

# Perspectives of Clonal Evolution at the Single-Cell Level

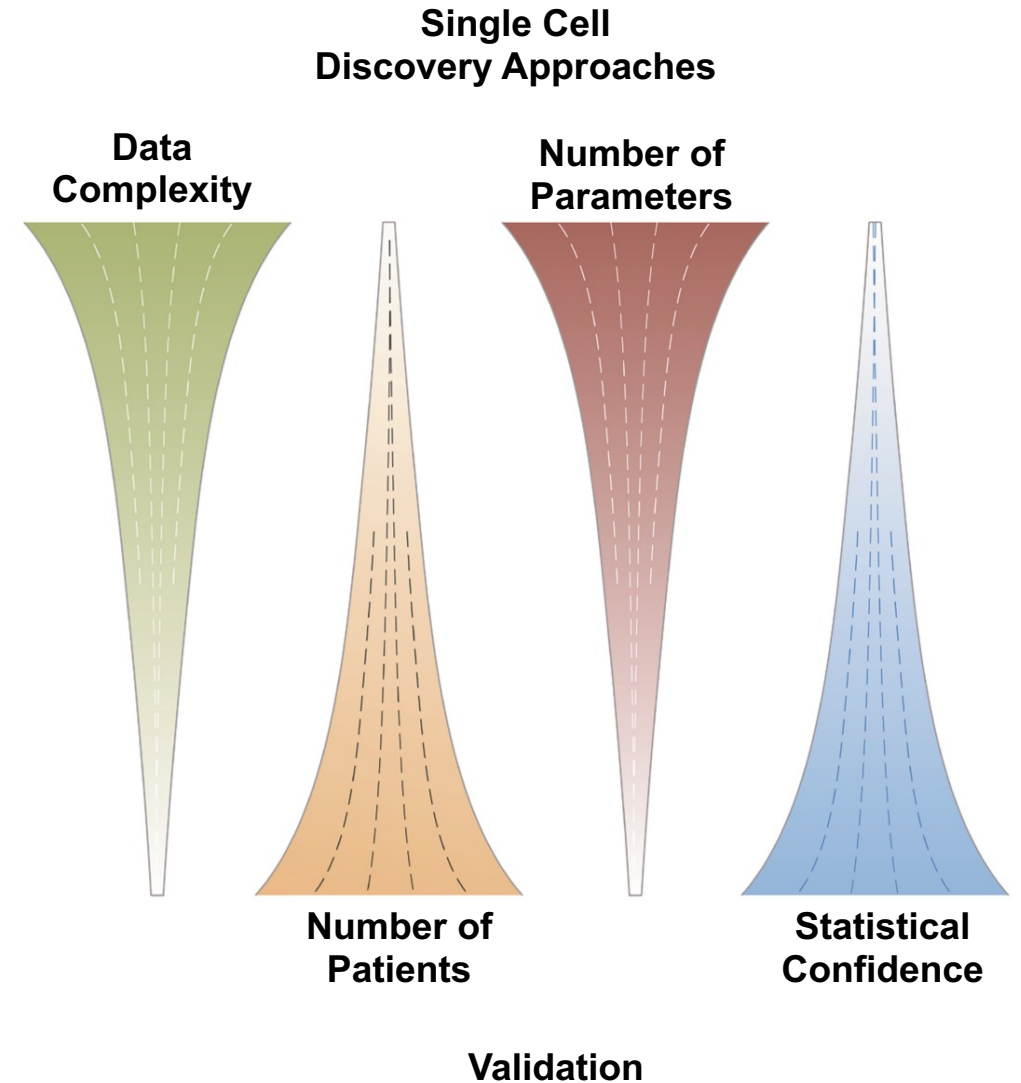
Satyen Gohil  
November 2023

# Disclosures

- Freeline: Consultancy
- Novalgen: Patents, Royalties and Consultancy
- Janssen: Speakers Fees
- AstraZeneca: Speakers Fees
- Beigene: Advisory Board, Travel
- Abbvie: Travel and Honorarium
- Electra: Advisory Board
- Takeda: Travel

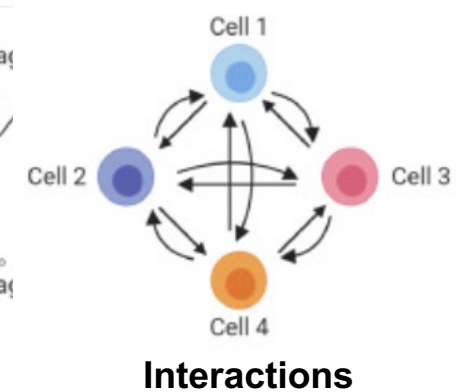
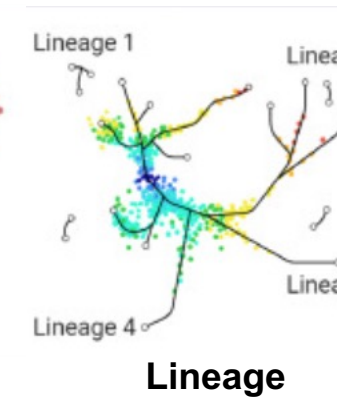
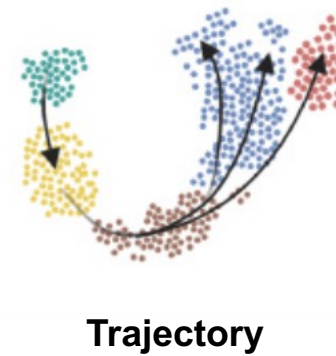
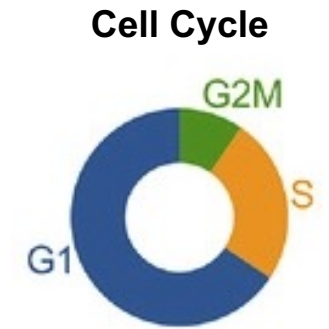
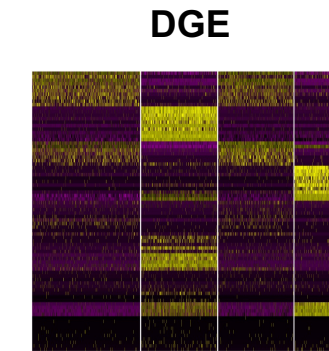
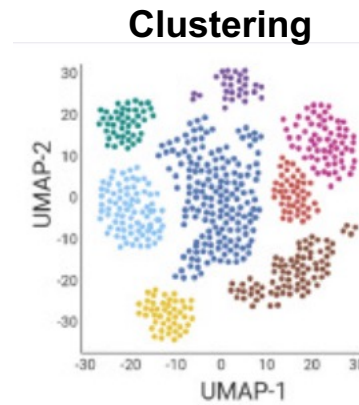
# Single Cell Analysis in CLL

- Longstanding & Established
- Exponential development of technology & bioinformatics
  - Increasing numbers of cells
  - Increasing numbers of parameters

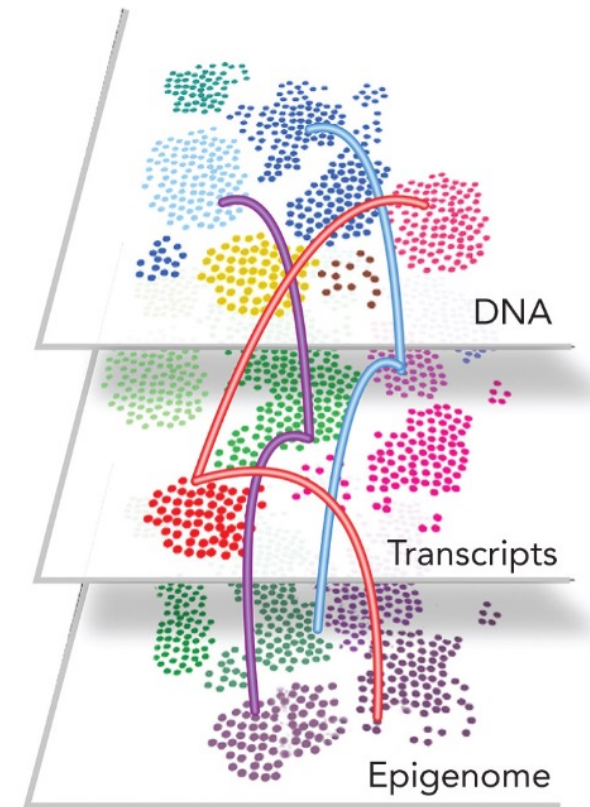
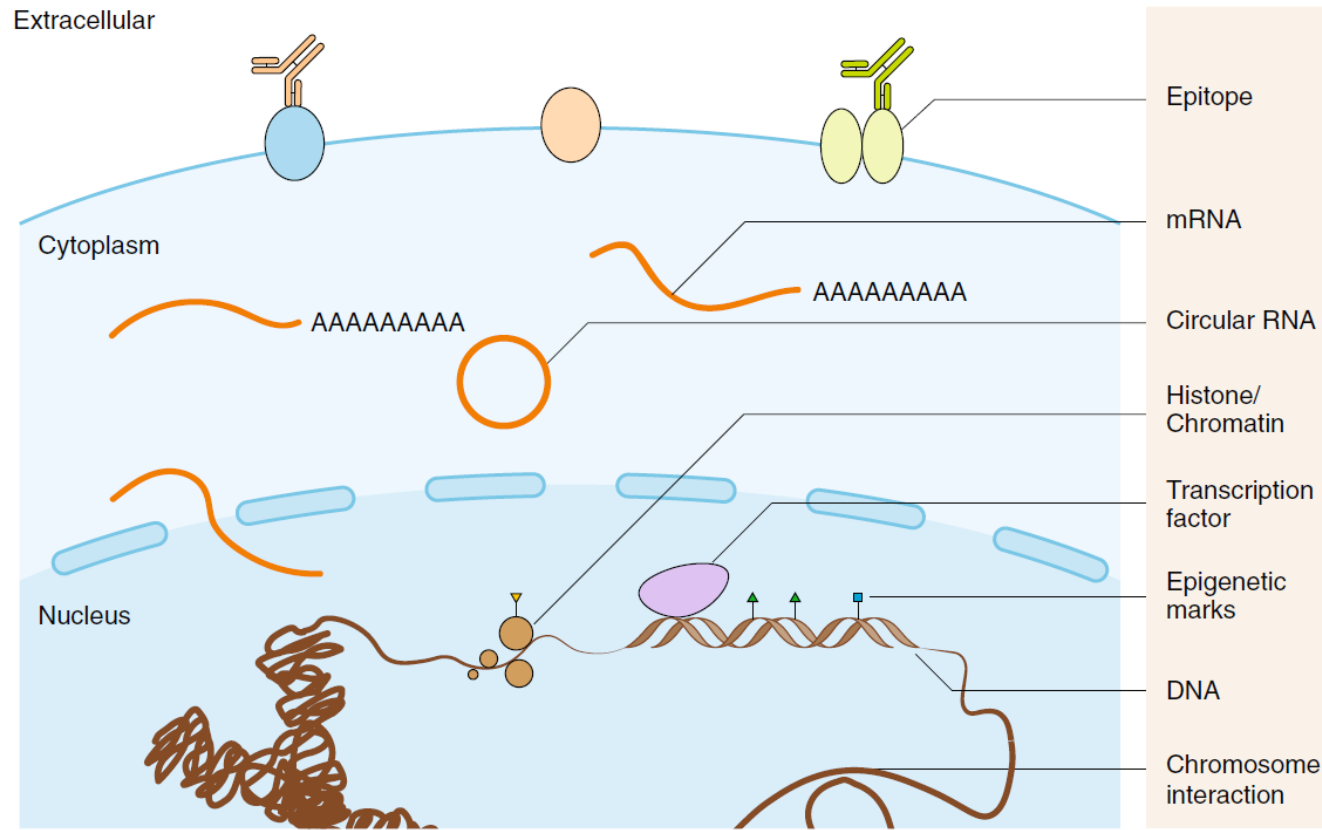


