Apport du méthylome dans la classification des hémopathies lymphoïdes B matures. Florence Nguyen-Khac
05/02/2026

ASH scientific spotlight session

American Society of Hematology, December 6-9, 2025, Orlando, Florida

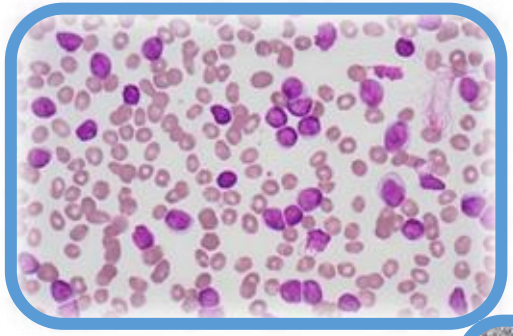
Epigenomic Frontiers in the Diagnosis of Hematological Malignancies

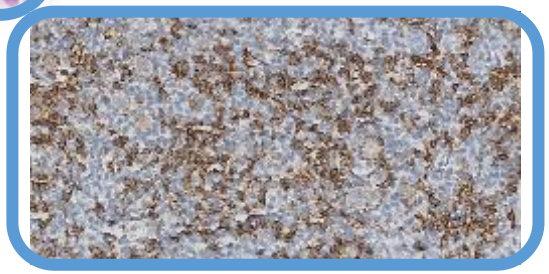
Prof. Florence Nguyen-Khac, MD, PhD
Hôpital Pitié-Salpêtrière, Sorbonne Université, Paris, France

Diagnosis of Hematological Malignancies

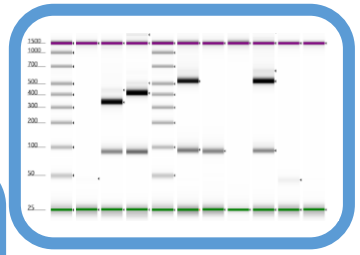
Clinical

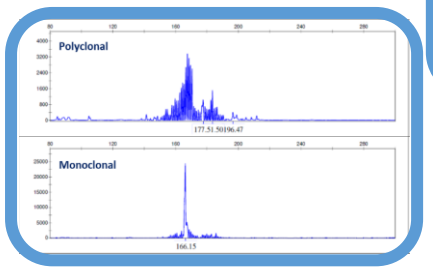
Morphology

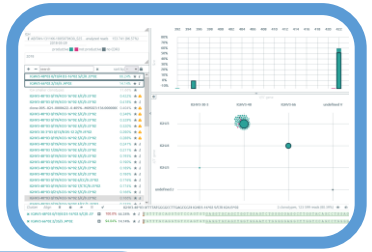


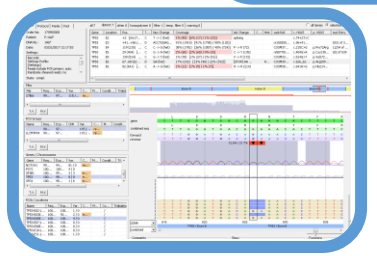


Molecular

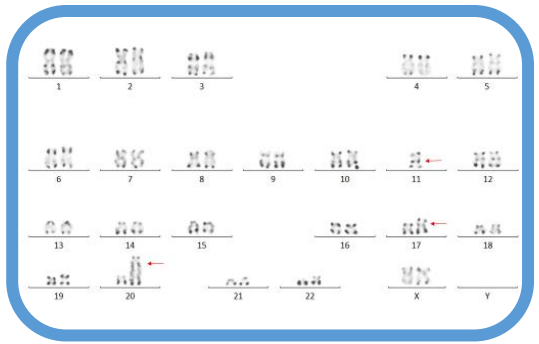


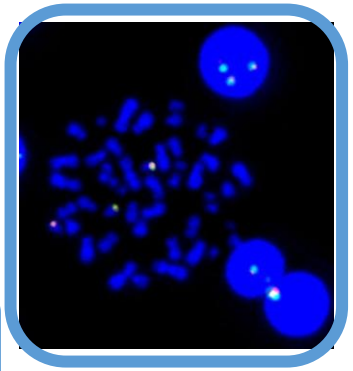


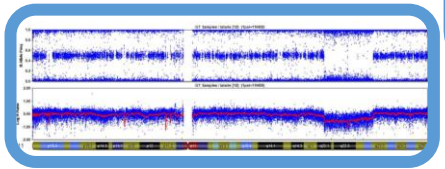


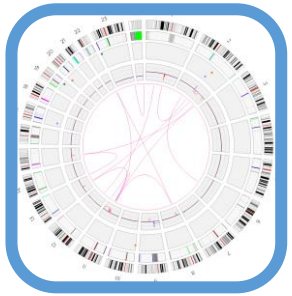


Cytogenetic

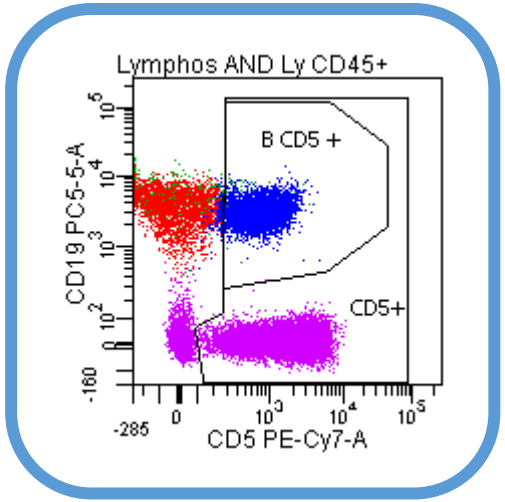








Immunophenotype



Diagnosis: why a classification?

« Diseases must be described, defined and named, before they can be diagnosed, treated and studied
WHO approach classification incorporates all available information – morphology, immunophenotype, genetic features and clinical features – to define the diseases »

WHO classification, Revised 4th Edition, 2017

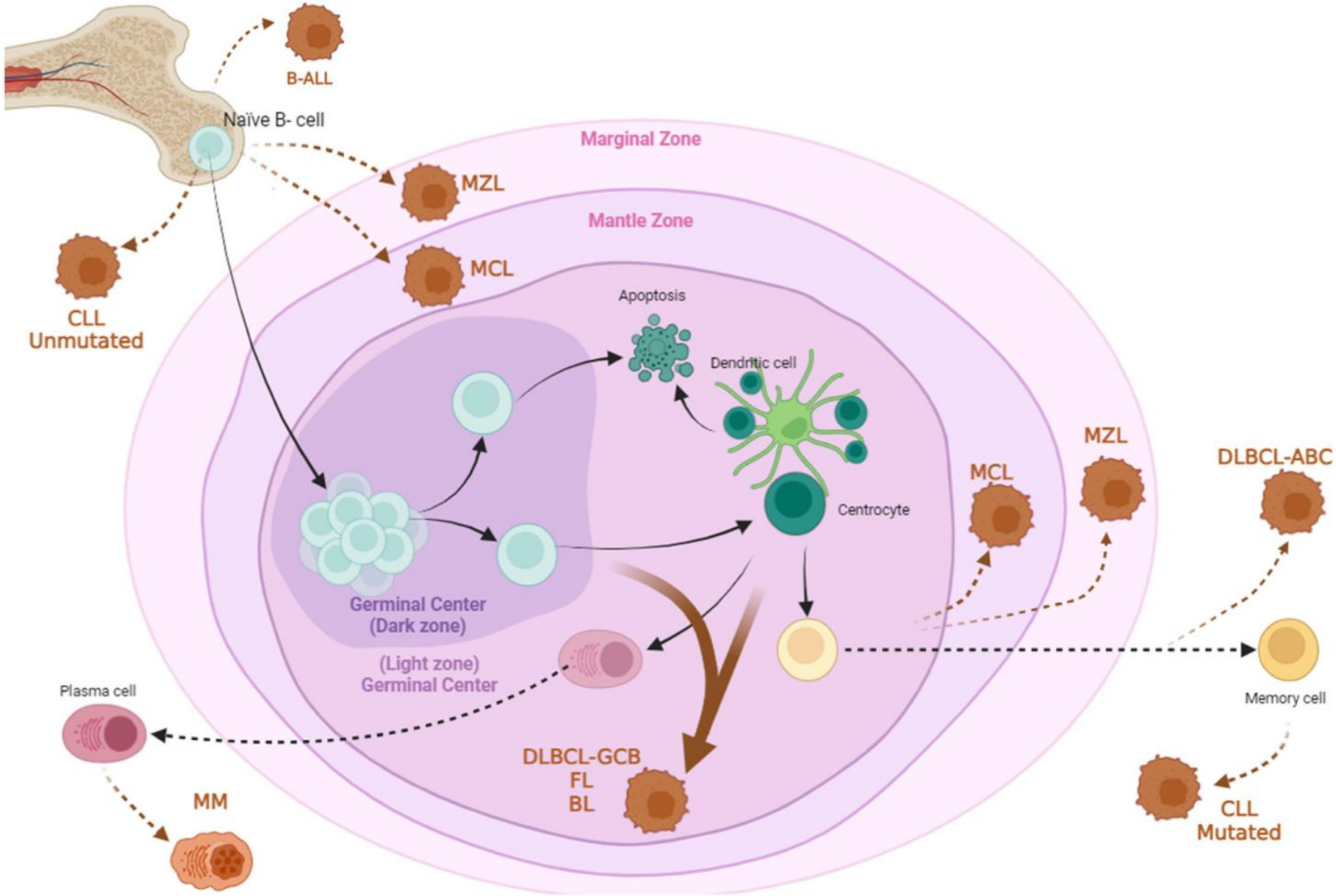
« Fundamental for the treatment of individual patients, monitoring of global disease incidence, and investigating all aspects of disease causation, prevention and therapy »

Alaggio et al., Leukemia 2022, WHO-HAEM5

« The explosion of genomic data is having an impact on our understanding of these diseases and is starting to be introduced into routine clinical practice for diagnosis and management strategies »

