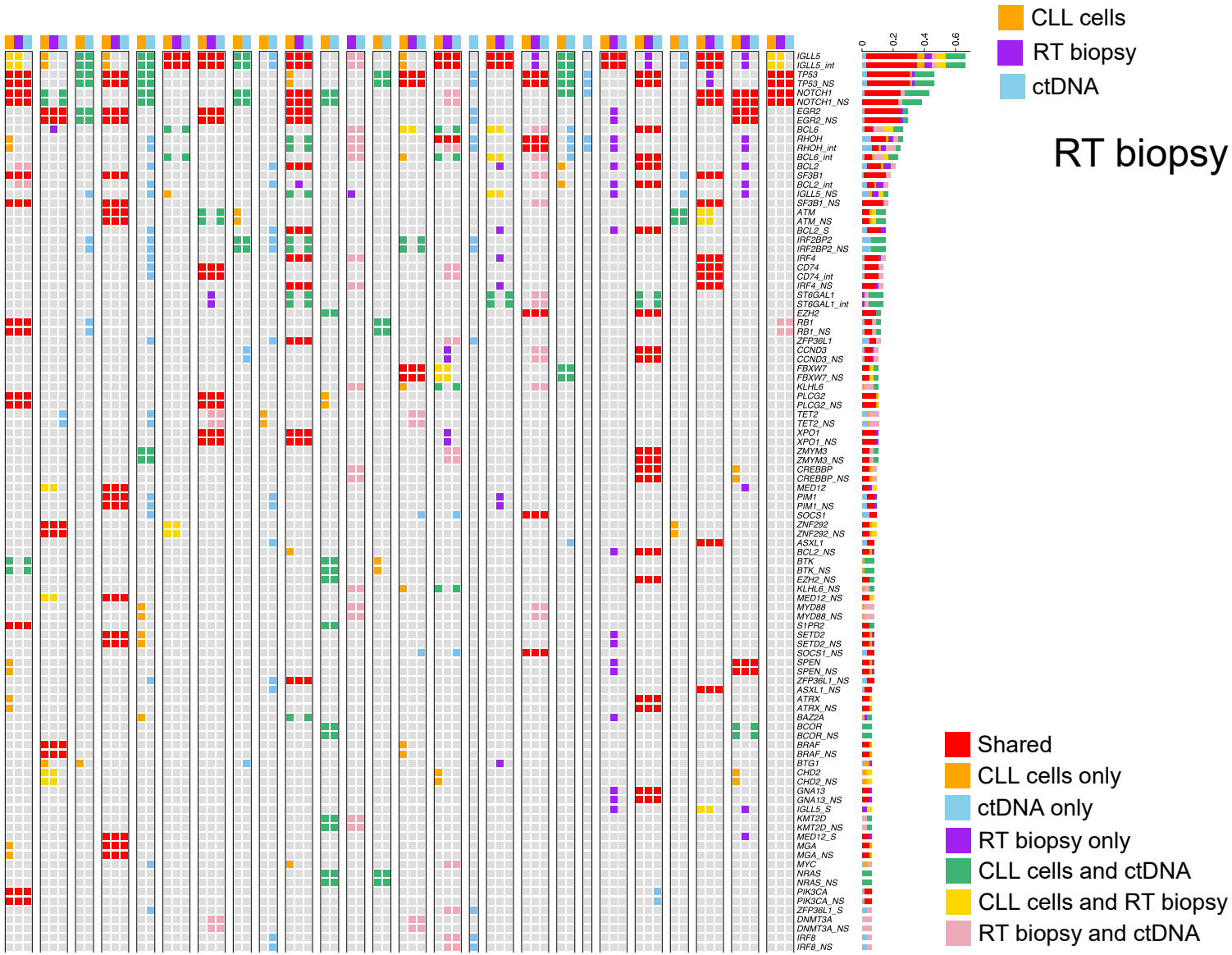


Nadeu F et al Nat Med 2022

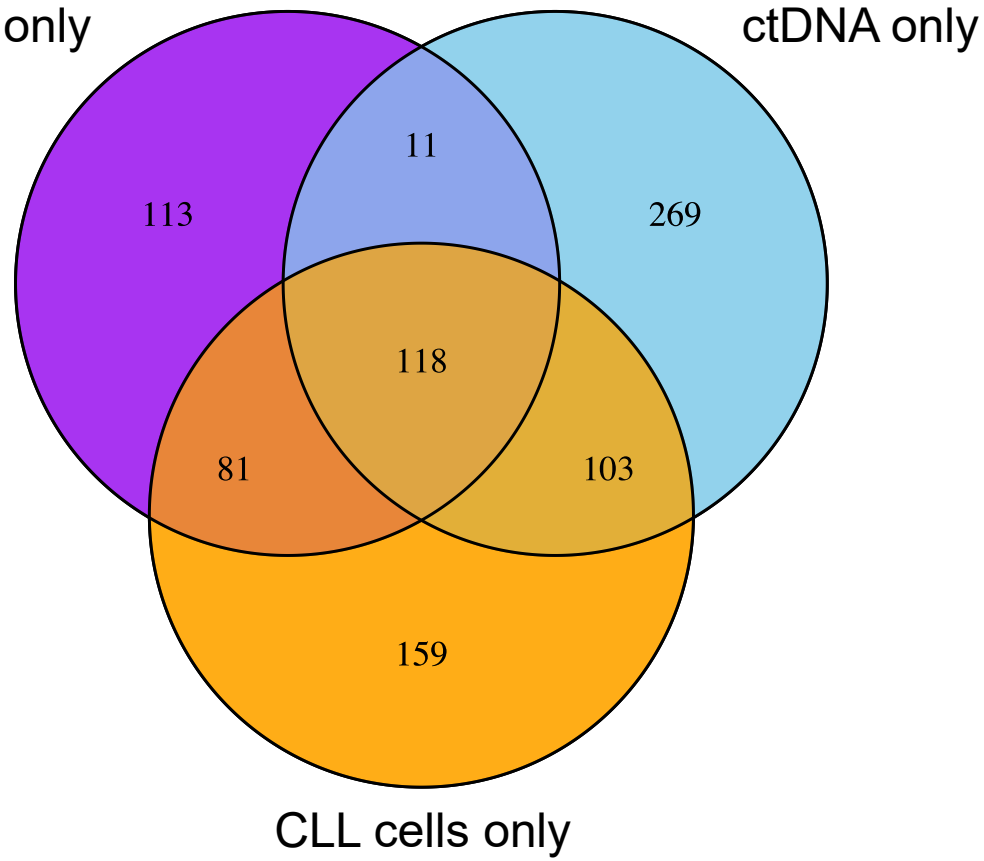
Somatic mosaicism

RT is a somatic mosaic



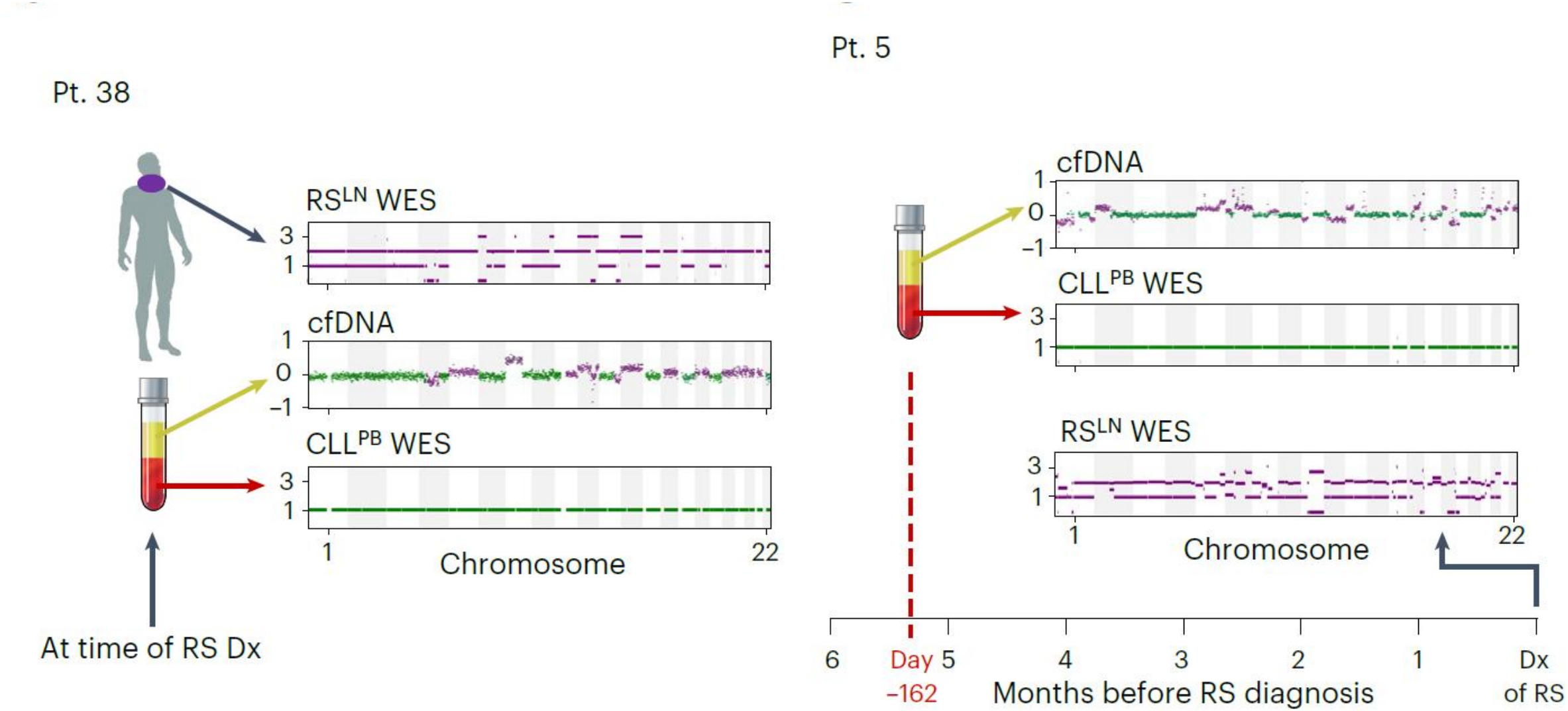
CLL cells
RT biopsy
ctDNA

Shared
CLL cells only
ctDNA only
RT biopsy only
CLL cells and ctDNA
CLL cells and RT biopsy
RT biopsy and ctDNA



Tedeschi A, et al. Lancet Oncol 2024

Liquid biopsy for early diagnosis



Parry et al, Nat Med 2023

Summary

- RT should be carefully differentiated from A-CLL, Pseudo-Richter, clonally unrelated LBCL and cHL
- The BCR signaling in RT is likely attenuated
- DNA-damage, cell cycle, NOTCH are the most frequently affected pathways and hit of acquired mutations
- Early seeding of RT clones prompts the development of diagnostic tests for their detection in circulating cells during the CLL phase
- Somatic mosaicism of RT prompts the development of diagnostic tests for its detection in ctDNA during the CLL phase