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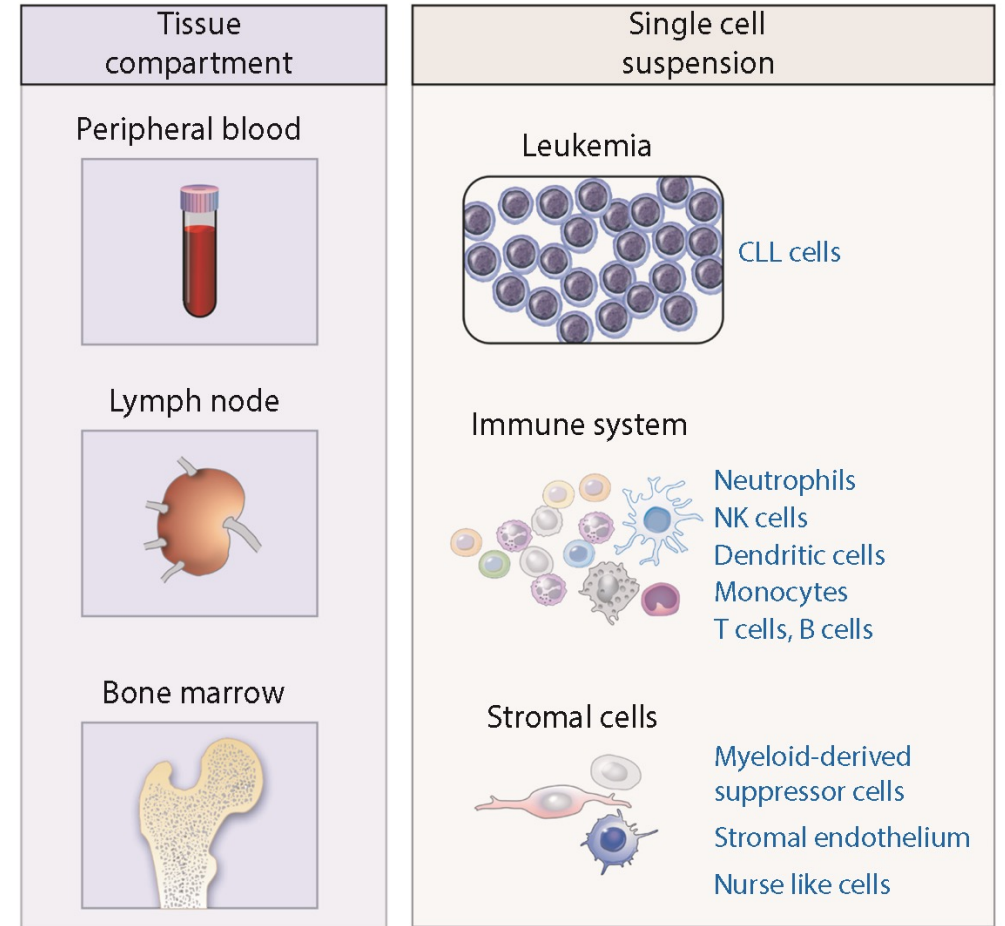
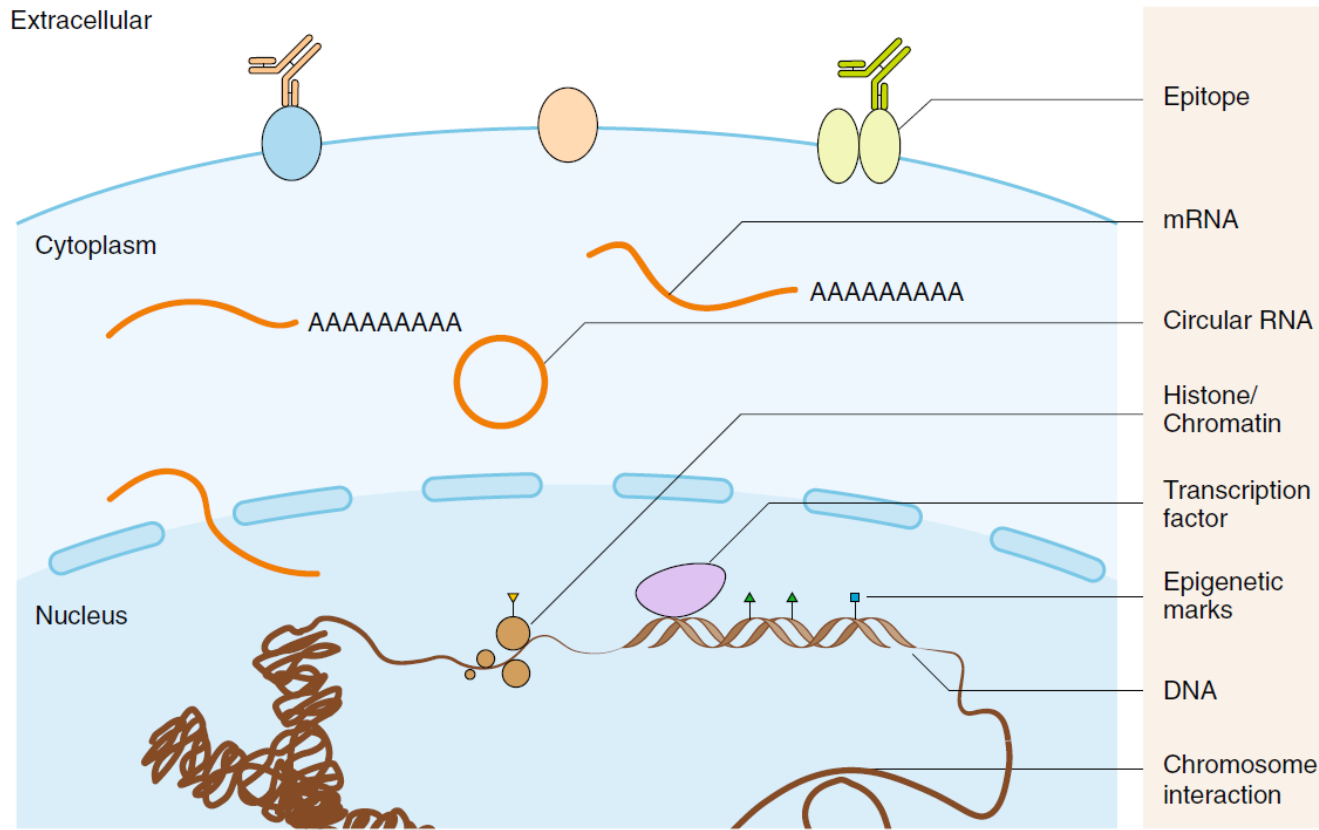
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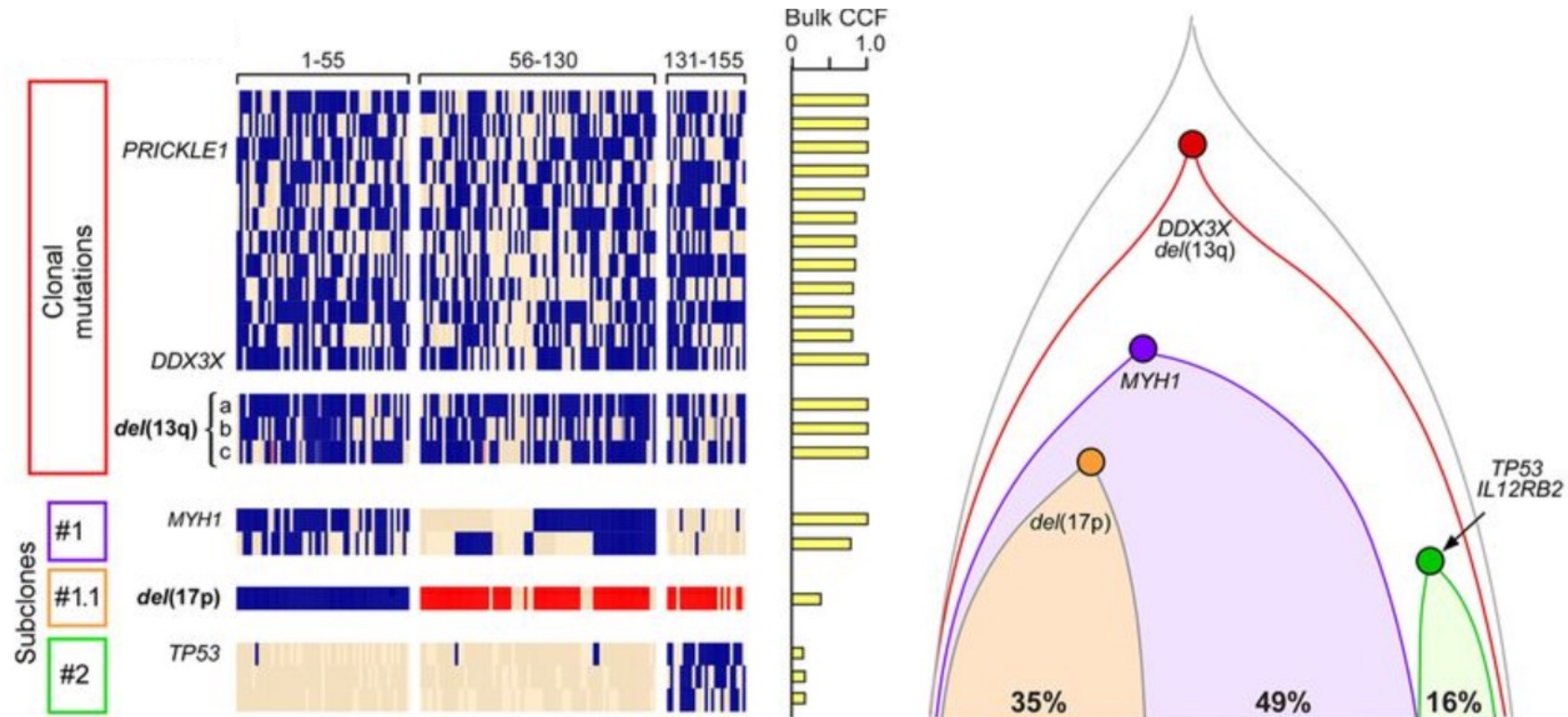
# Accurate Determination of CLL Phylogeny