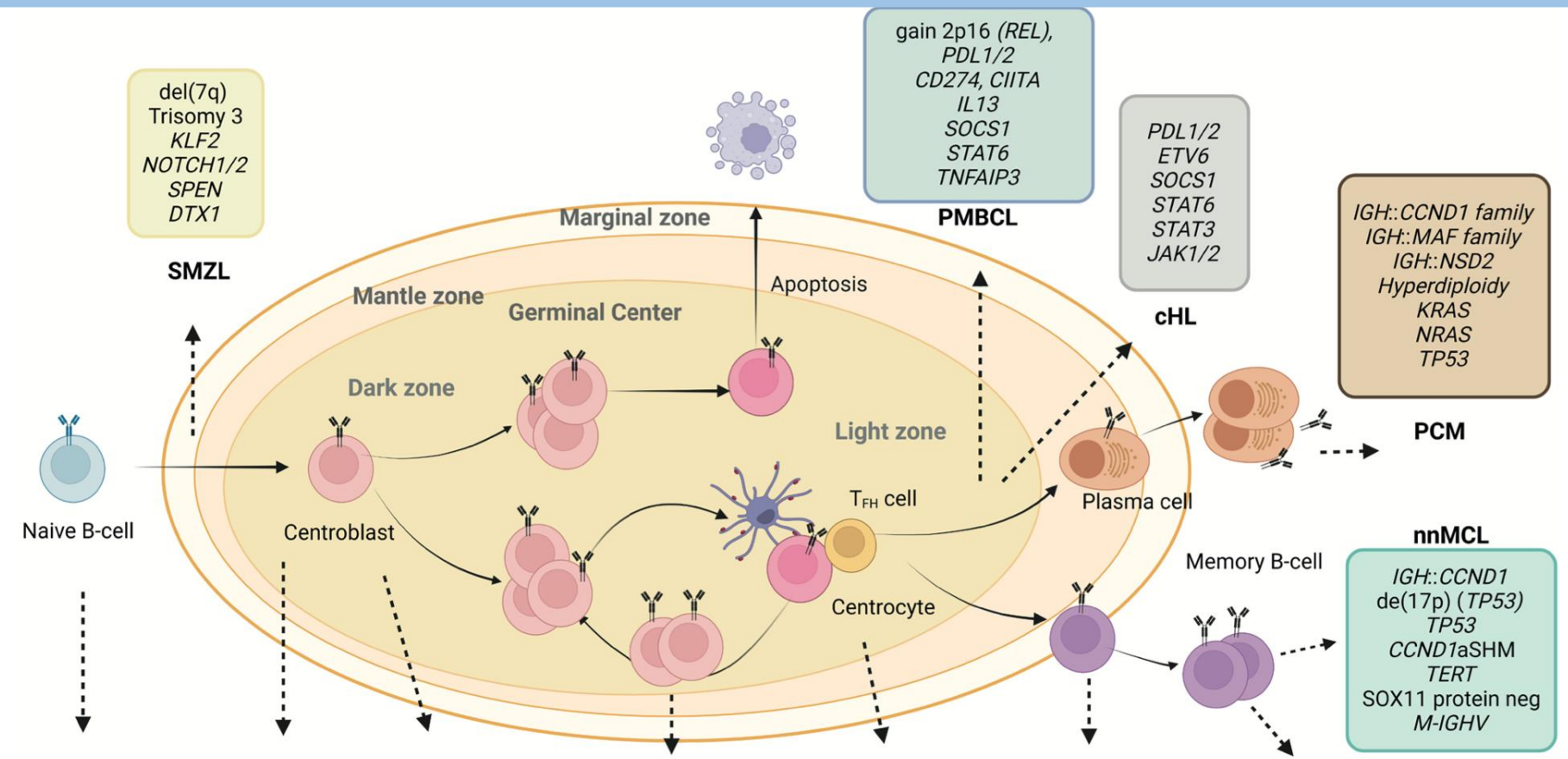
Campo et al., Blood 2022, ICC

Cellular origins of B-cell malignancies



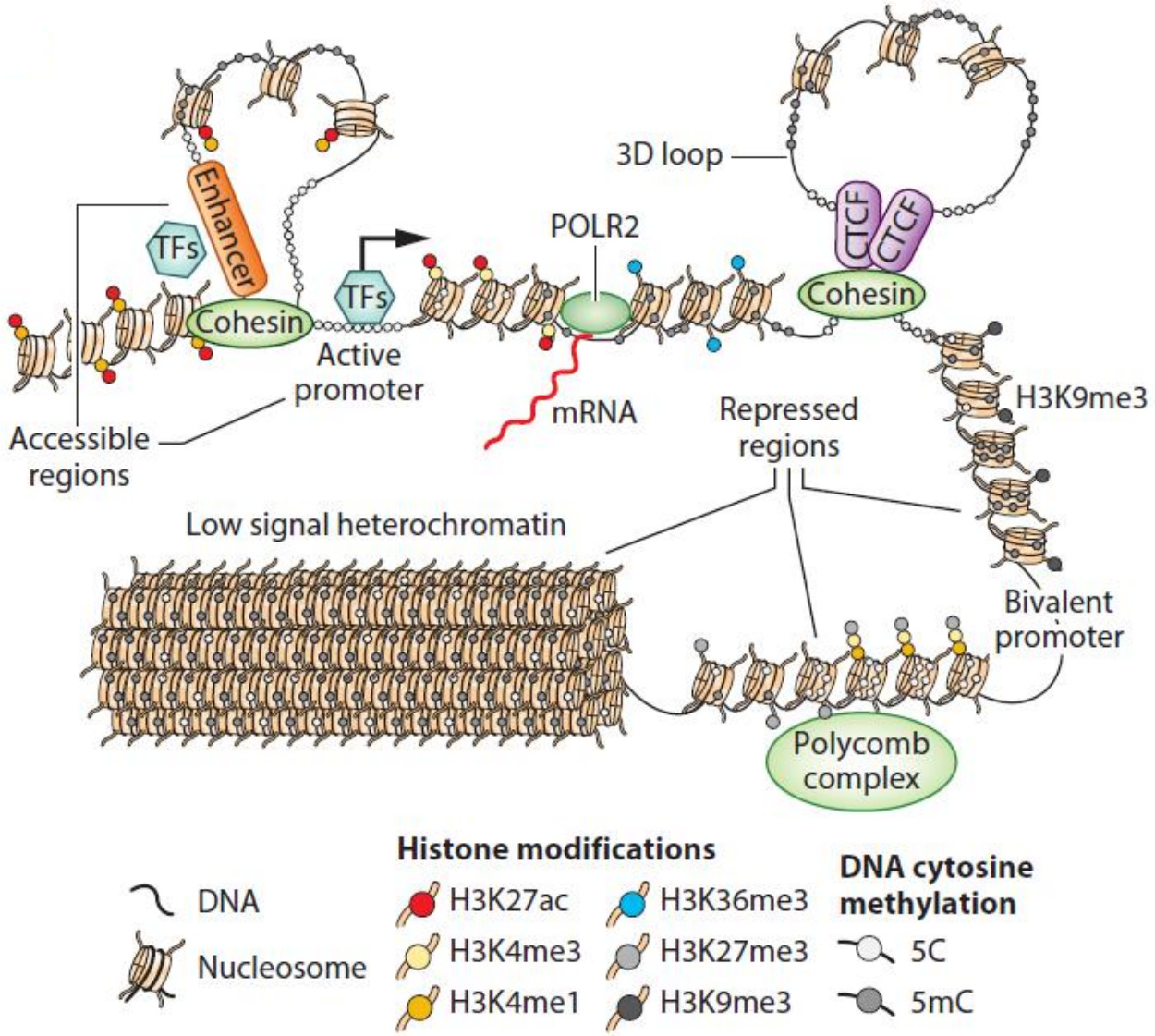
Jimenez et al., Int. J. Mol. Sci. 2024

Genomic aberrations in mature B-cell malignancies



Lopez et al., Med Genet 2024

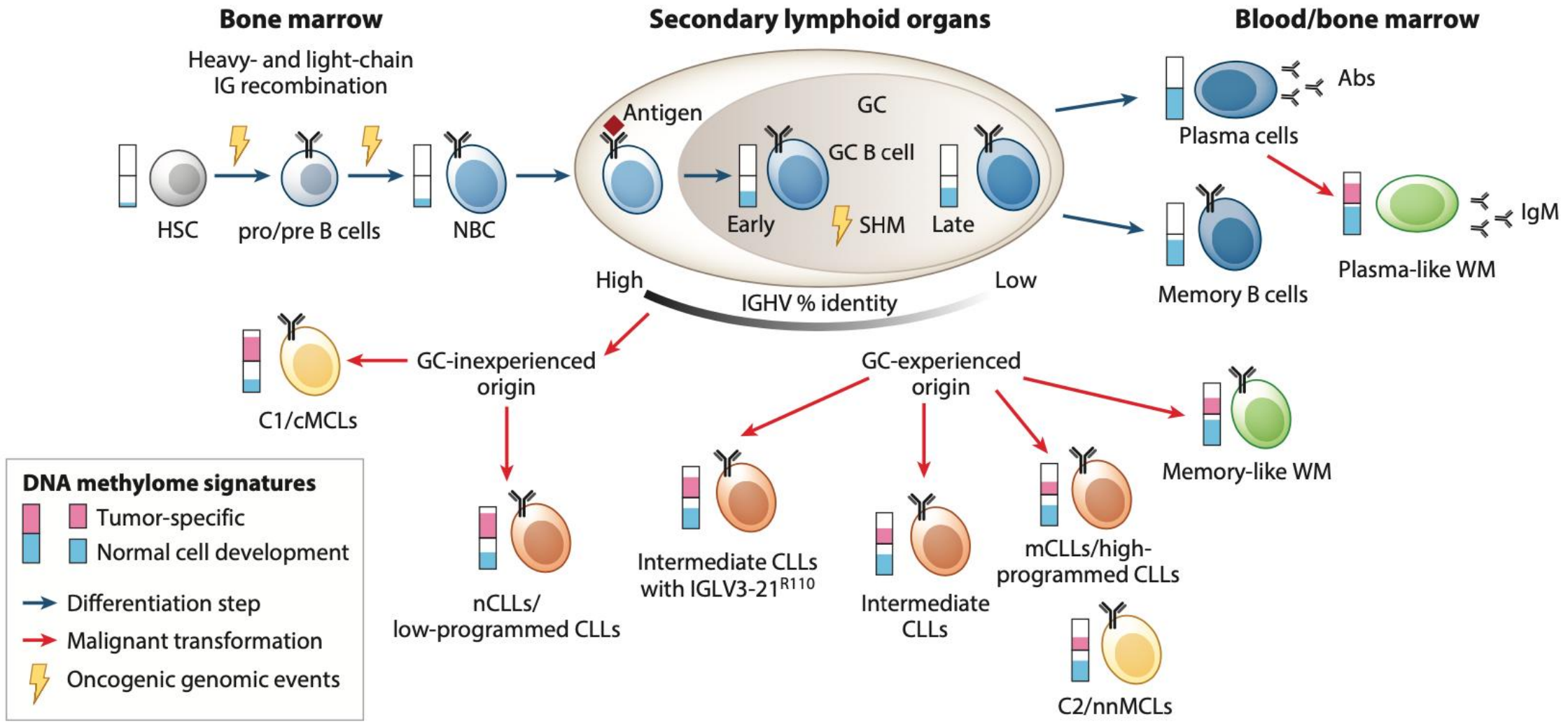
The epigenetic landscape of gene regulation



- DNA methylation
- Histone modifications
- Chromatin accessibility
- 3D structure of the genome

Duran-Ferrer & Martin-Subero, Annu. Rev. Pathol. Mech. Dis. 2024

DNA methylation as a molecular mark to trace cell identity



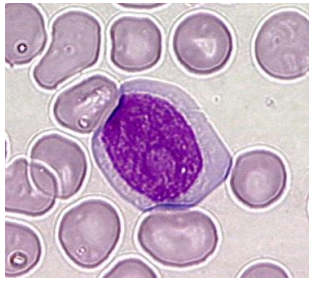
Duran-Ferrer & Martin-Subero, Annu. Rev. Pathol. Mech. Dis. 2024

DNA methylation

- **DNA methylation and classification**
  - **B-cell prolymphocytic leukemia (B-PLL)**

- **DNA methylation and prognosis**
  - **B-cell prolymphocytic leukemia (B-PLL)**
  - **UM-IGHV chronic lymphocytic leukemia (CLL)**
  - **Waldenström's Macroglobulinemia (WM)**

DNA methylation and classification



B-cell prolymphocytic leukemia (B-PLL)

Very rare

Very aggressive

Rapide increasing lymphocyte count

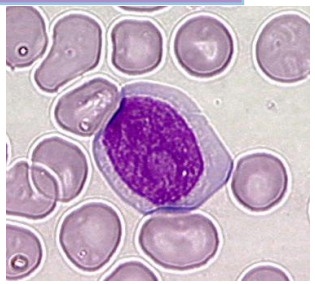
Massive splenomegaly

DNA methylation and classification: B-cell prolymphocytic leukemia (B-PLL)

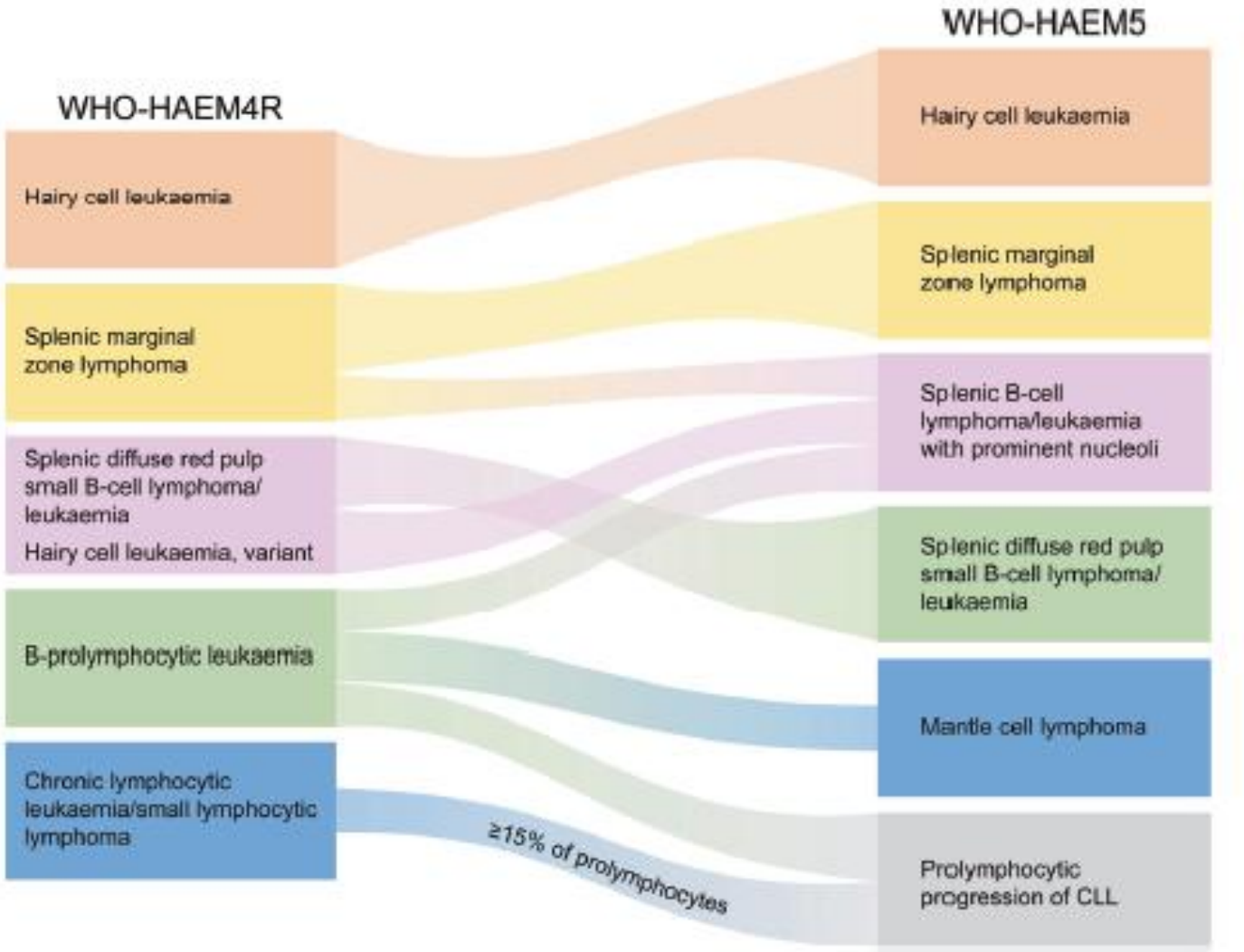
- First description *(Catovsky et al., Lancet 1973; Galton et al., Br J Haematol 1974)*
- Could be a CLL transformation *(Enno et al., Br J Haematol 1979)*
- **FAB classification:** >55% lymphoïd cells in the blood *(Bennett et al., J Clin Pathol 1986)*
- **WHO classification:**

- 2001: >55% lymphoïd cells in the blood. Cases of transformed CLL and CLL with increased prolymphocytes are excluded
- 2008: … and lymphoid proliferations with very similar morphology but carrying the t(11;14) are excluded
- 2017: … and lymphoid proliferations with relatively similar morphology but with a t(11;14) or SOX11 expression are excluded

DNA methylation and classification: B-cell prolymphocytic leukemia (B-PLL)



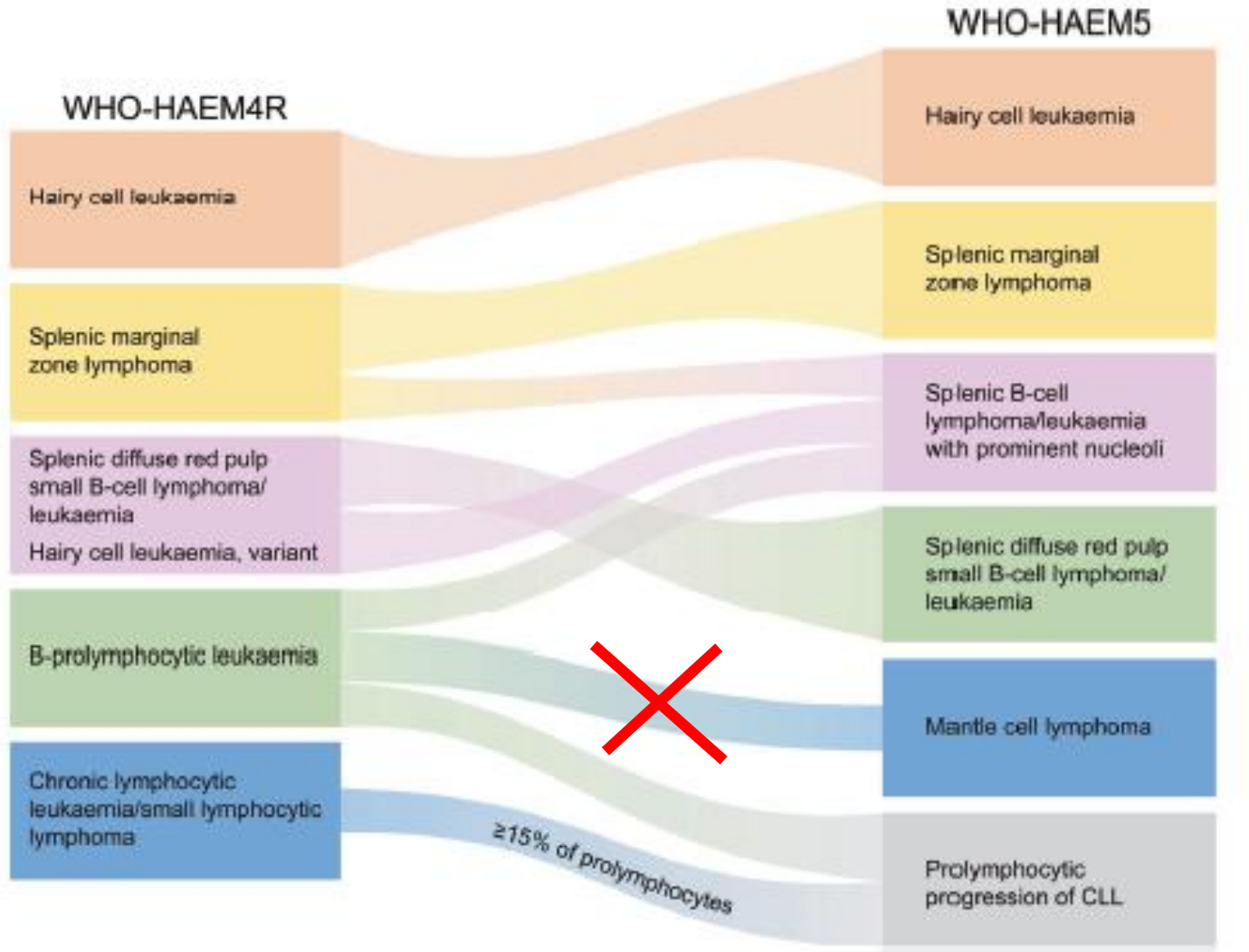
➢WHO classification 2022: B-PLL is no longer recognized



Alaggio et al., Leukemia 2022

DNA methylation and classification: B-cell prolymphocytic leukemia (B-PLL)

➢WHO classification 2022: B-PLL is no longer recognized

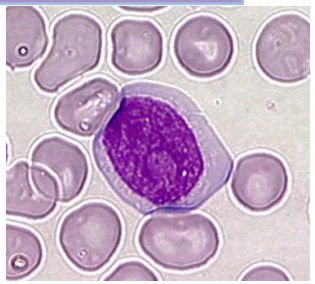


CD5- B-PLL
HCLv

CD5+ B-PLL

Alaggio et al., Leukemia 2022

DNA methylation and classification: B-cell prolymphocytic leukemia (B-PLL)



➢**ICC 2022: B-PLL is an entity**

Table 1. International Consensus Classification of mature lymphoid and histiocytic/dendritic cell neoplasms

Mature B-cell neoplasms

- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- Monoclonal B-cell lymphocytosis
  - Chronic lymphocytic leukemia type
  - Non-chronic lymphocytic leukemia type
- B-cell prolymphocytic leukemia
- Splenic marginal zone lymphoma
- Hairy cell leukemia
- *Splenic B-cell lymphoma/leukemia, unclassifiable*
  - *Splenic diffuse red pulp small B-cell lymphoma*
  - *Hairy cell leukemia-variant*
- Lymphoplasmacytic lymphoma
  - Waldenström macroglobulinemia

➡ Criteria WHO-HAEM4R

Campo et al., Blood 2022

DNA methylation and classification: B-cell prolymphocytic leukemia (B-PLL)

➢Genomic

34 B-PLL patients

		B-PLL	CLL	Mantle cell lymphoma[1,2]	Splenic marginal zone Lymphoma[3]
Chromosomal abnormalities	Complex Karyotype ≥ 3	++++	++	+++	+++
	Translocation t(11;14)	-	-	++++	+
	MYC Translocation	++++	+	+	+
	MYC gain	++	+	++	
	17p deletion (*TP53*)	+++	+	++	++
	13q deletion	++	++++	+++	+
	Trisomy 18	++	+	+	++
	Trisomy 3	++	+	++	+++
	Trisomy 12	++	++	+	++
	7q Deletion	-	-	-	+++
	11q deletion (*ATM*)	+	++	++	-
Gerne mutations	*TP53*	+++	++	++	++
	MYD88	++	+	-	+
	BCOR	++	+	-	-
	FAT1	++	+	+	-
	SF3B1	++	++	-	-
	MYC	++	+	-	-
	CCND1	-	-	+++	-
	ATM	-	++	+++	-
	NOTCH1	-	++	+	++
	NOTCH2	+	+	+	+++

Sarkozy et al., Genes Chromosome Cancer 2014; Halldorsdottir et al., Genes Chromosome Cancer 2011; Salido et al., Blood, 2010; Chapiro et al., Blood 2019

DNA methylation and classification: B-cell prolymphocytic leukemia (B-PLL)

➢Immunogenetic: IGHV genes

B-PLL	CLL	MCL	SMZL
IGHV3, IGHV4	IGHV1–69, IGHV4-34	IGHV3-21, IGHV4-34	IGHV1-2*04 (30%), IGHV4-34
IGHV-M (79%)	IGHV-M (GI<98%) (54%)	IGHV-UM or minimally M (GI>97%) (87%)	IGHV-M (minimally for IGHV1-2*04)

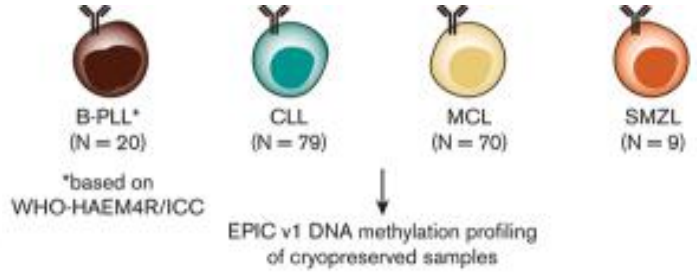
GI : germline identity

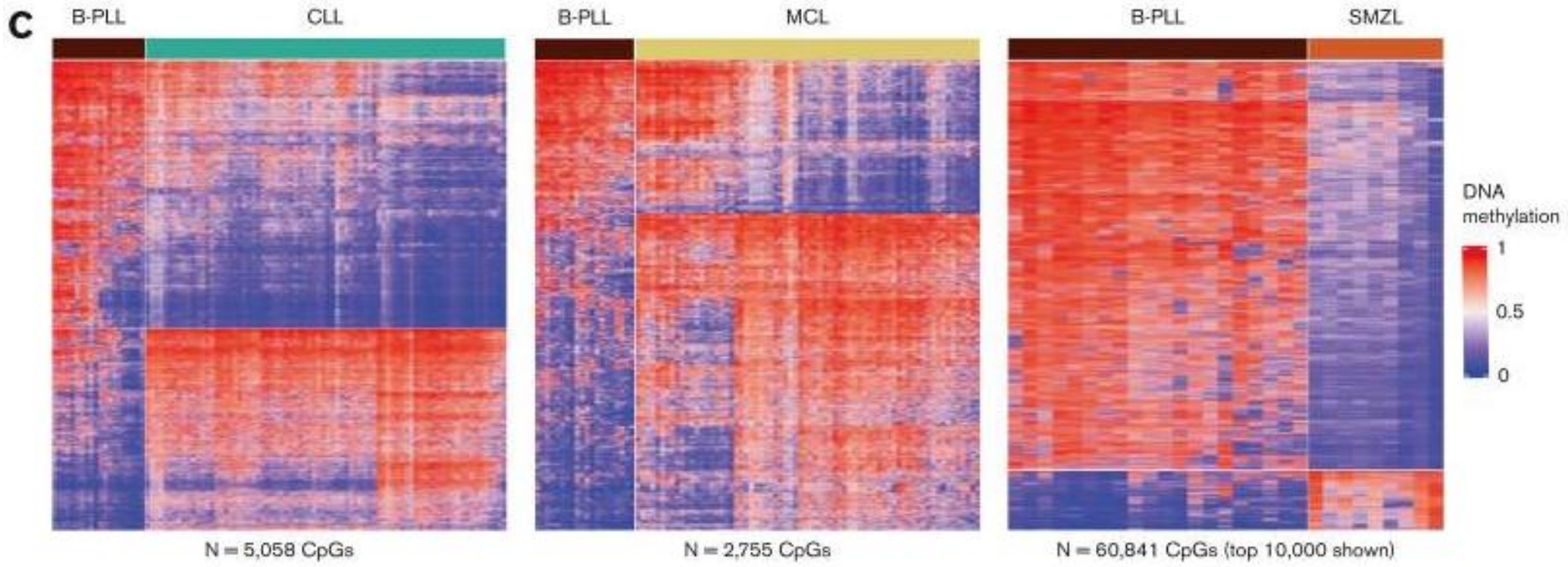
Davi et al., Blood 1996; Hadzidimitriou, Blood 2011; Bikos, Leukemia 2012; Chapiro et al.; Blood 2019; Agathangelidis et al., Blood 2021

DNA methylation and classification: B-cell prolymphocytic leukemia (B-PLL)

➢Epigenetic: DNA methylation

20 B-PLL patients



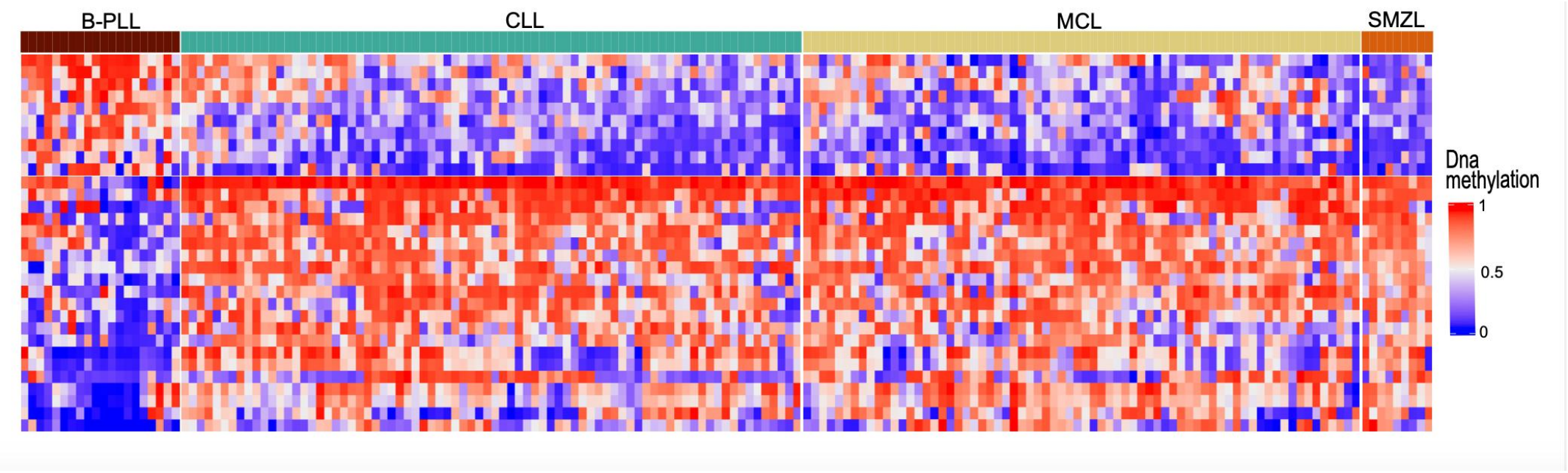


→differentially methylated (DM) CpGs between B-PLL and CLL, MCL and SMZL

Charalampopoulou et al., Blood Adv 2024

DNA methylation and classification: B-cell prolymphocytic leukemia (B-PLL)

➢Epigenetic: DNA methylation

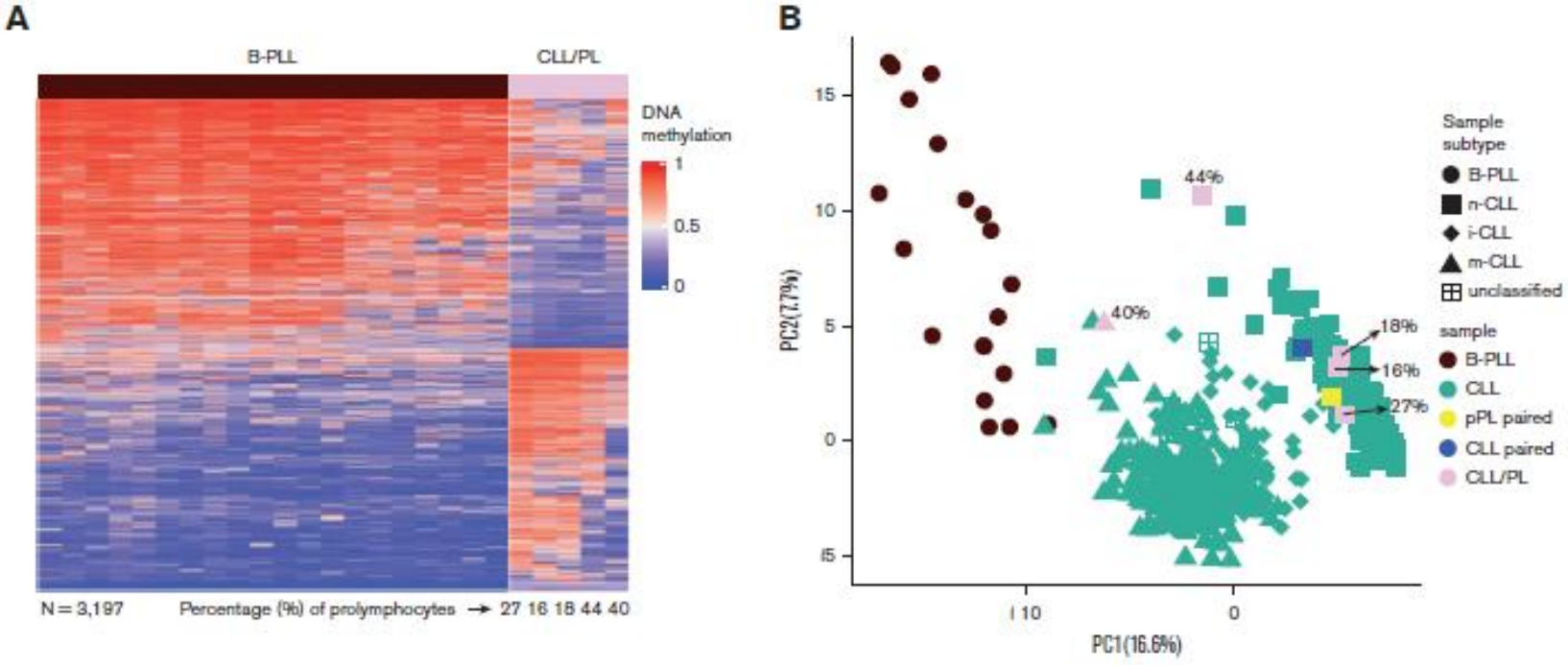


→ signature of 31 CpGs that can differentiate B-PLL vs CLL, MCL, and SMZL

Charalampopoulou et al., Blood Adv 2024

DNA methylation and classification: B-cell prolymphocytic leukemia (B-PLL)

➢Epigenetic: DNA methylation



→*de novo* B-PLLs are epigenetically distinct from CLLs with prolymphocytoid cells or even a prolymphocytic progression of a previous CLL

Charalampopoulou et al., Blood Adv 2024

DNA methylation

➢ **DNA methylation and classification**

❑ **B-cell prolymphocytic leukemia (B-PLL)**

→B-PLL has a differential DNA methylation signature compared with CLL, CLL/PL, MCL, and SMZL

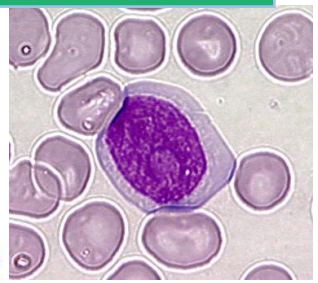
→Independent *de novo* disease

DNA methylation

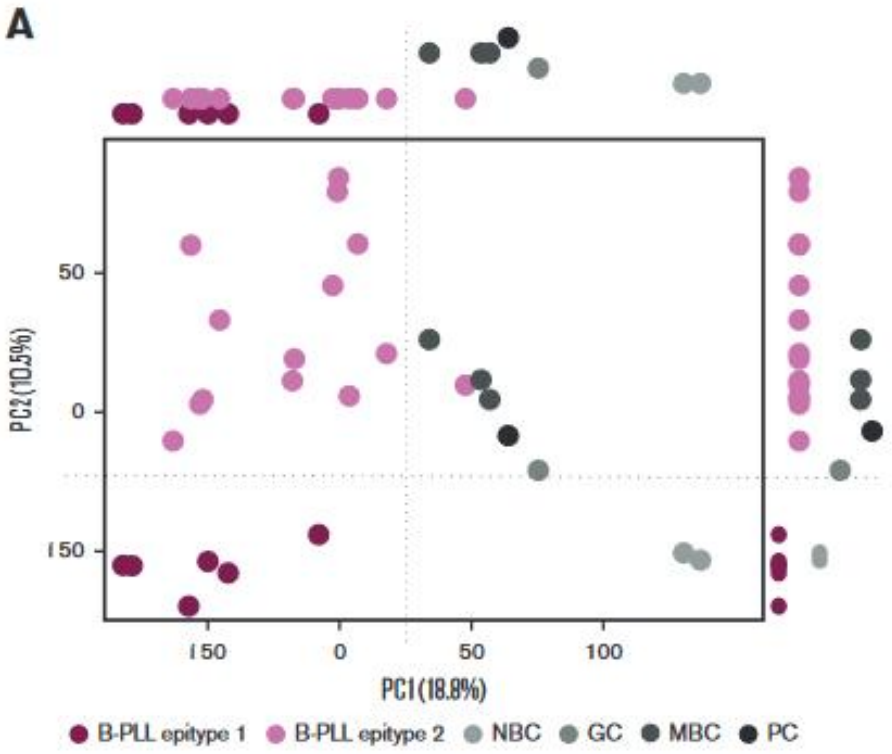
- DNA methylation and classification
  - B-cell prolymphocytic leukemia (B-PLL)

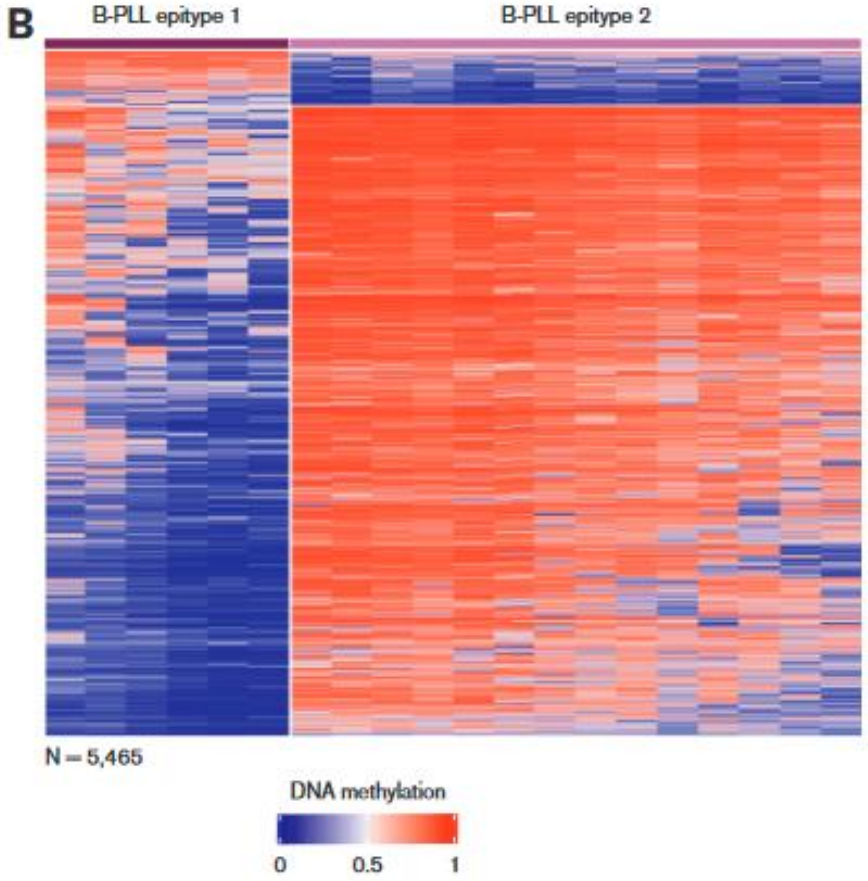
- **DNA methylation and prognosis**
  - **B-cell prolymphocytic leukemia (B-PLL)**
  - **UM-IGHV chronic lymphocytic leukemia (CLL)**
  - **Waldenström's Macroglobulinemia (WM)**