- Patient with del(13q), del(17p), *MYH1* and *TP53* mutations



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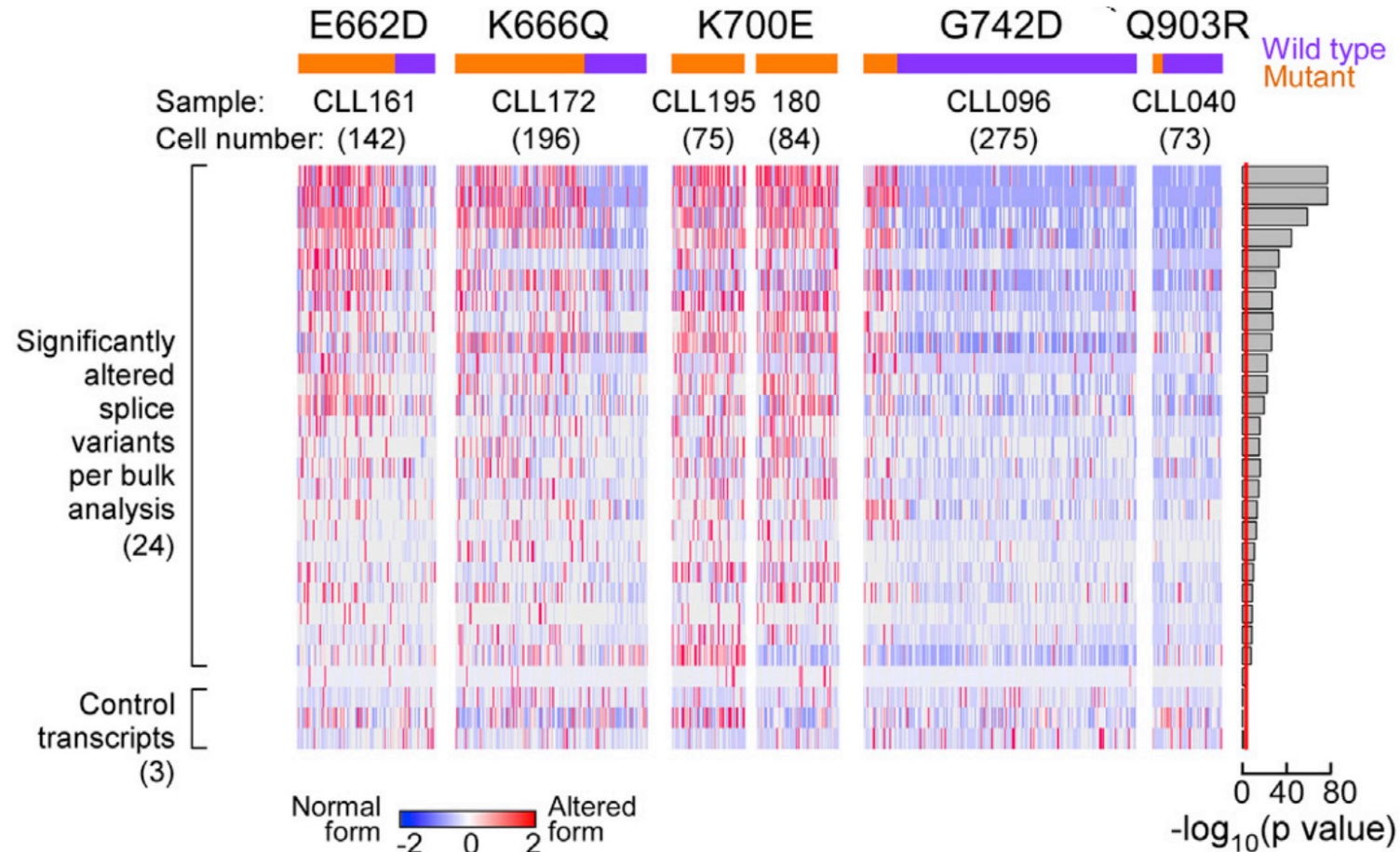
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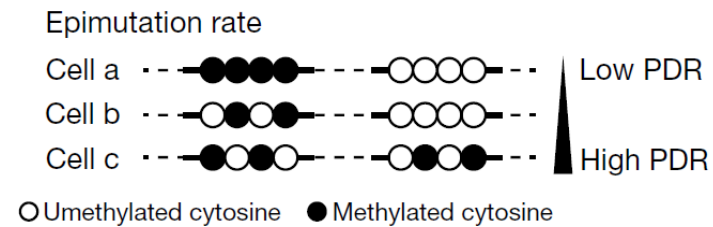
# Linking Mutations to Transcriptional State

- Consequence of *SF3B1* mutations



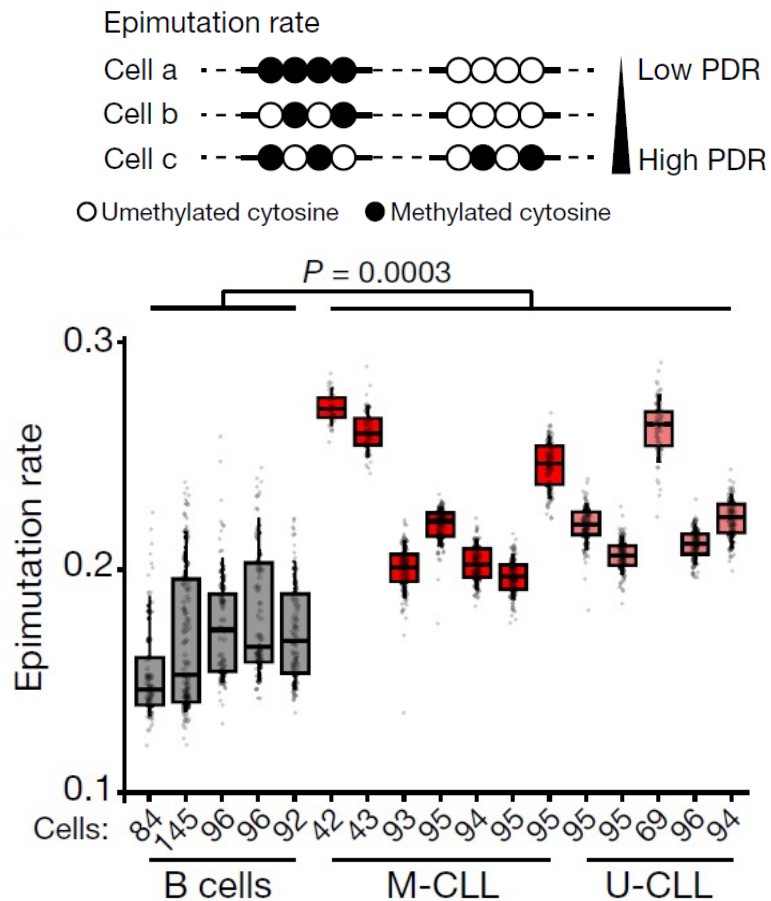
# Epigenetic states at a single cell level