DNA methylation and prognosis



❖ **B-cell prolymphocytic leukemia (B-PLL)**

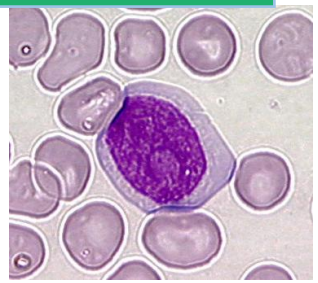




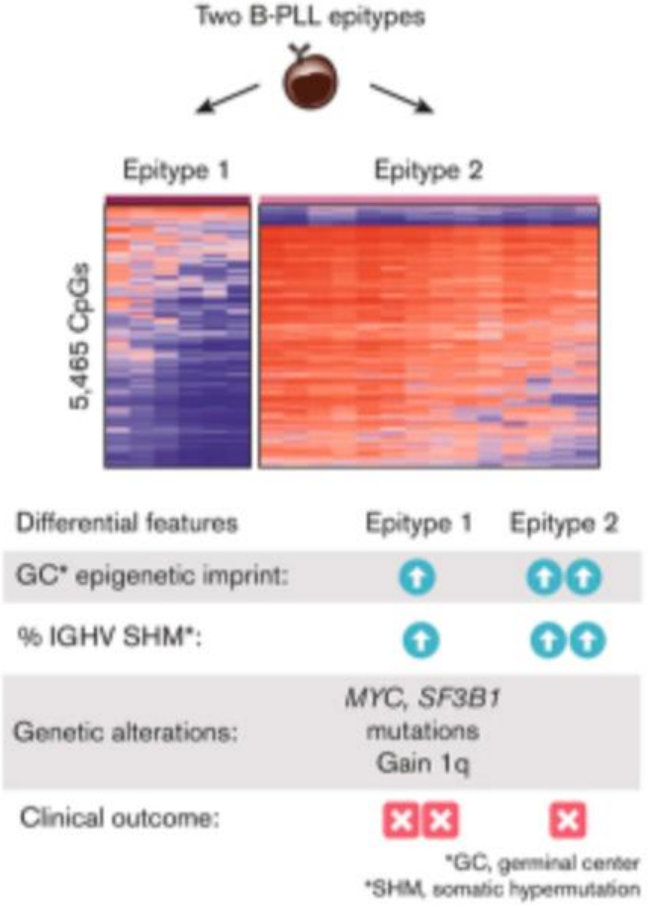
→ Two epitypes, one closer to Naive B cells, the other closer to GC-experienced B cells

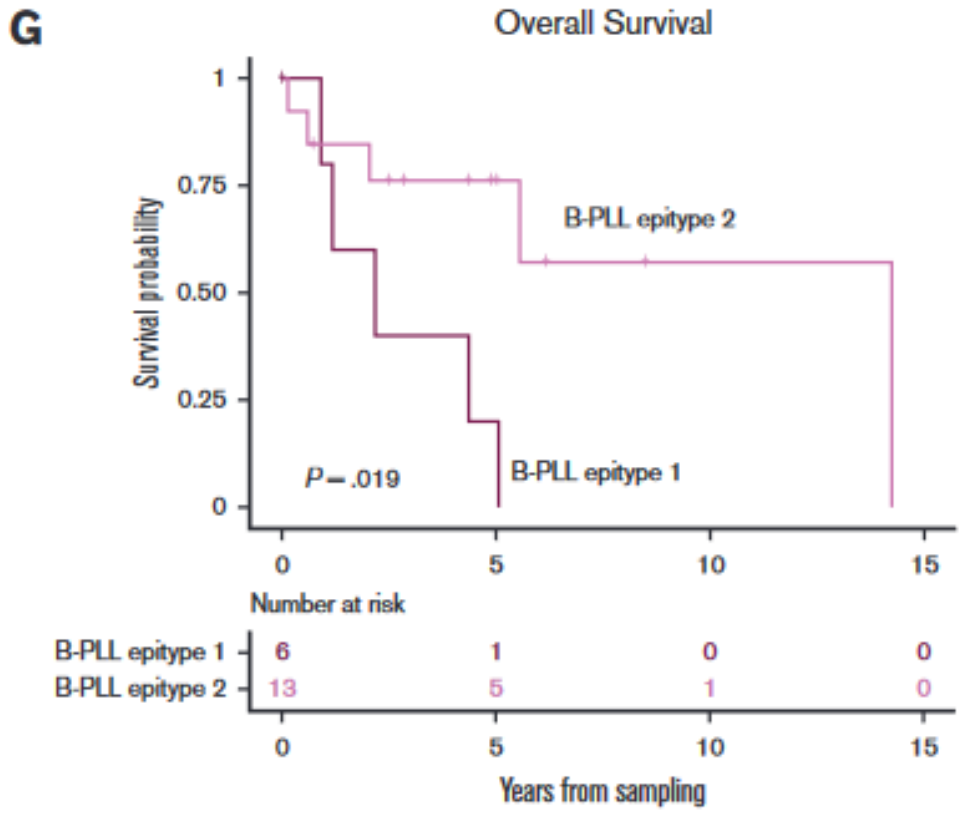
Charalampopoulou et al., Blood Adv 2024

DNA methylation and prognosis



❖ B-cell prolymphocytic leukemia (B-PLL)

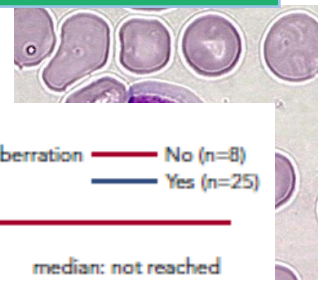




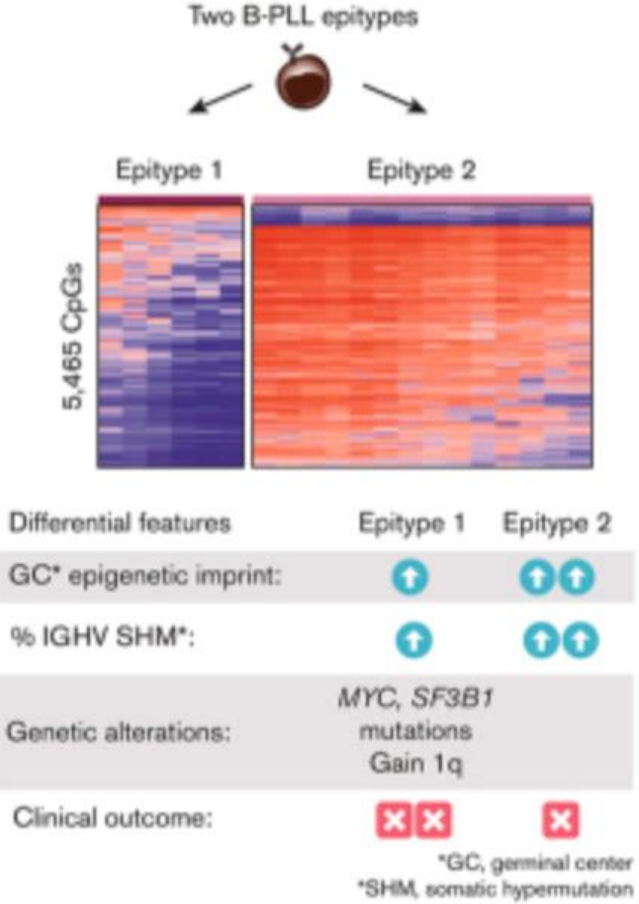
→ B-PLL epitype 1 is associated with a poorer outcome

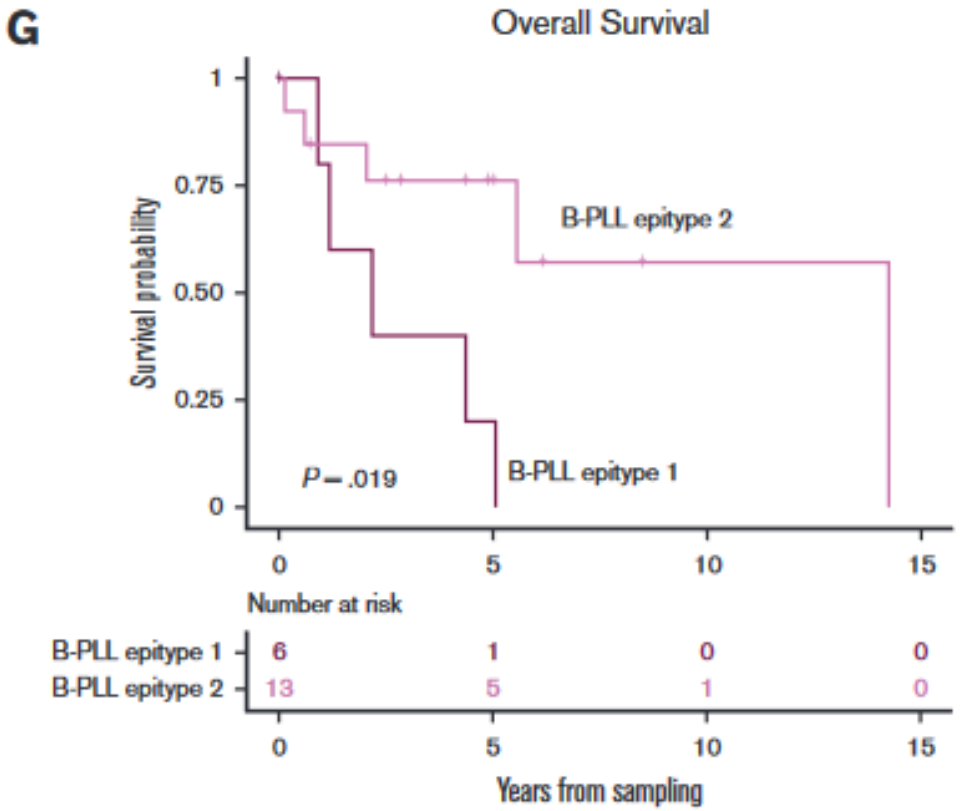
Charalampopoulou et al., Blood Adv 2024

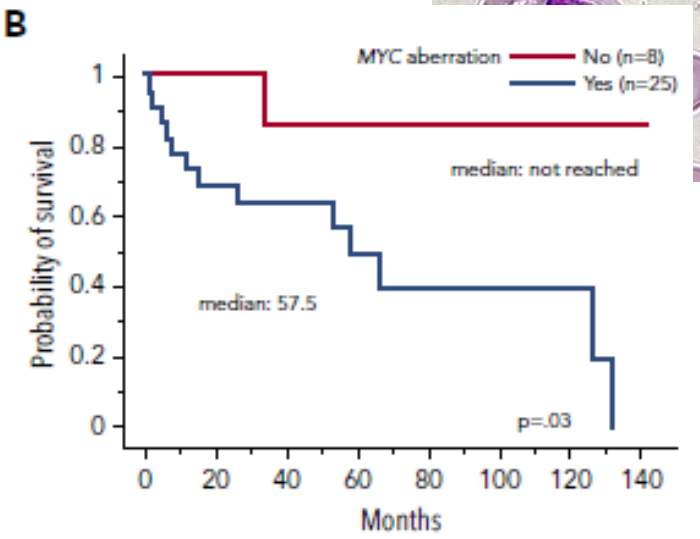
DNA methylation and prognosis

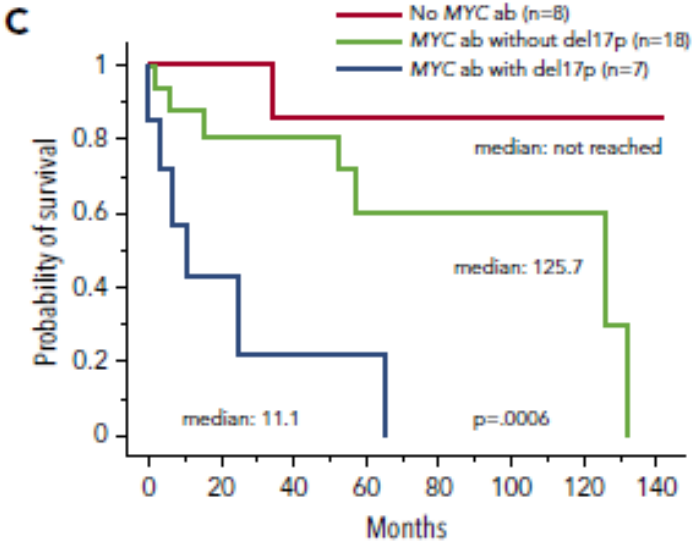


❖ B-cell prolymphocytic leukemia (B-PLL)





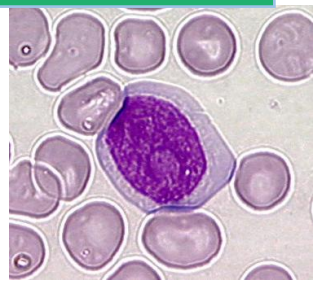




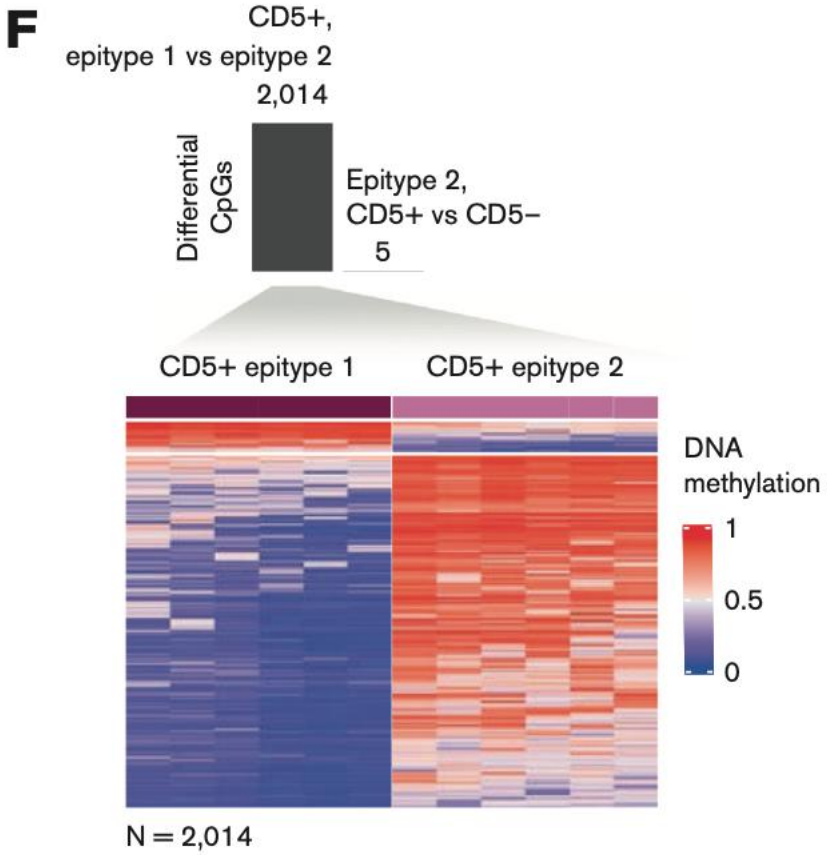
Charalampopoulou et al., Blood Adv 2024

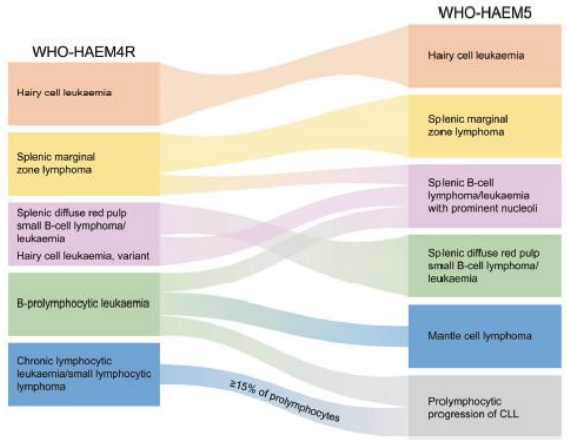
Chapiro et al., Blood 2019

DNA methylation and prognosis…and classification



❖ **B-cell prolymphocytic leukemia (B-PLL)**

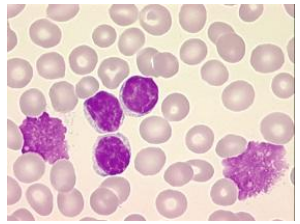




→ Segregation based on the CD5 expression is not supported by a differential DNA methylation signature

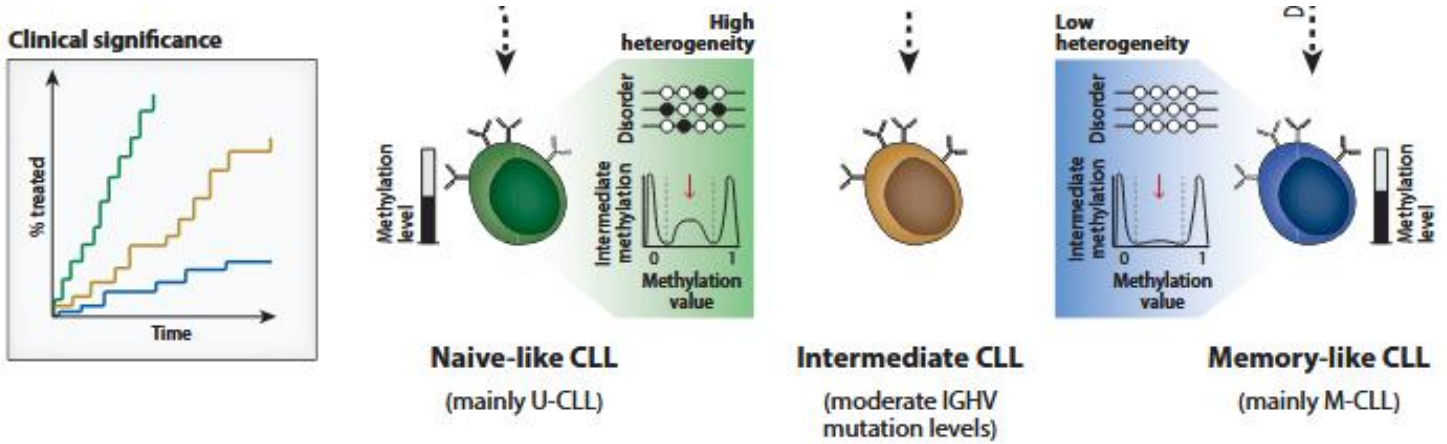
Charalampopoulou et al., Blood Adv 2024

DNA methylation and prognosis



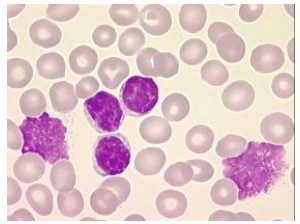
❖ UM-IGHV Chronic lymphocytic leukemia (CLL)

Monoclonal B-cell count > 5G/L
CD5+, CD23+
Binet/Rai Stages
del13q, tri12, del11q (*ATM*)
del17p/*TP53*mut, HCK (>= 5 chromosomal abnormalities)
IGHV mutated (-M)/unmutated (-UM)
3 epigenetic subtypes have different clinical outcomes



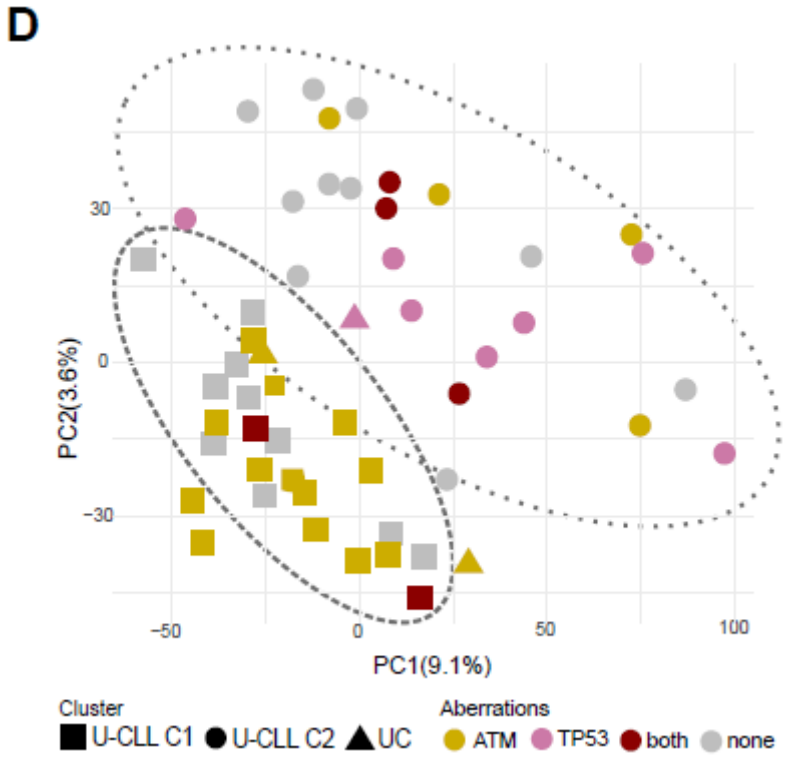
Nadeu et al., Annu. Rev. Pathol. Mech. Dis. 2020.

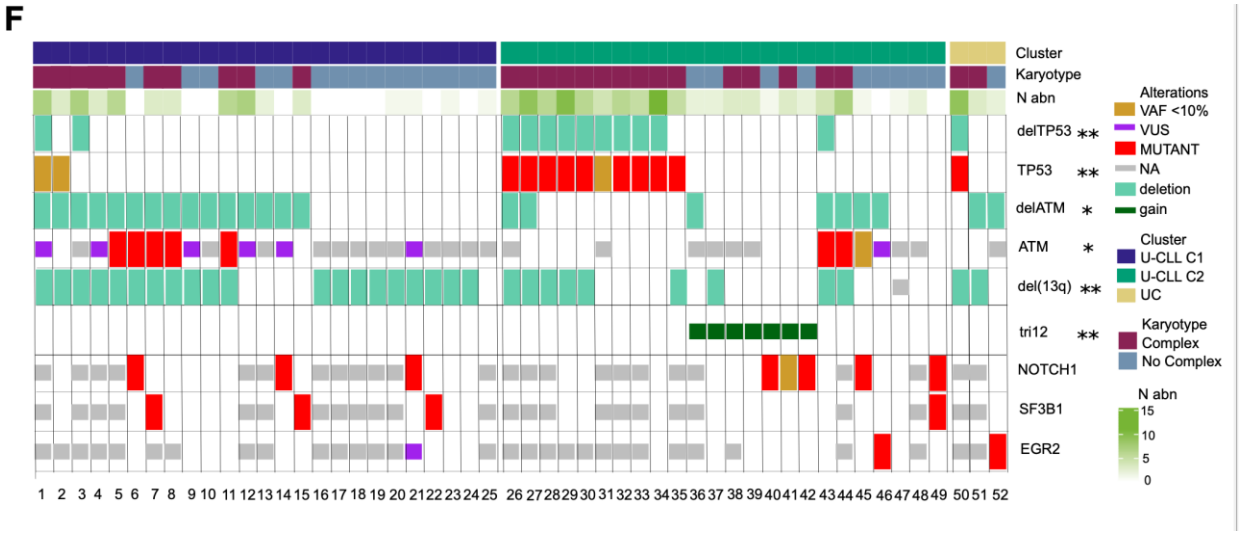
DNA methylation and prognosis



❖ UM-IGHV Chronic lymphocytic leukemia (CLL)

52 UM-IGHV CLL patients



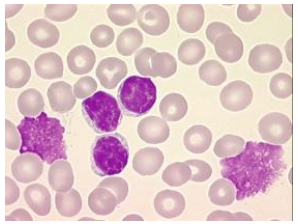


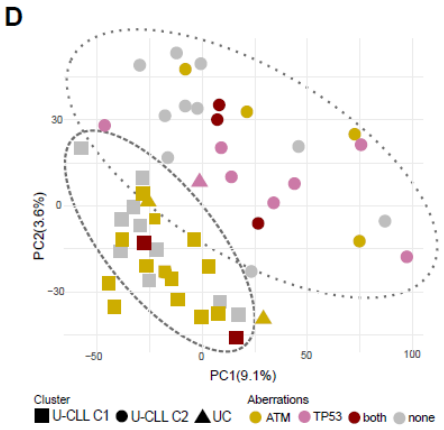
→ Two epitypes inside UM-IGHV CLL

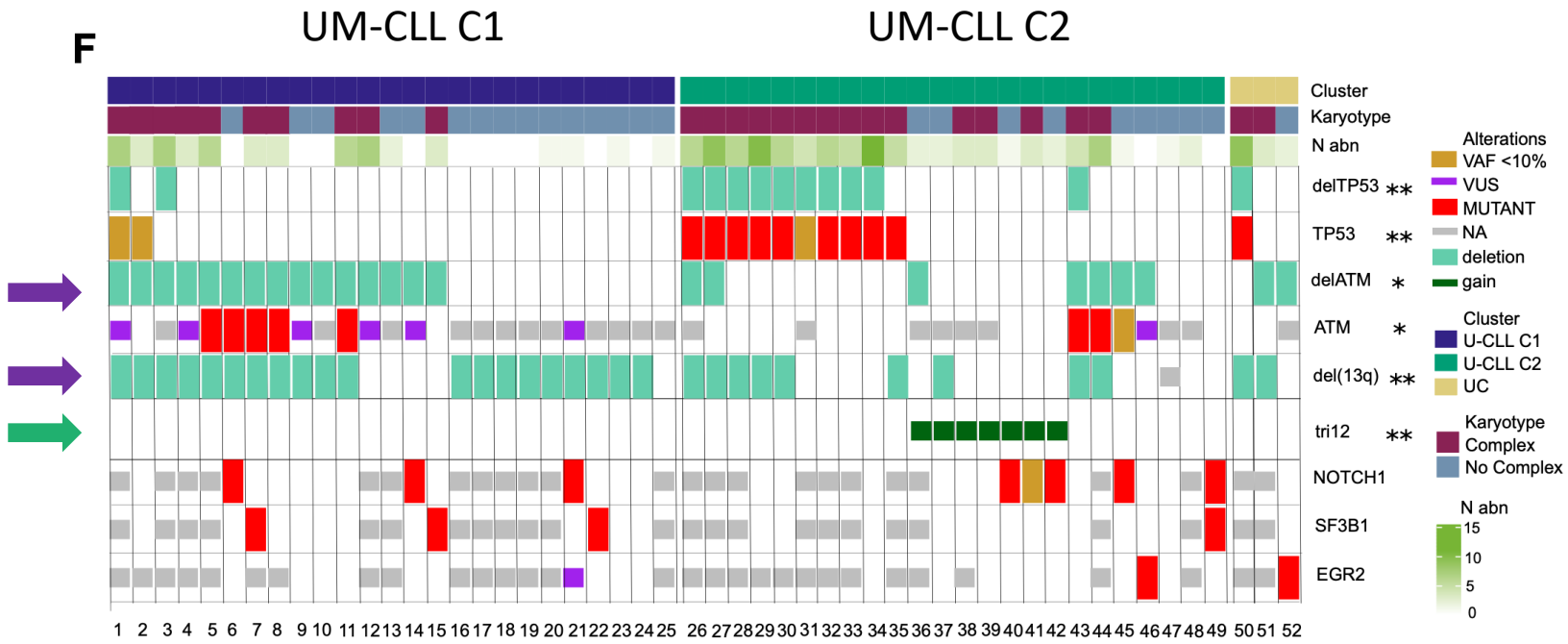
Charalampopoulou et al., Hemasphere, in press

DNA methylation and prognosis

❖ UM-IGHV Chronic lymphocytic leukemia (CLL)





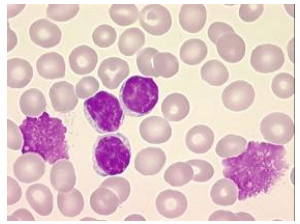


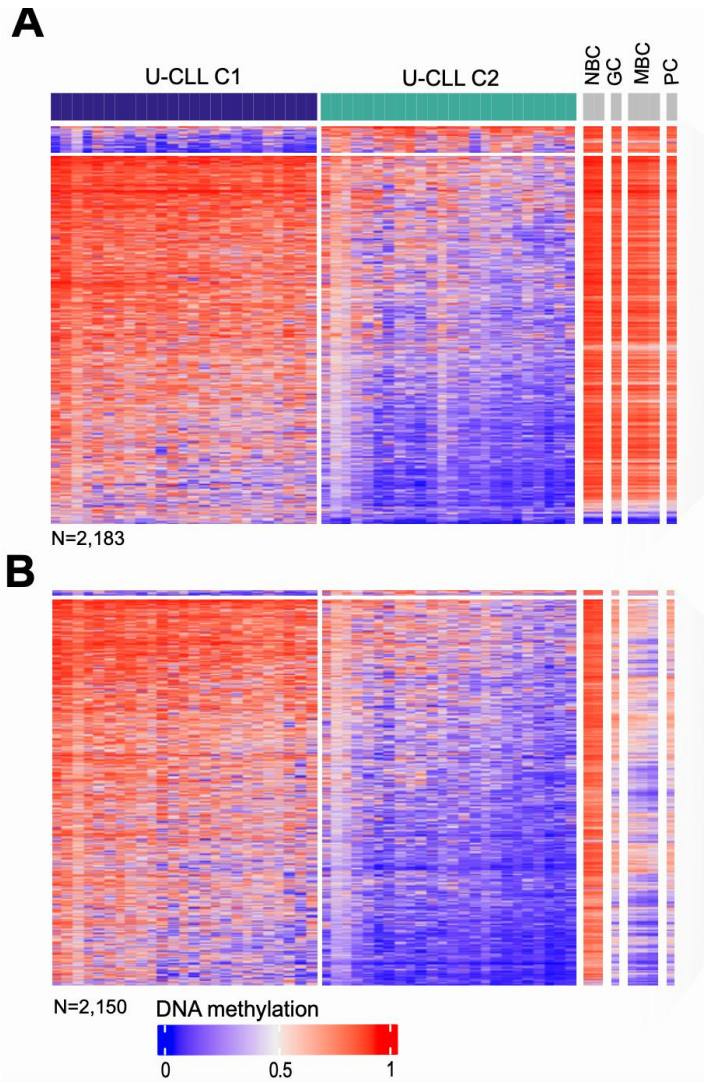
→Two epitypes of UM-IGHV CLL with distinct genetic alterations

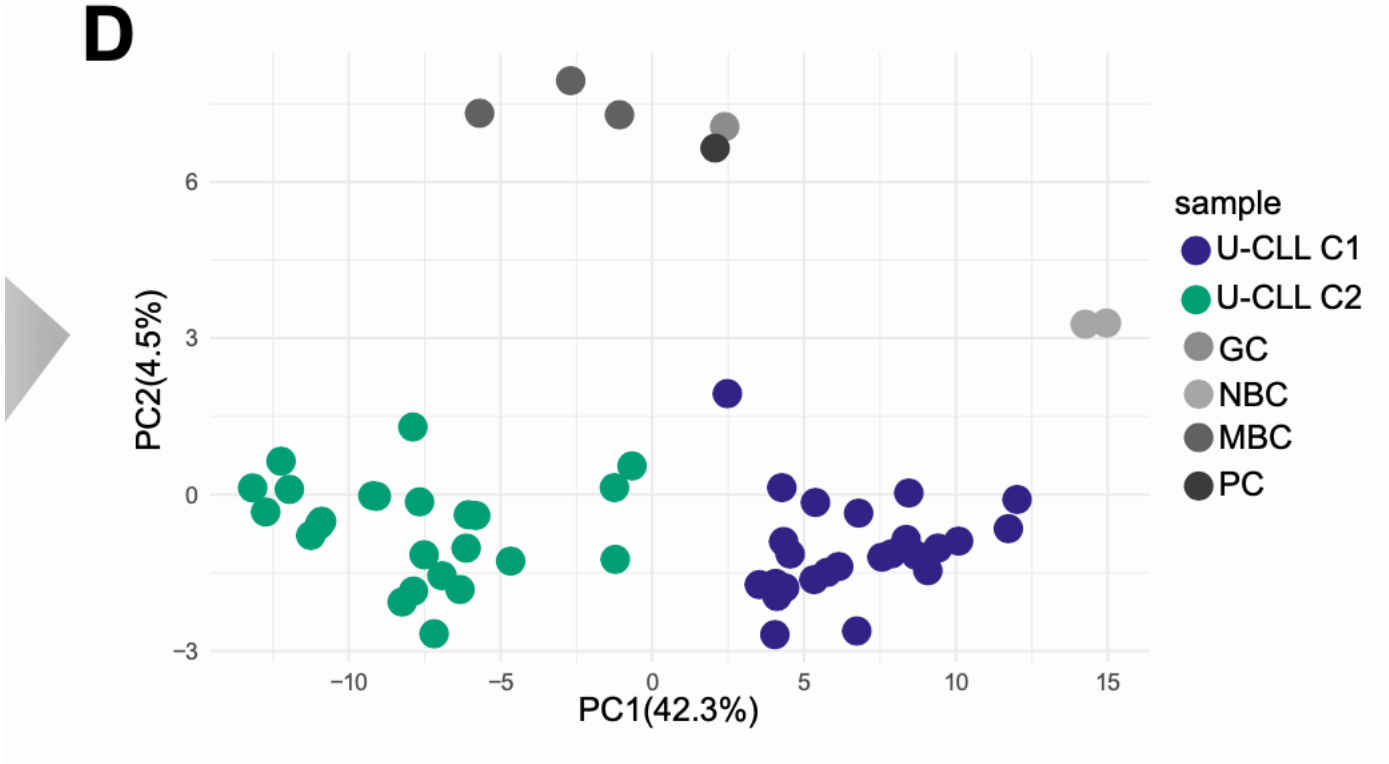
Charalampopoulou et al., Hemasphere, in press

DNA methylation and prognosis

❖ UM-IGHV Chronic lymphocytic leukemia (CLL)