- Reduced representation bisulfite sequencing



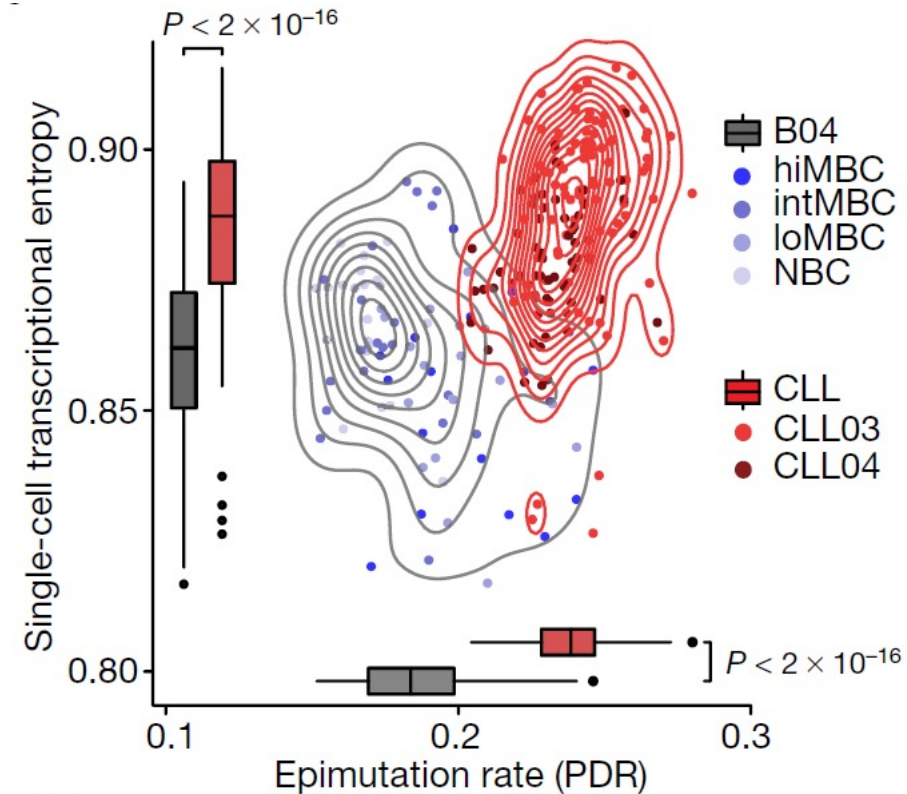
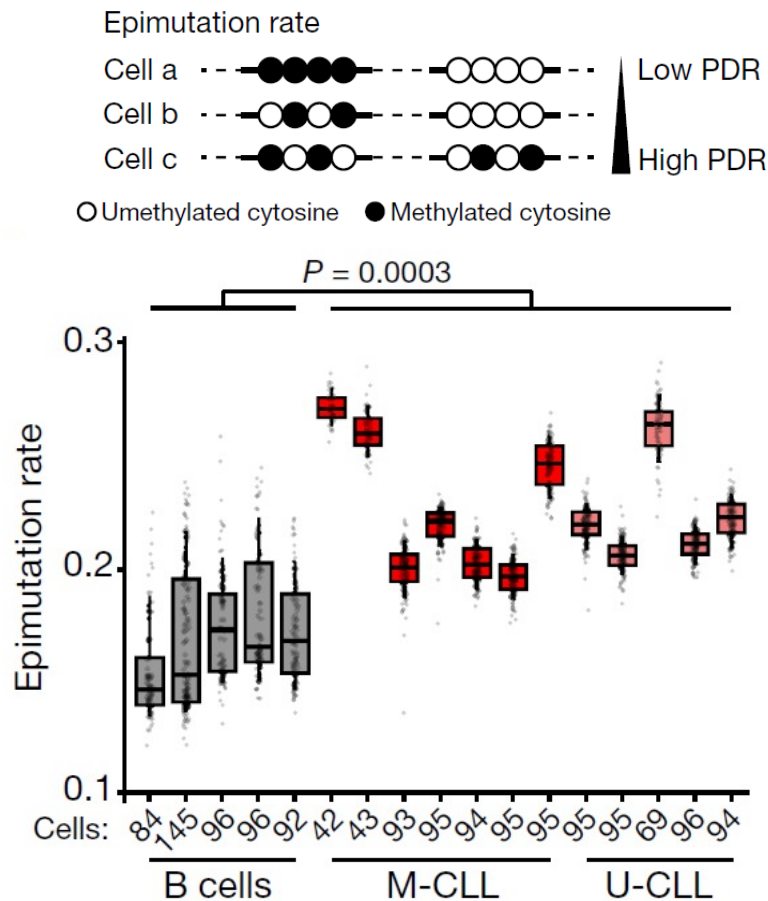
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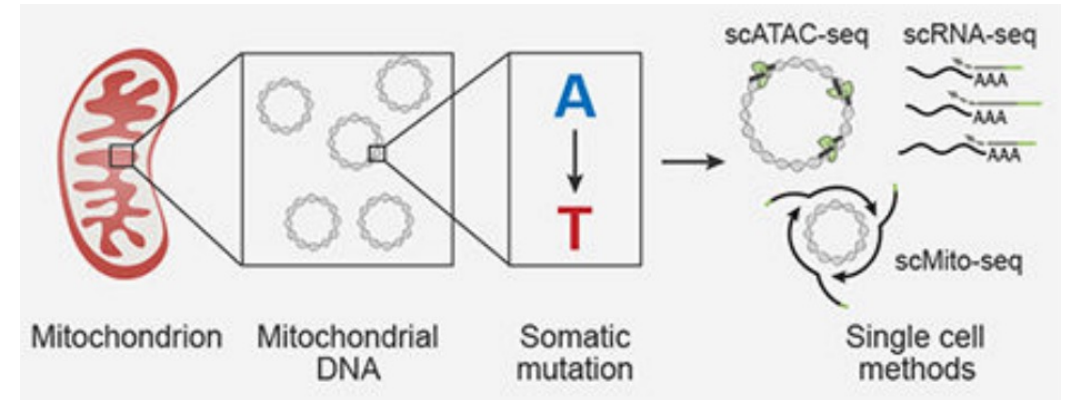
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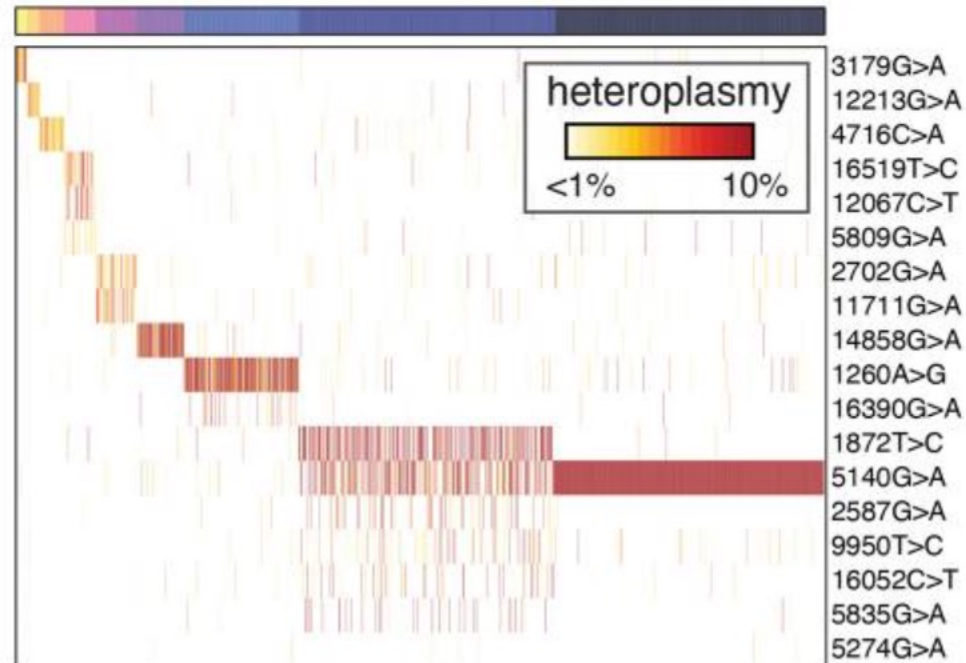
# Single Cell Mitochondrial DNA Sequencing

- Mitochondrial genome 16.6kb
- Increased mutation rate
- Large numbers of mitochondria/cell
- In vivo clone tracking

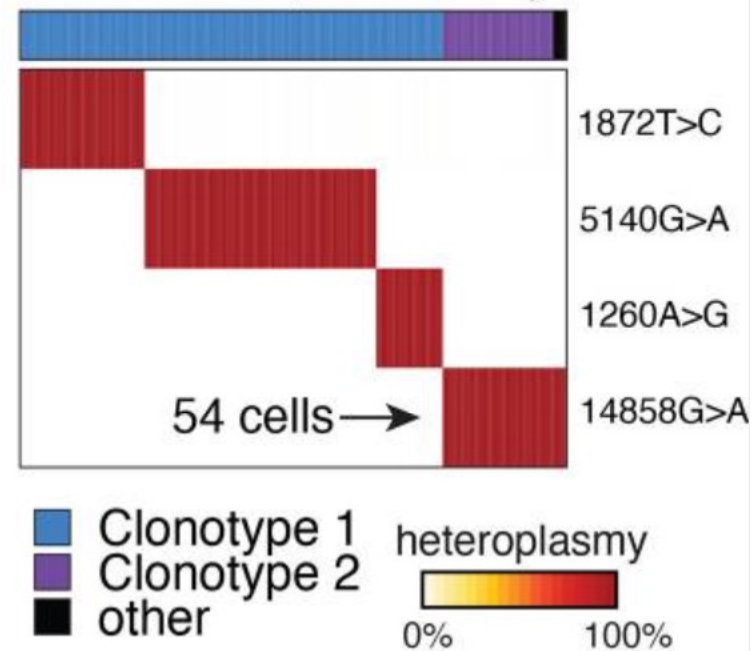


# Single Cell Mitochondrial DNA Sequencing

2,246 Patient 1 B-cells; 9 clusters

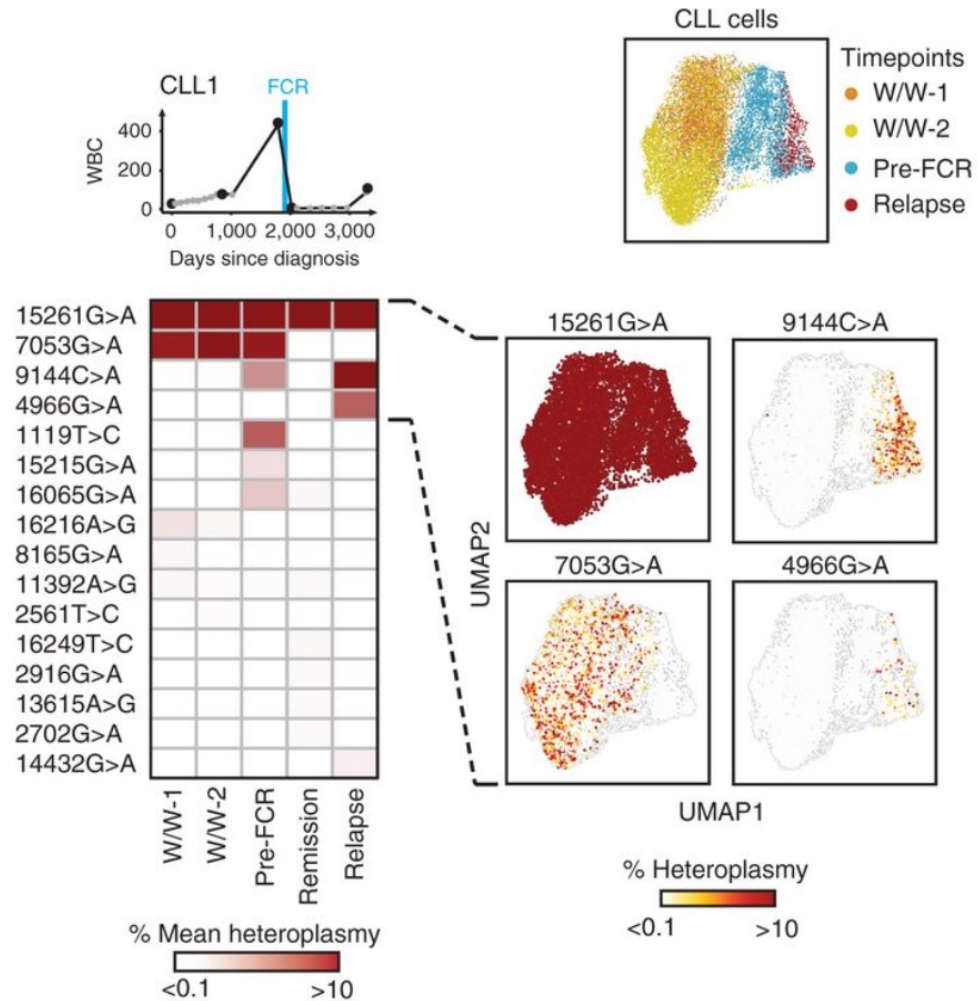


238 B-cells (scRNA-seq)



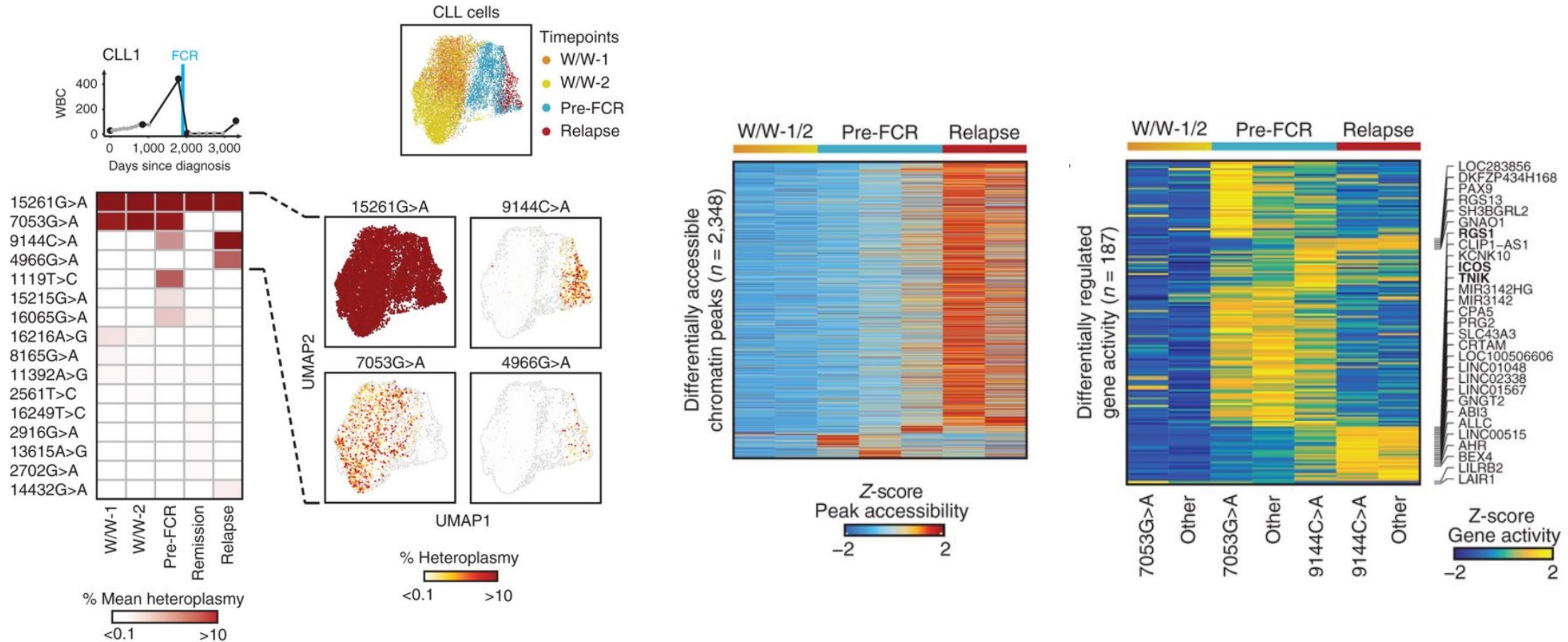


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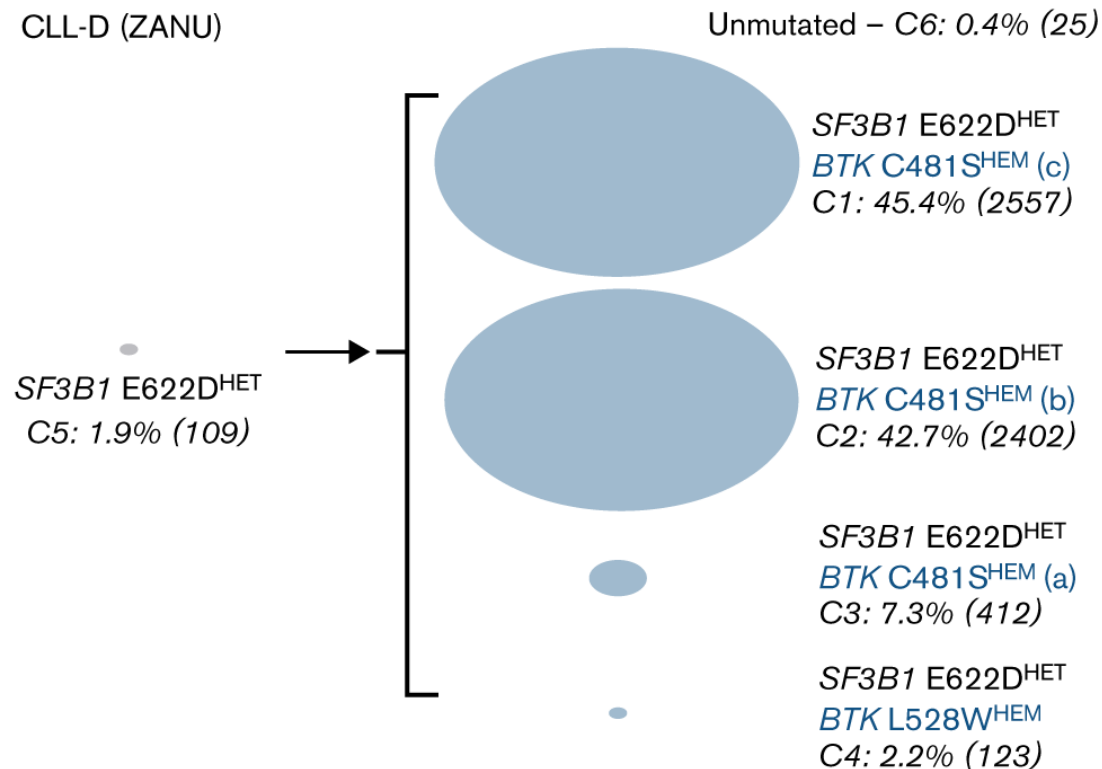