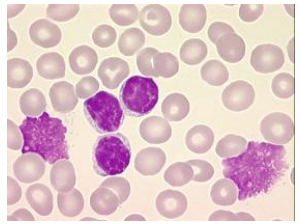




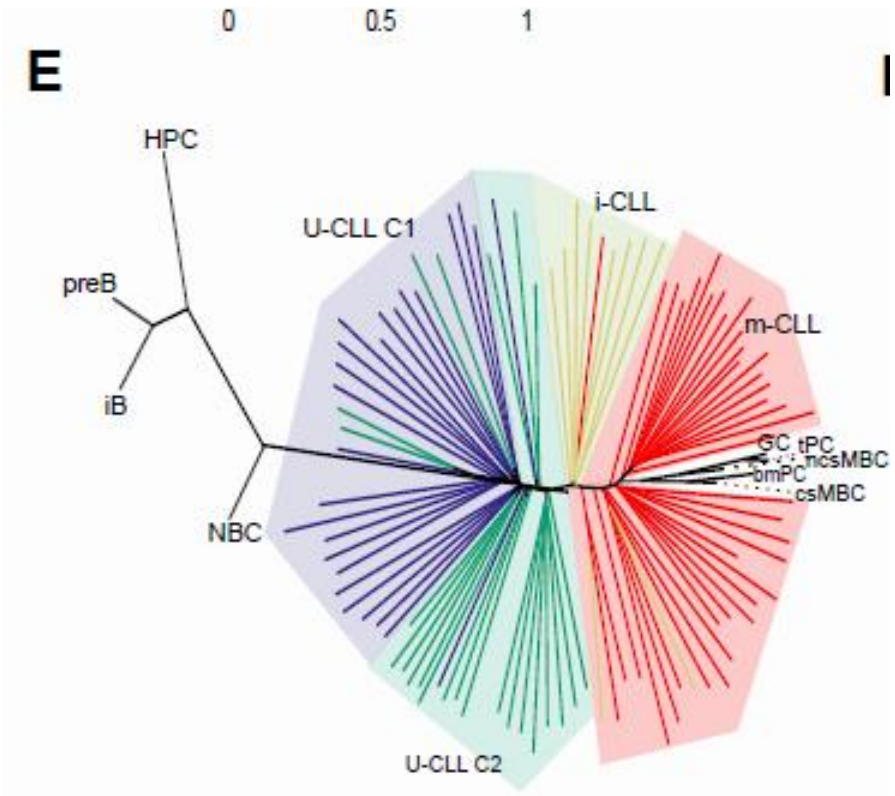


Charalampopoulou et al., Hemasphere, in press

DNA methylation and prognosis



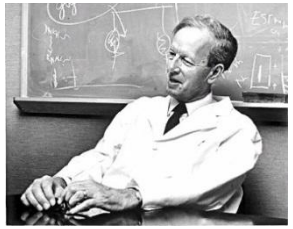
❖ UM-IGHV Chronic lymphocytic leukemia (CLL)



→ a continuum of CLL that can be segregated into 4 epitypes
→the two UM-CLL clusters: no OS or TTT differences

Charalampopoulou et al., Hemasphere, in press

DNA methylation and prognosis

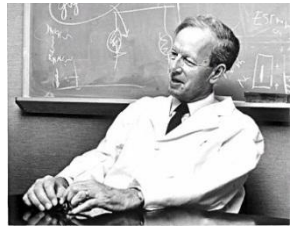


❖ Waldenström's Macroglobulinemia (WM)

Lymphoplasmacytic lymphoma with

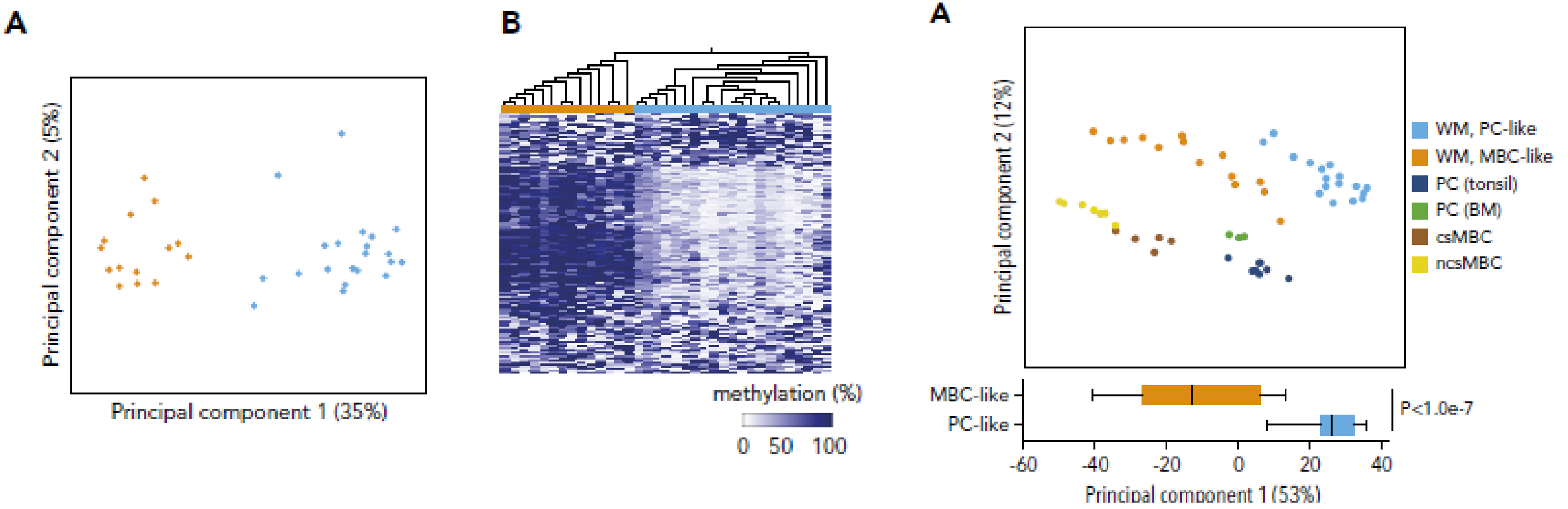
- Bone marrow involvement: small lymphocytes, plasmacytoid lymphocytes, plasma cells ; increased mast cells
- IgM monoclonal gammopathy
- 6q deletion ~50%
- MYD88 L265P > 90%, CXCR4 mut > 40%

DNA methylation and prognosis



❖ Waldenström's Macroglobulinemia (WM)

35 WM MYD88mut patients

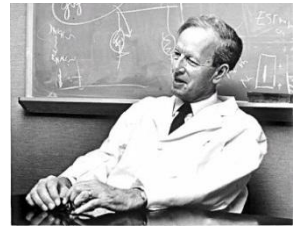


→ 2 groups with distinct methylation profiles : MBC-like and PC-like

orange: MBC (memory B-cell)-like; blue: PC (plasma cell)-like

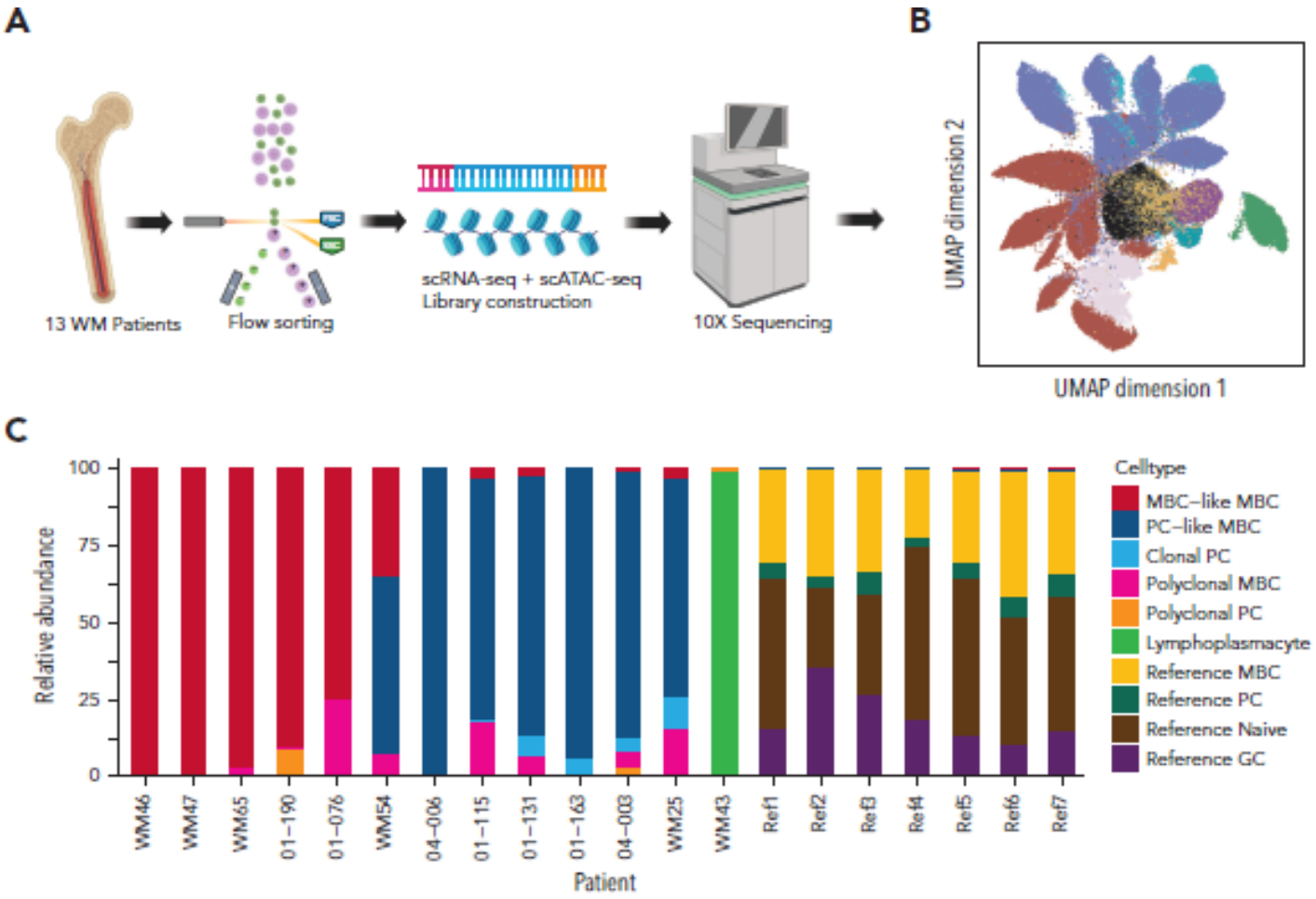
Roos-Weil et al., Blood 2020

DNA methylation and prognosis



❖ Waldenström's Macroglobulinemia (WM)

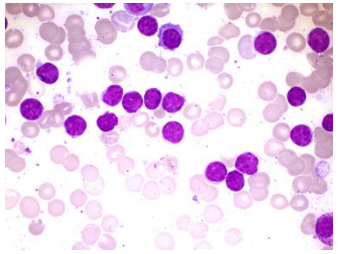
13 WM MYD88mut patients

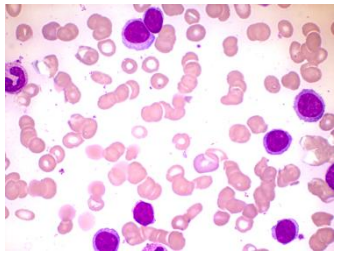


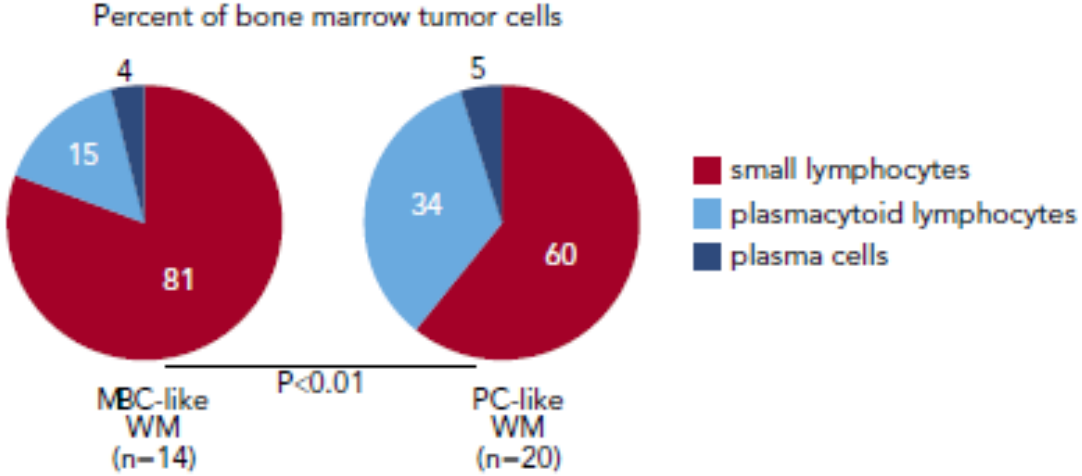
Gagler, Ghamlouch et al., Blood 2025

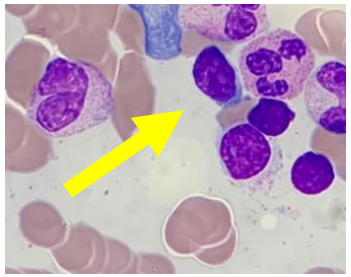
DNA methylation and prognosis

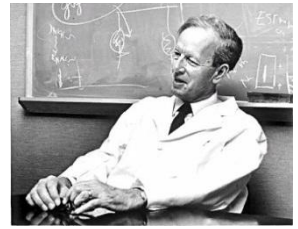
❖ Waldenström's Macroglobulinemia (WM)



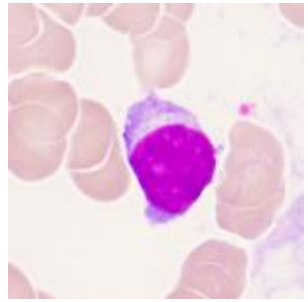




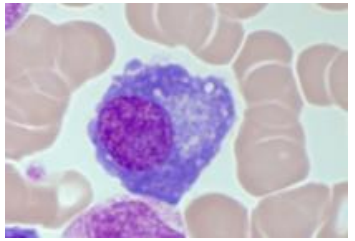




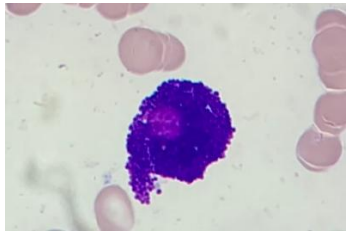
Small lymphocyte



Plasmacytoid lymphocyte



Plasma cell

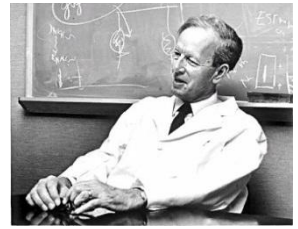


Mast cell

→WM subgroups show differential morphological features

Roos-Weil et al., Blood 2020

DNA methylation and prognosis



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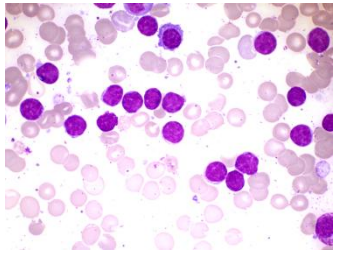
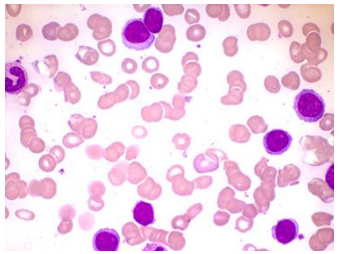
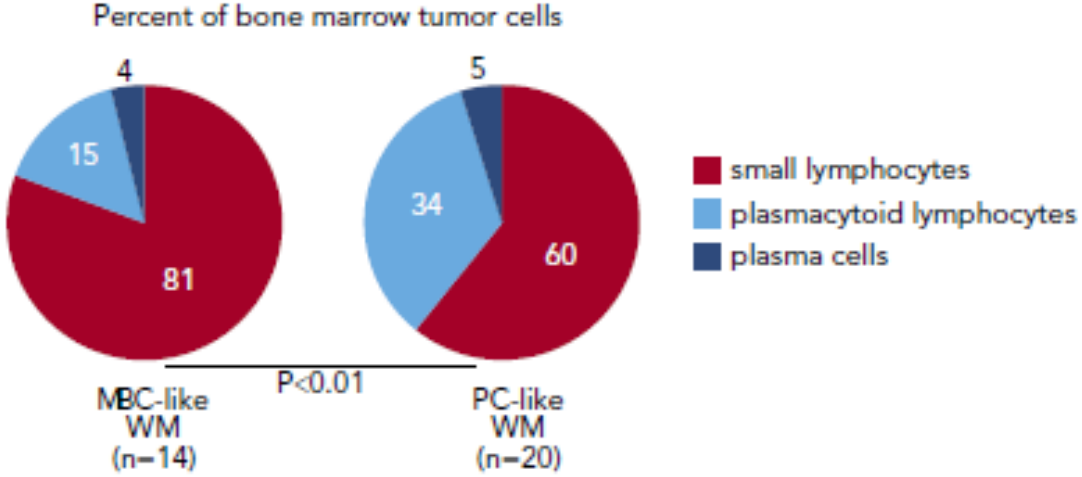
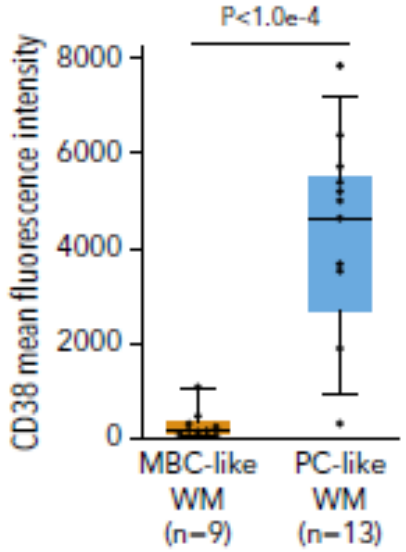




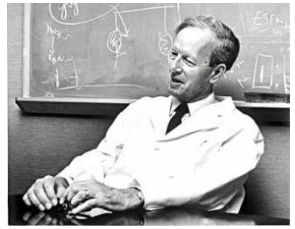




→WM subgroups show differential morphological and imunophenotypic features

Roos-Weil et al., Blood 2020

DNA methylation and prognosis



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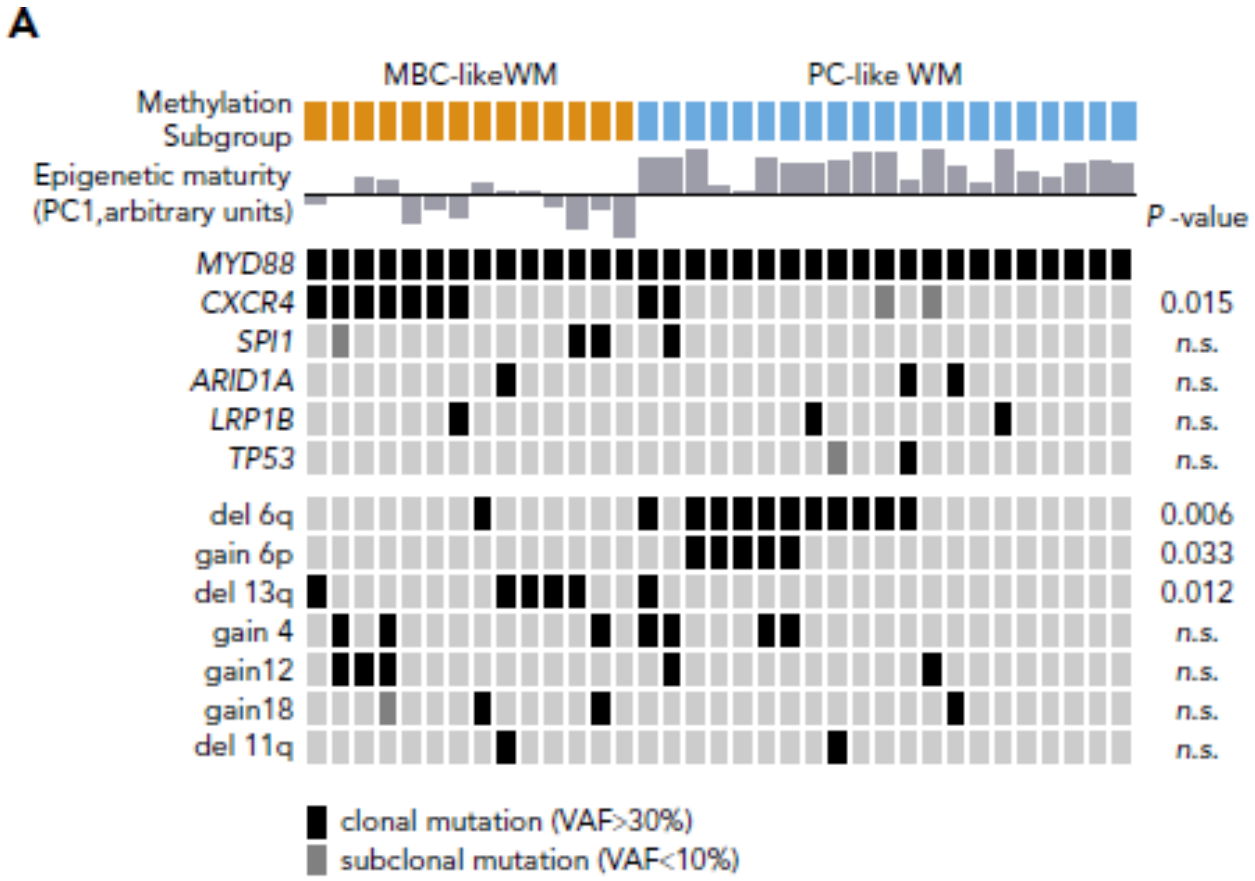
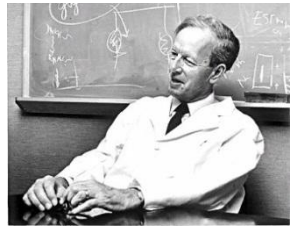


Roos-Weil et al., Blood 2020

DNA methylation and prognosis



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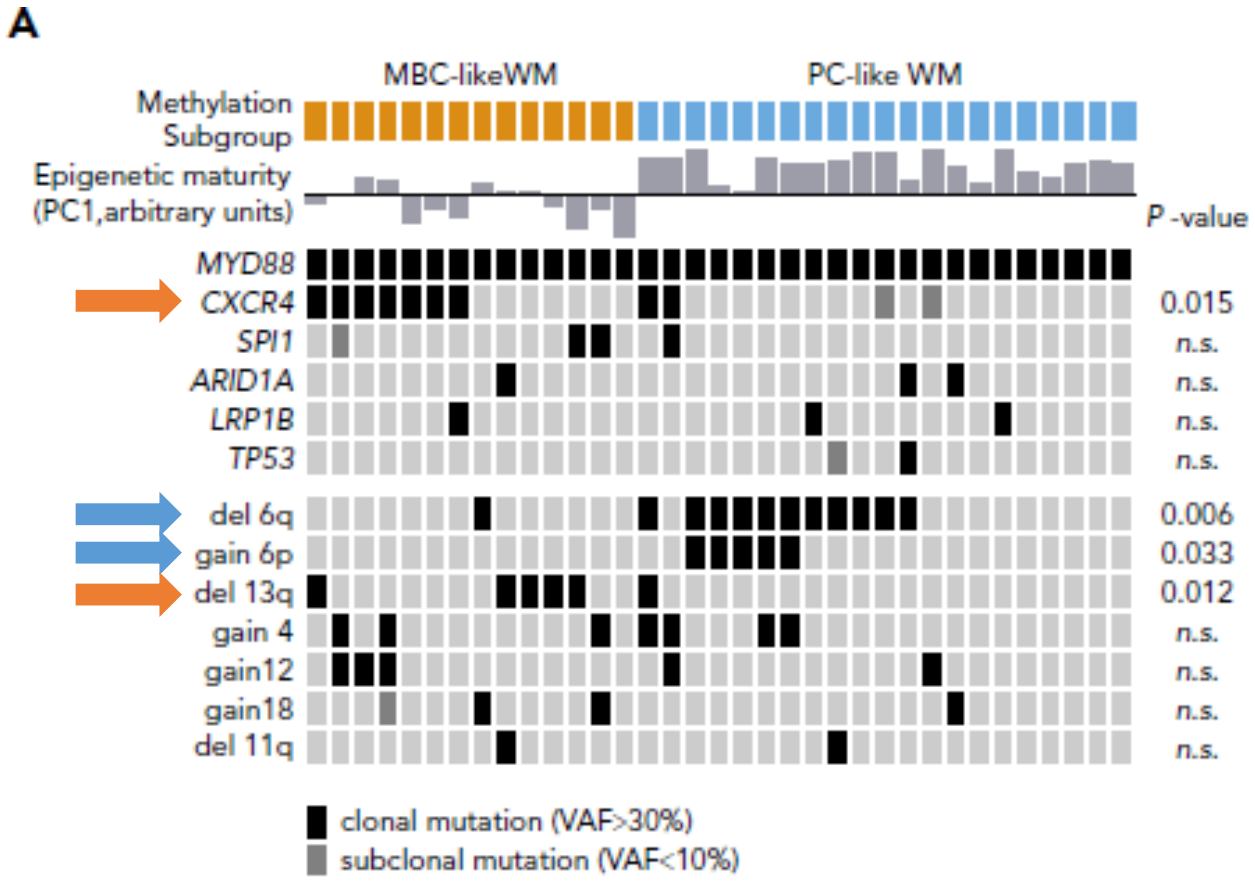
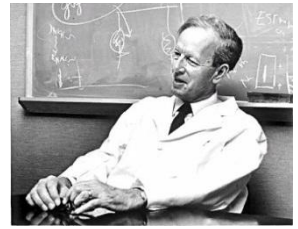


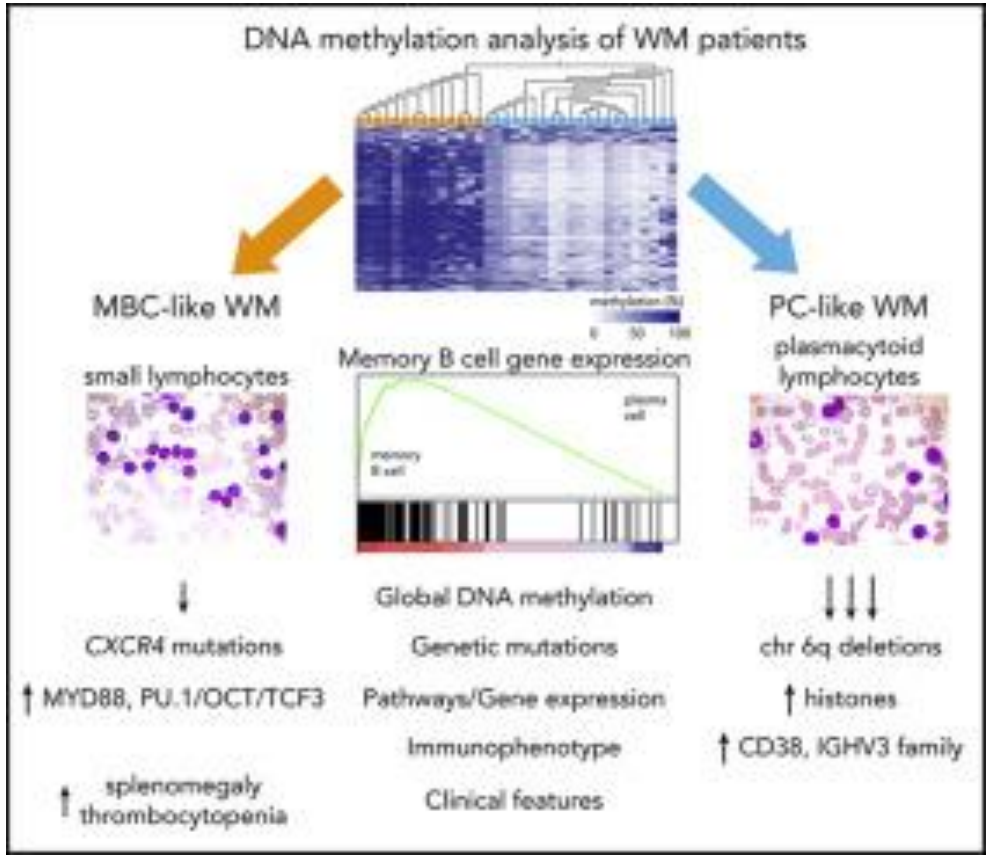
→WM subgroups show differential genomic aberrations

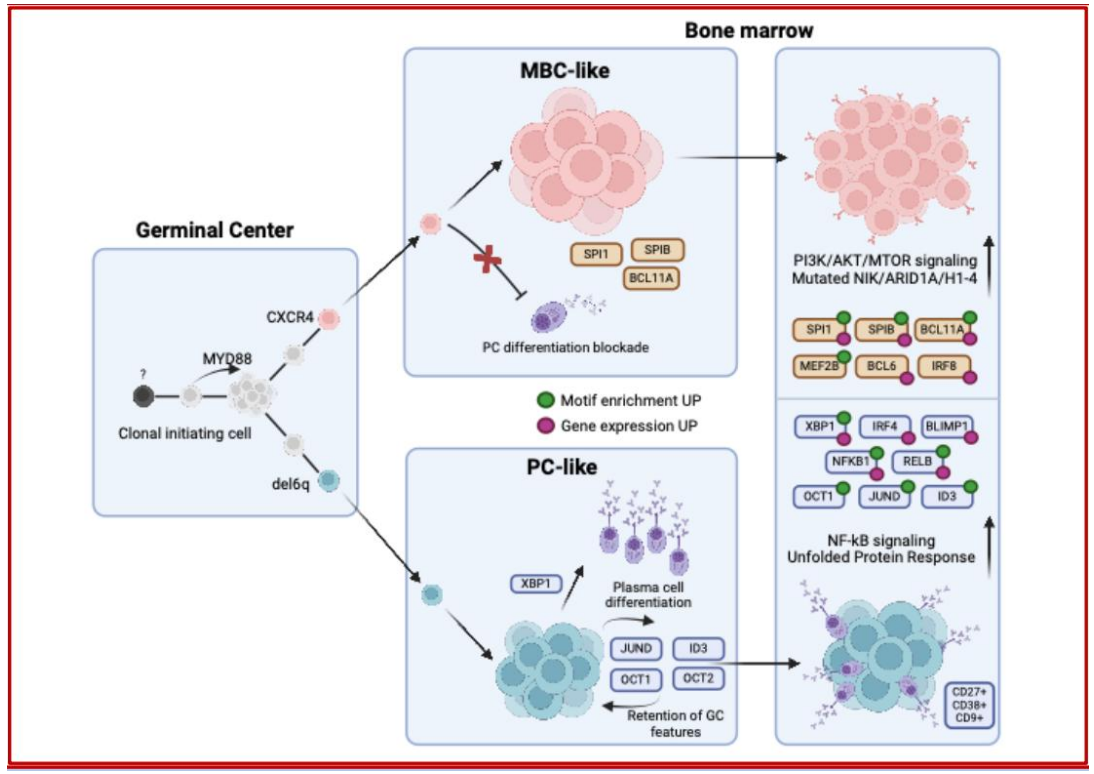
Roos-Weil et al., Blood 2020

DNA methylation and prognosis

❖ Waldenström's Macroglobulinemia (WM)





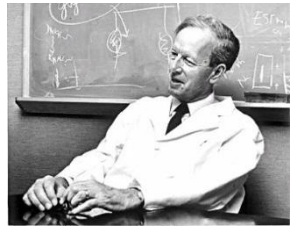


→ 2 subtypes

→Potential subtype-specific therapeutic approaches?

Roos-Weil et al., Blood 2020; Gagler, Ghamlouch et al., Blood 2025

DNA methylation and prognosis



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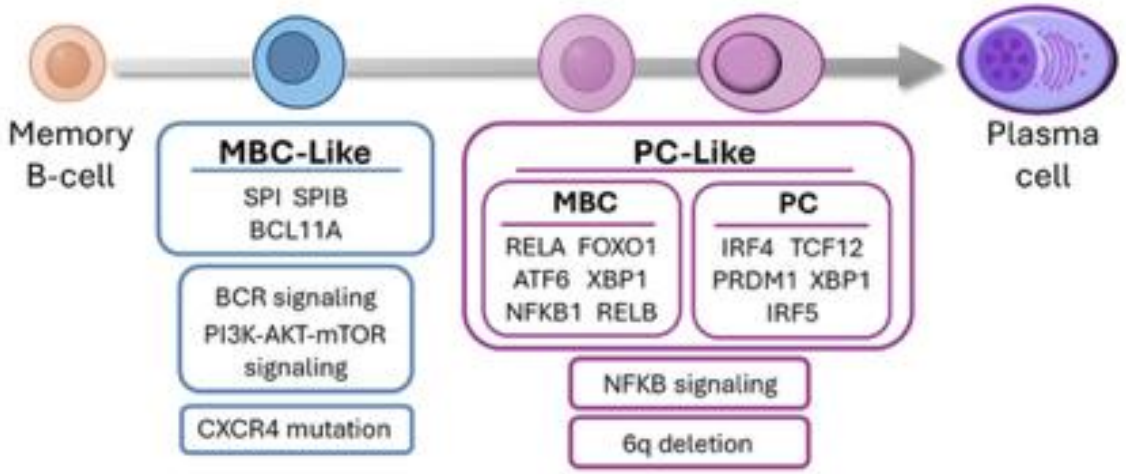