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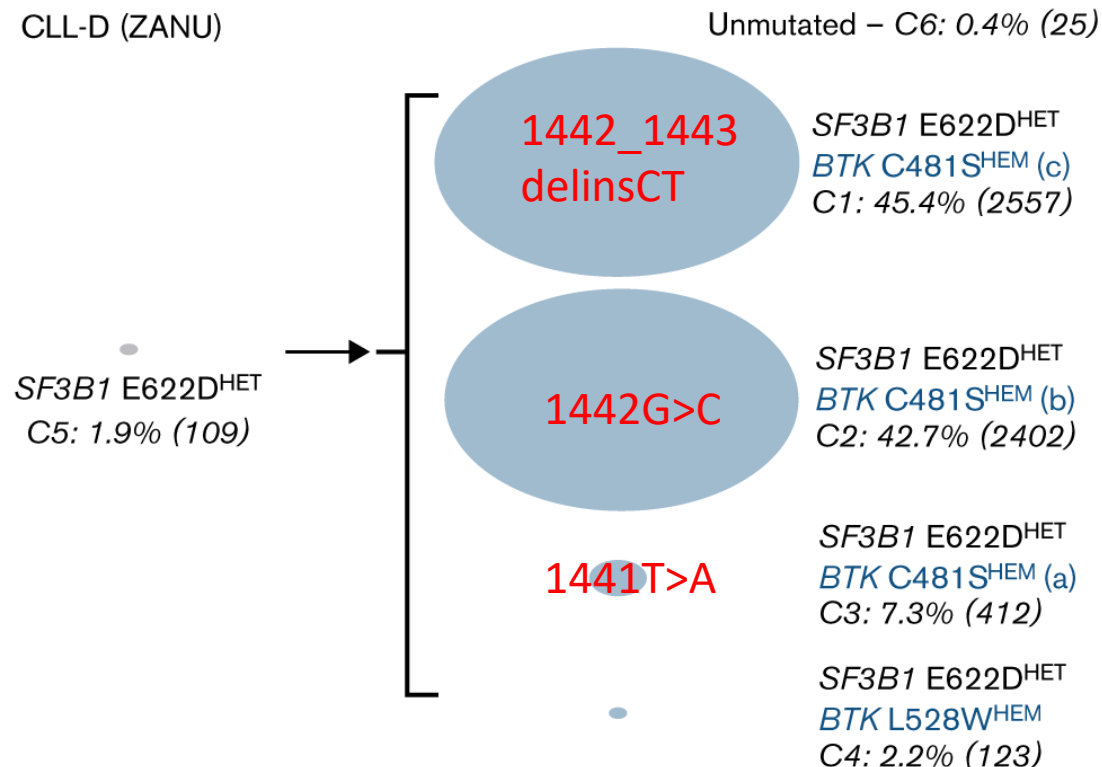
# Therapeutic Resistance: BTKi/BCL2

- Multiple resistance mutations targeting same agent in mutually exclusive clones

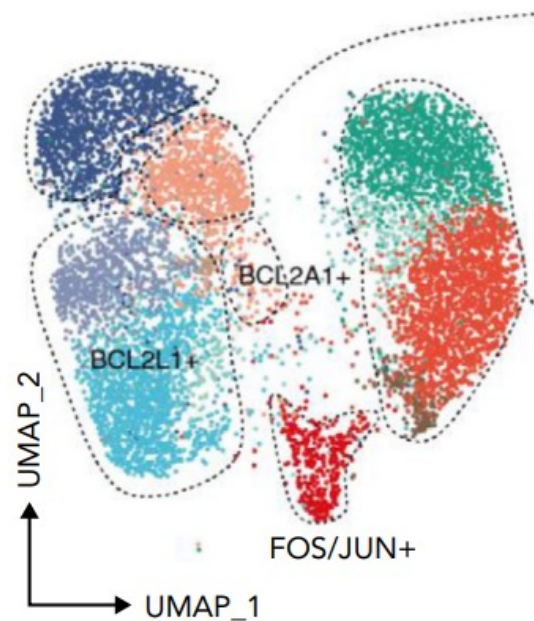


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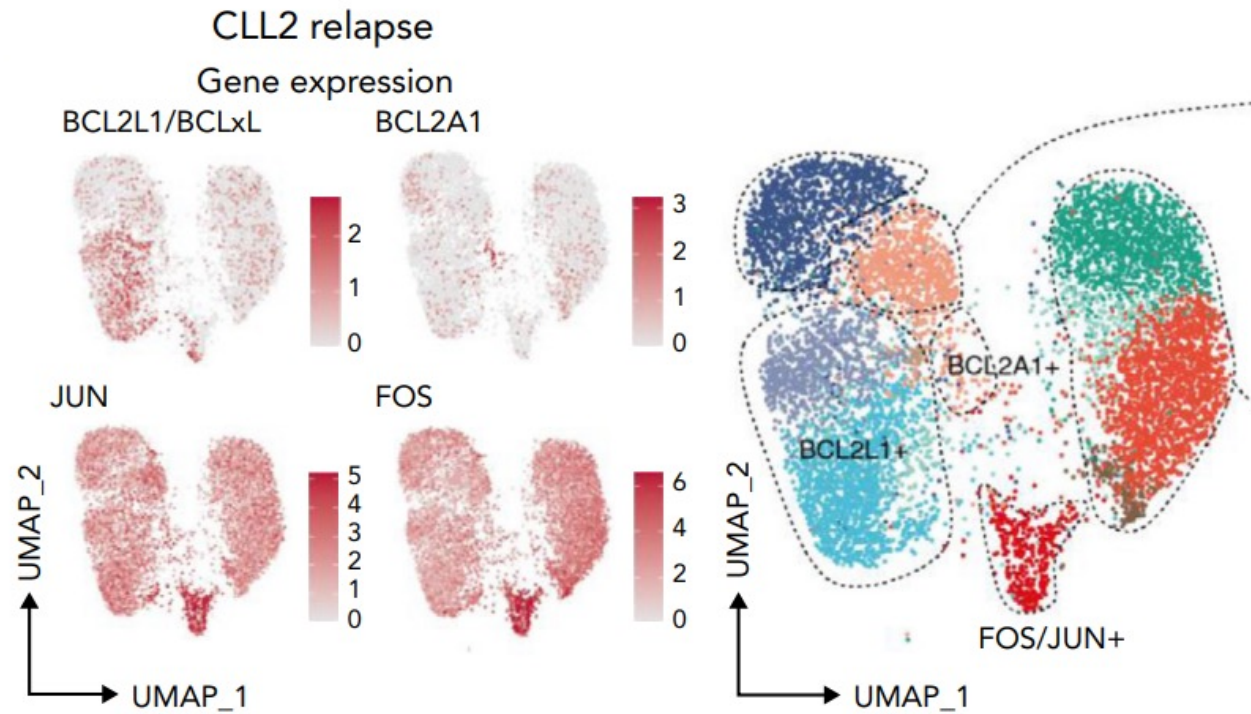
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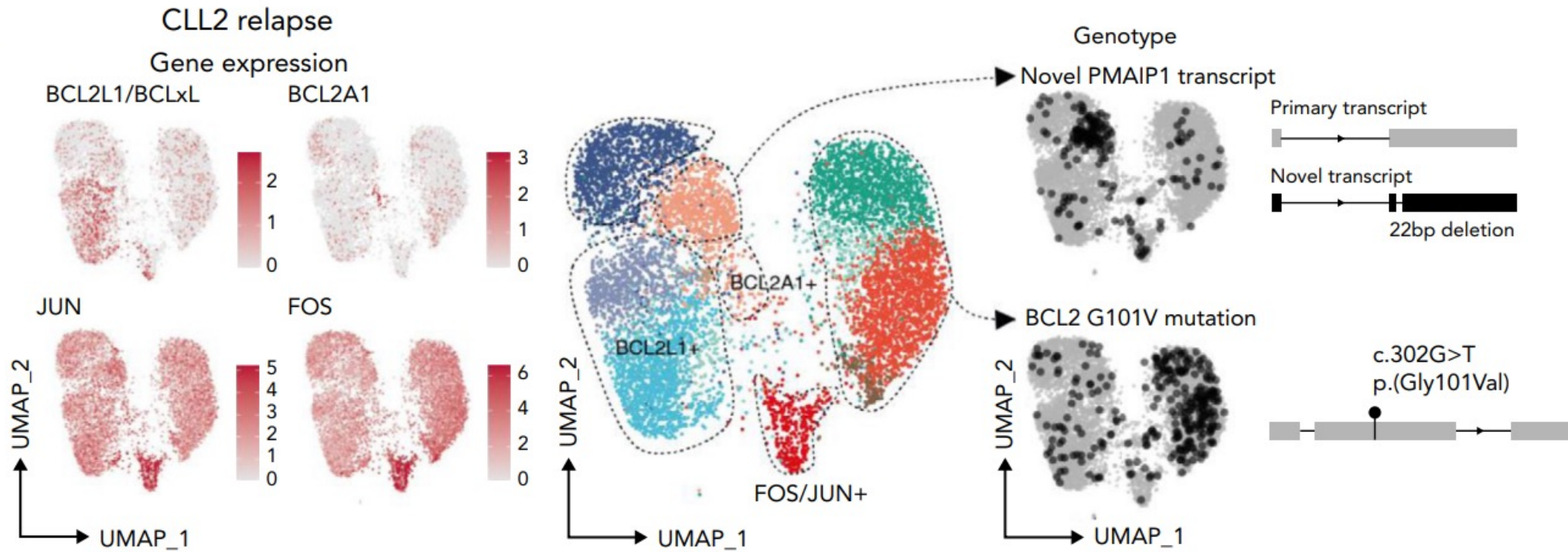


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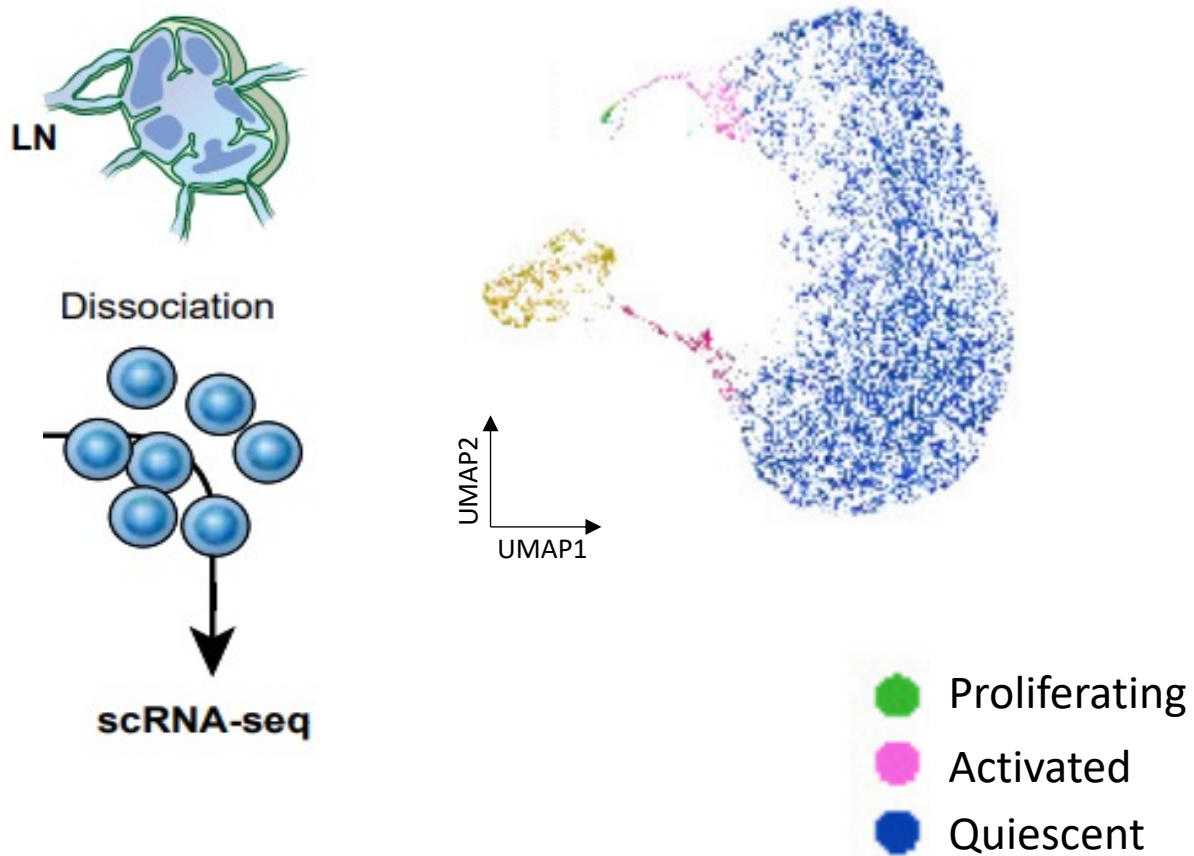




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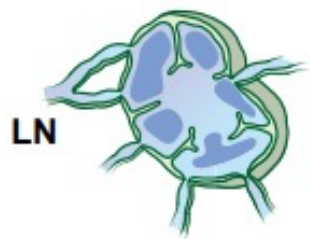


# Dissecting the Tumour Microenvironment



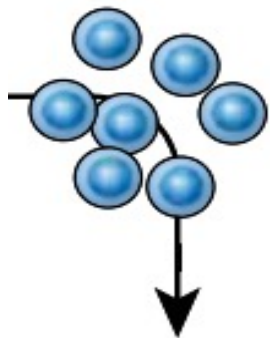


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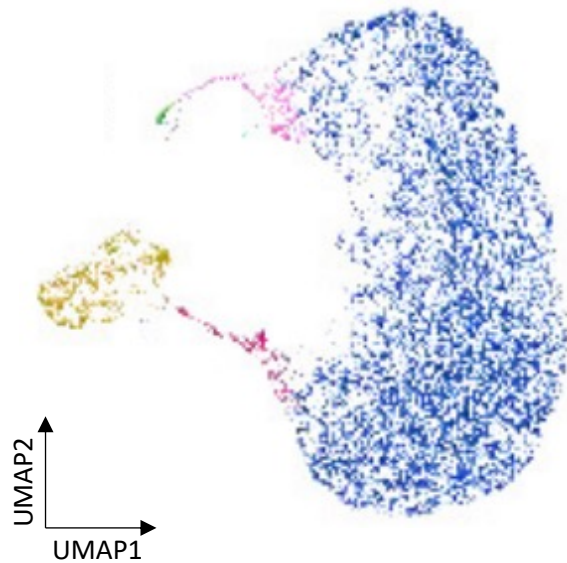


LN

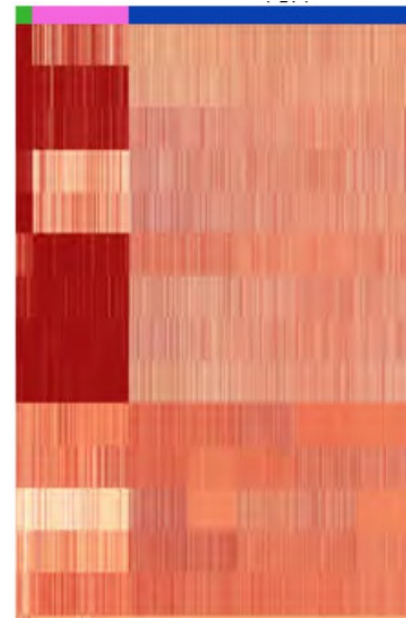
Dissociation



scRNA-seq

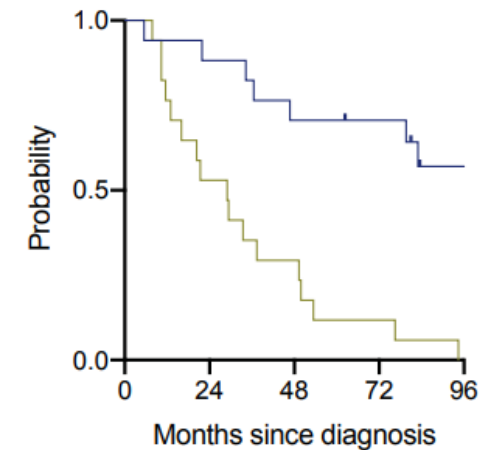


- Proliferating
- Activated
- Quiescent



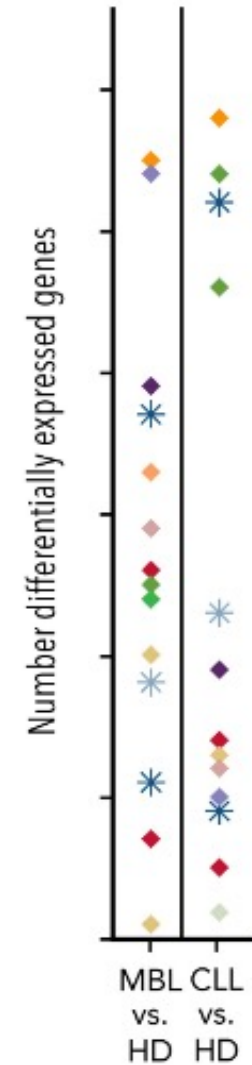
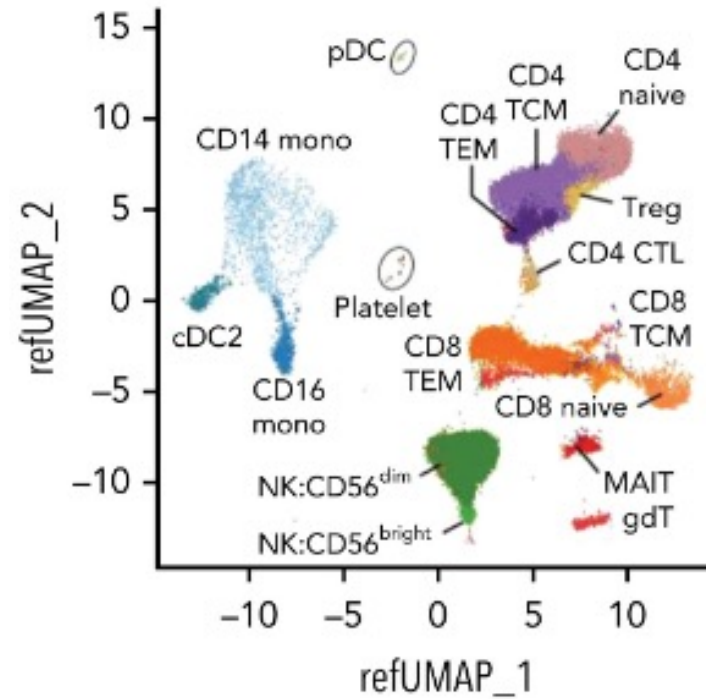
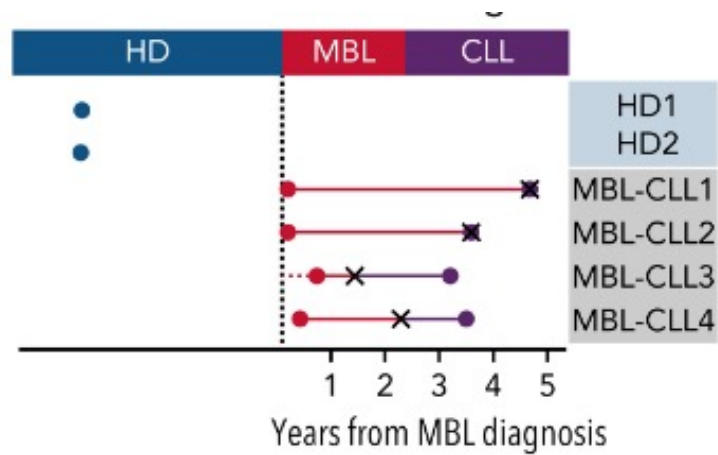
Cell identity

STMN1  
TUBB  
TUBA1B  
HIST1H4C  
HMGB2  
HSP90AB1  
PRDX1  
RAN  
ENO1  
IGLC3  
IGLC2  
KLF4  
IGHM  
CD74

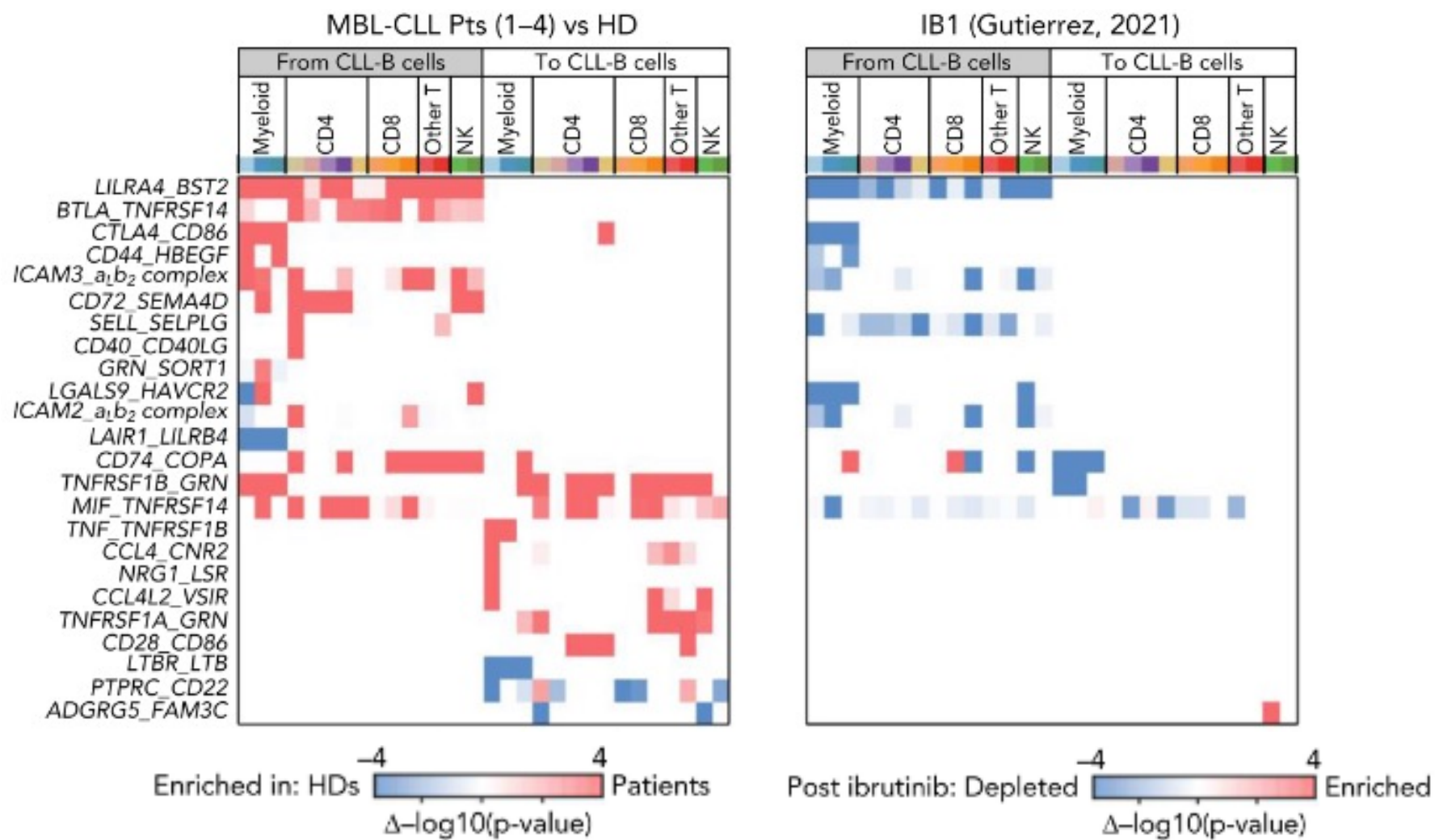


- Act. CLL < median
  - Act. CLL  $\geq$  median
- $p < 0.0001$

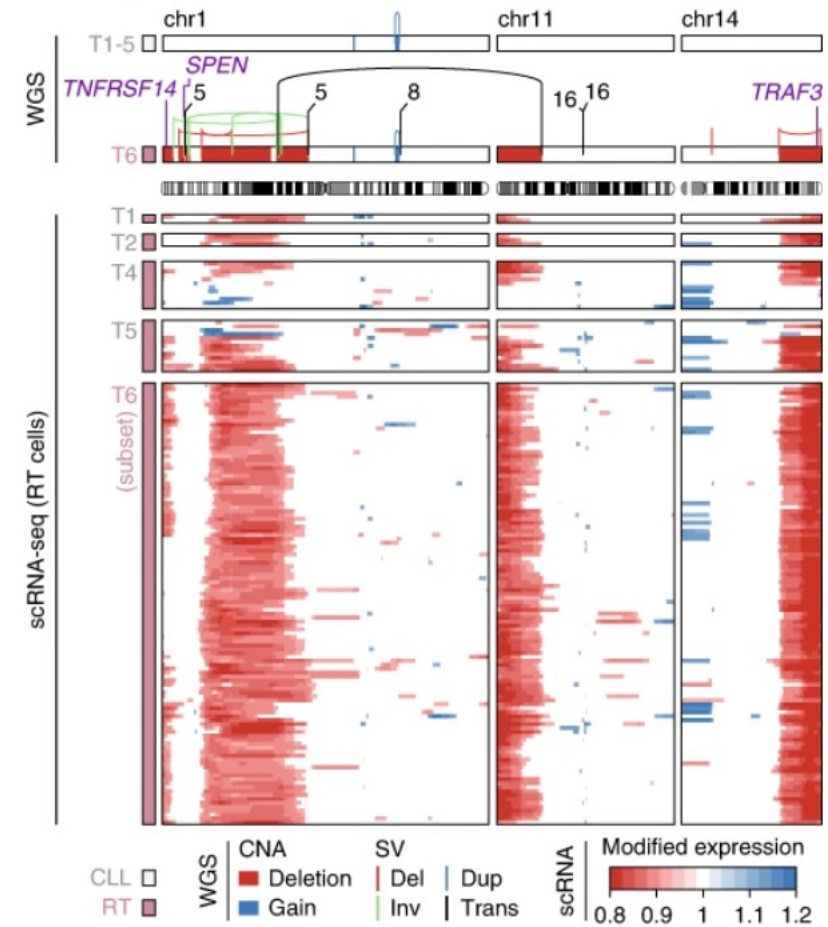
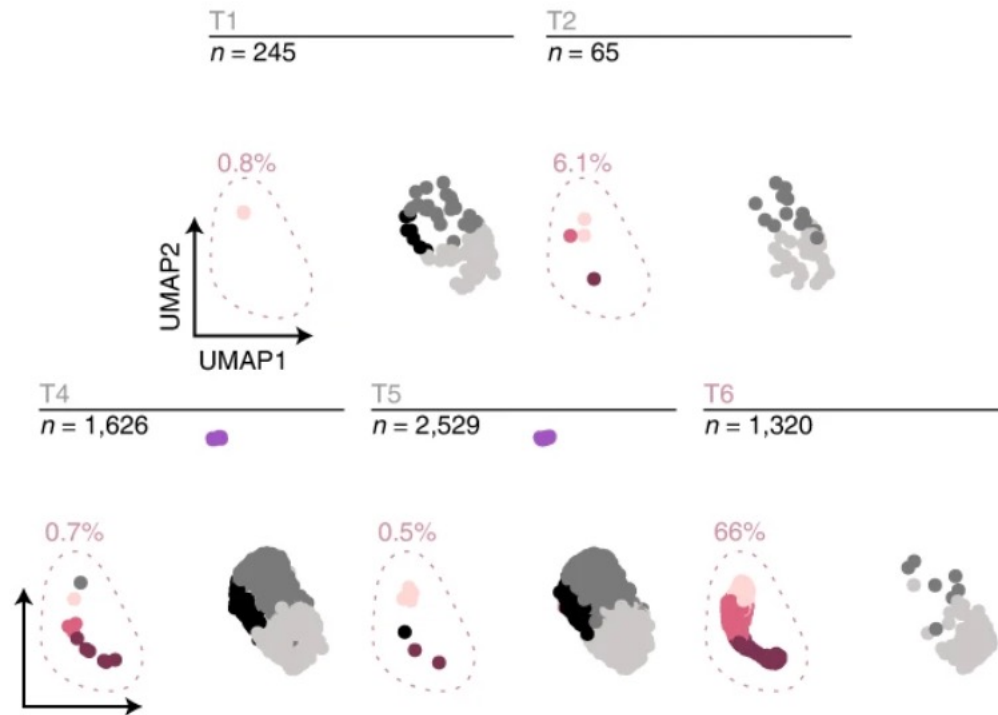
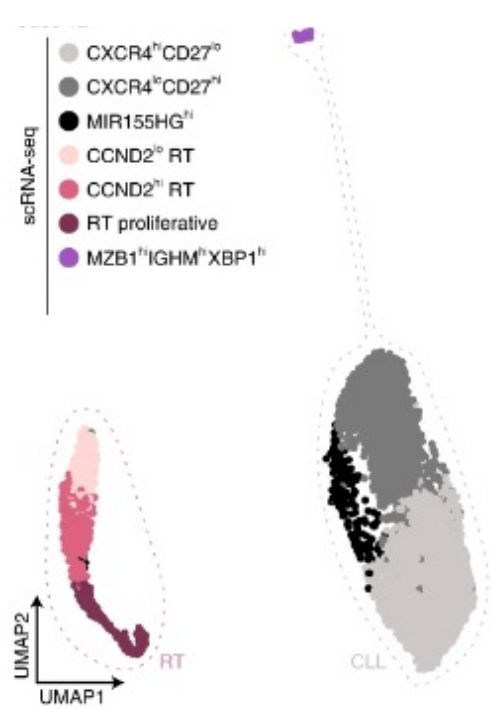
# Immune dysfunction in CLL



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# Insights into Richter Transformation



# Conclusions

- High Dimensional Single Cell Approaches provide unparalleled insights into leukemic and immune cells in CLL
- Ongoing advances will drive further understanding of CLL biology, response and resistance
- Spatial transcriptomics will provide the next tranche of insights