Gagler, Ghamlouch et al., Blood 2025

Fluctuating DNA methylation and prognosis

Article

Fluctuating DNA methylation tracks cancer evolution at clinical scale

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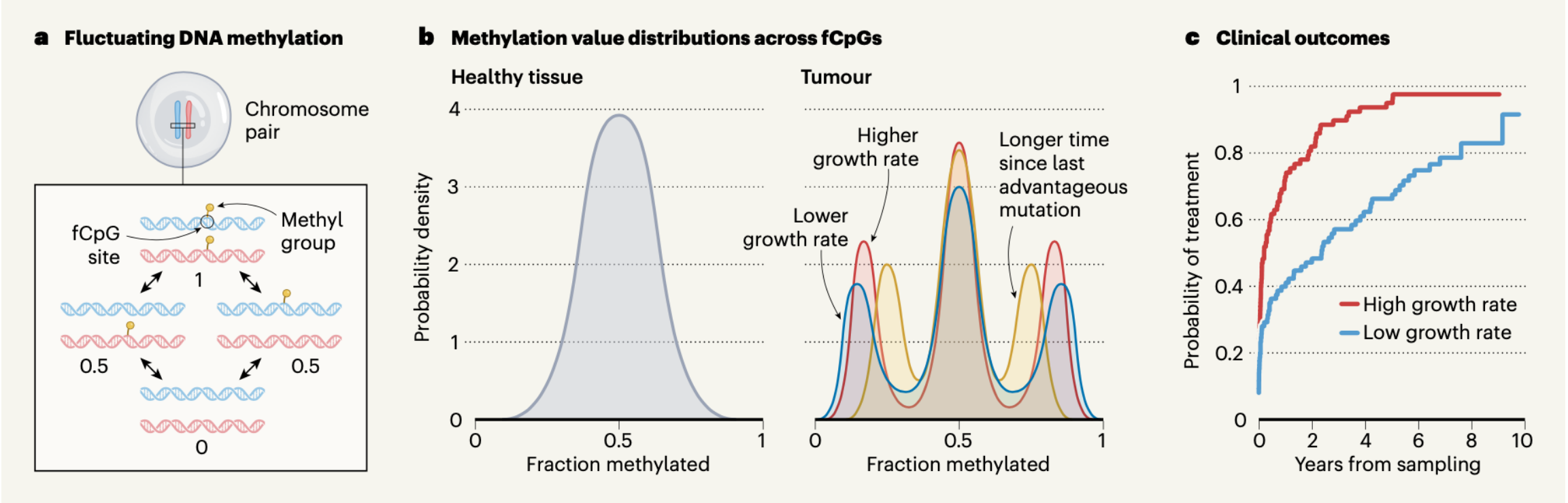
Calum Gabbutt[1,2,3,16], Martí Duran-Ferrer[4,5,16], Heather E. Grant[1], Diego Mallo[6], Ferran Nadeu[4,5], Jacob Househam[1,3], Neus Villamor[4,5,7], Madlen Müller[8], Simon Heath[8], Emanuele Raineri[8], Olga Krali[9], Jessica Nordlund[9], Thorsten Zenz[10,11], Ivo G. Gut[8,12], Elias Campo[4,5,7,12], Armando Lopez-Guillermo[4,5,7], Jude Fitzgibbon[3], Chris P. Barnes[13], Darryl Shibata[14], José I. Martin-Subero[4,5,12,15,17] & Trevor A. Graham[1,3,17]

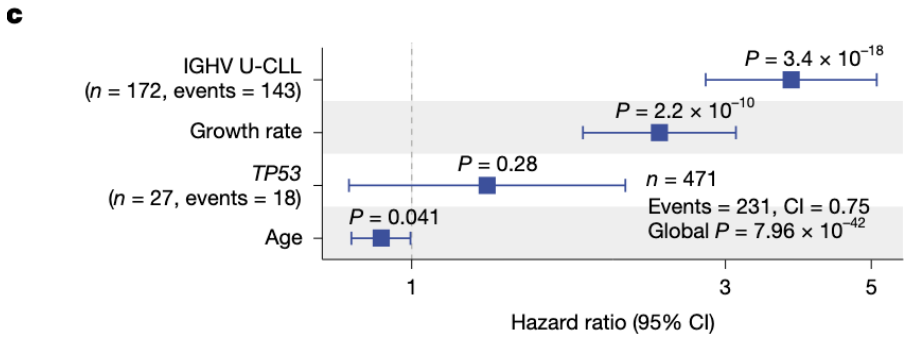
Gabbutt et al., Nature 2025

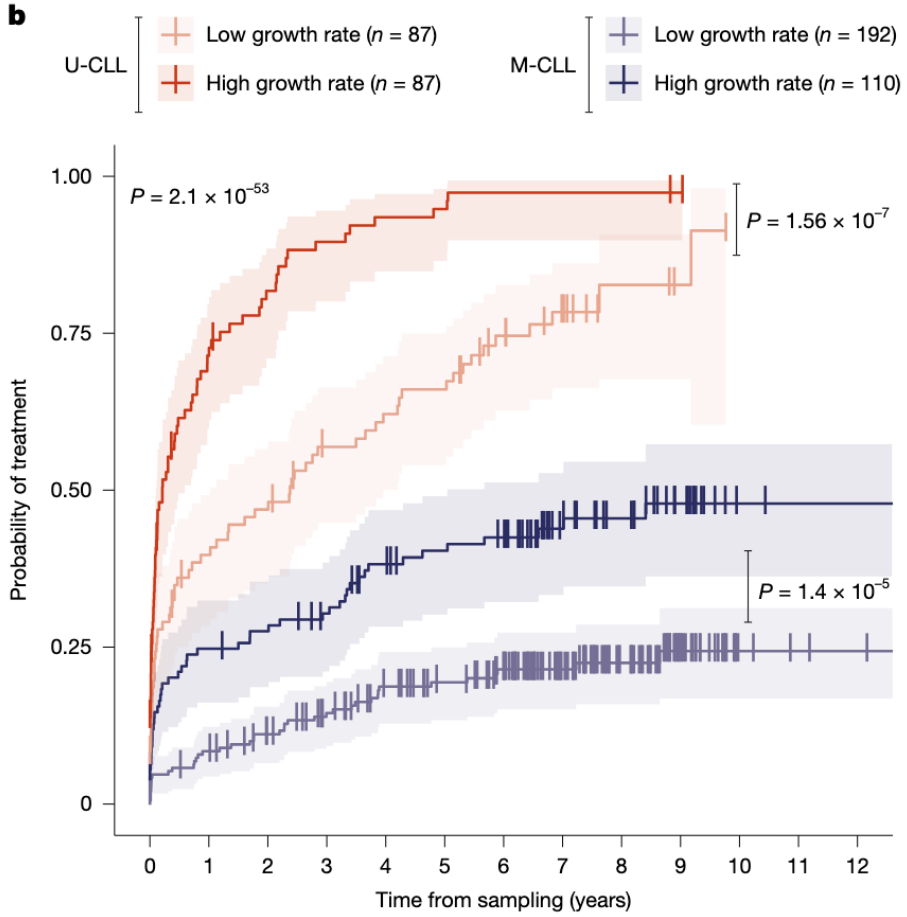


P Lutsik, News&views, Nature 2025

Fluctuating DNA methylation and prognosis

CLL





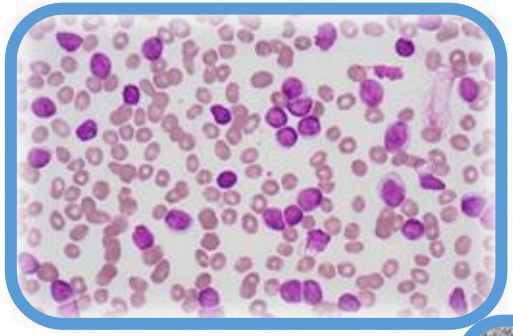
→The evolutionary history of a tumor is prognostic of clinical outcome

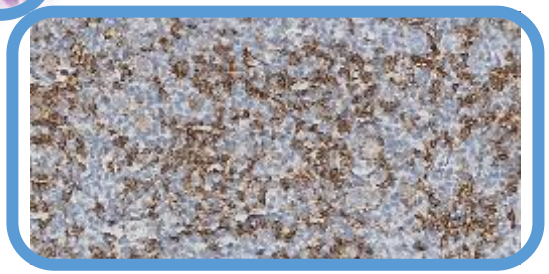
Gabbutt et al., Nature 2025

Diagnosis of Hematological Malignancies

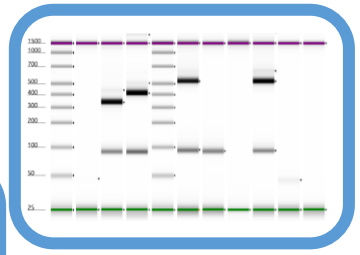
Clinical

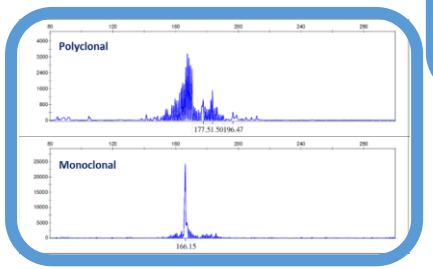
Morphology

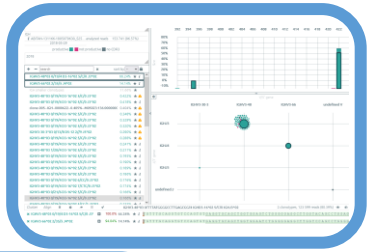


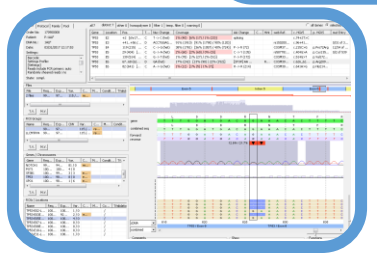


Molecular

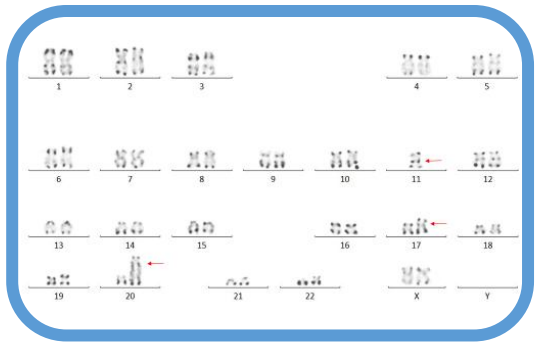


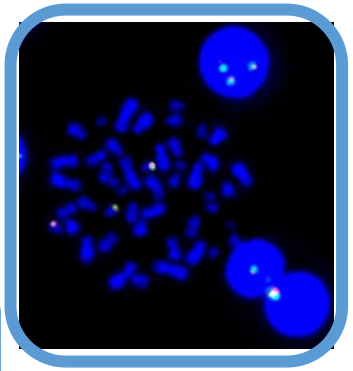


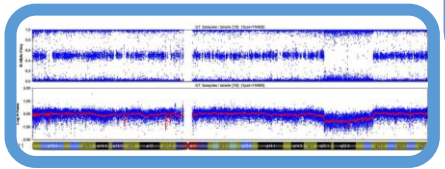


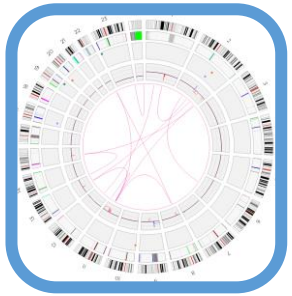


Cytogenetic

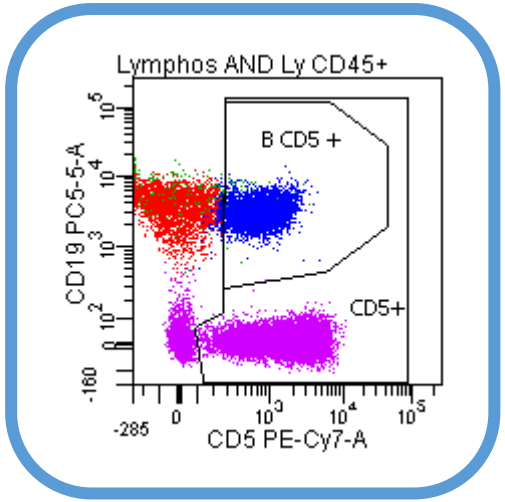






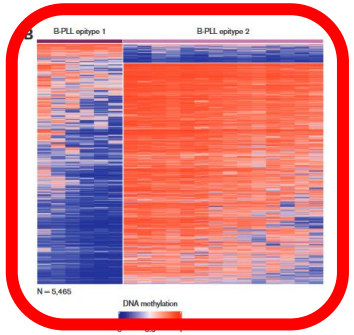


Immunophenotype



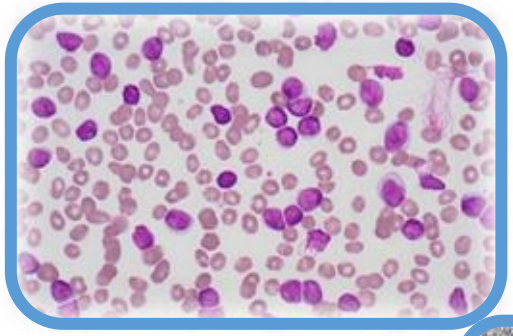
Diagnosis of Hematological Malignancies

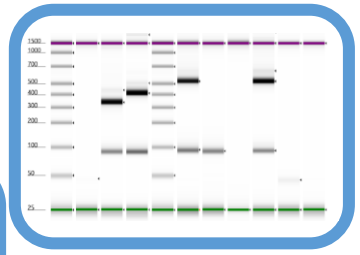
Epigenetic

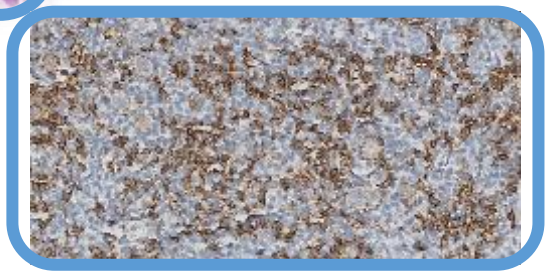


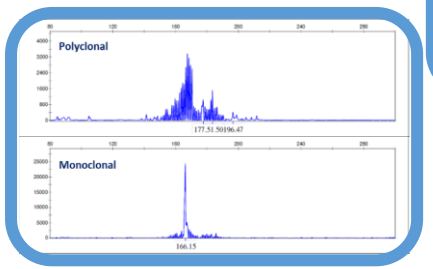
Clinical

Morphology

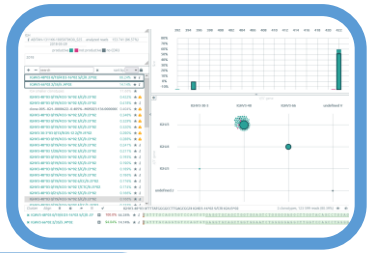


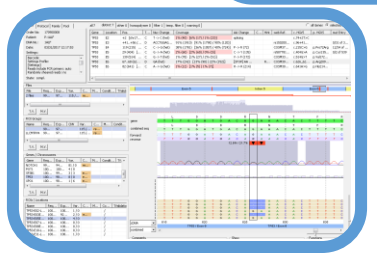




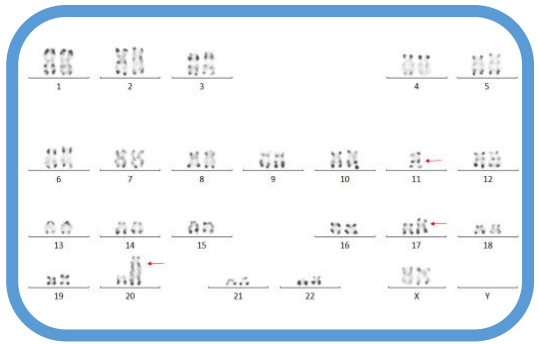


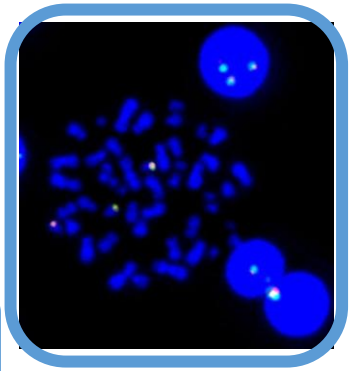
Molecular

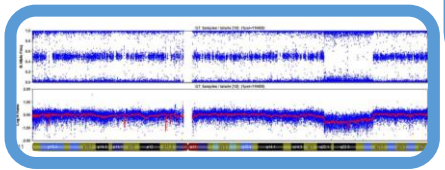


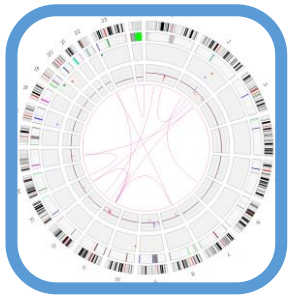


Cytogenetic

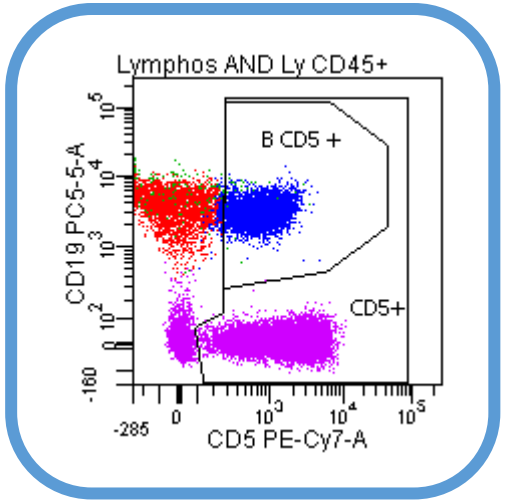








Immunophenotype



Epigenetic

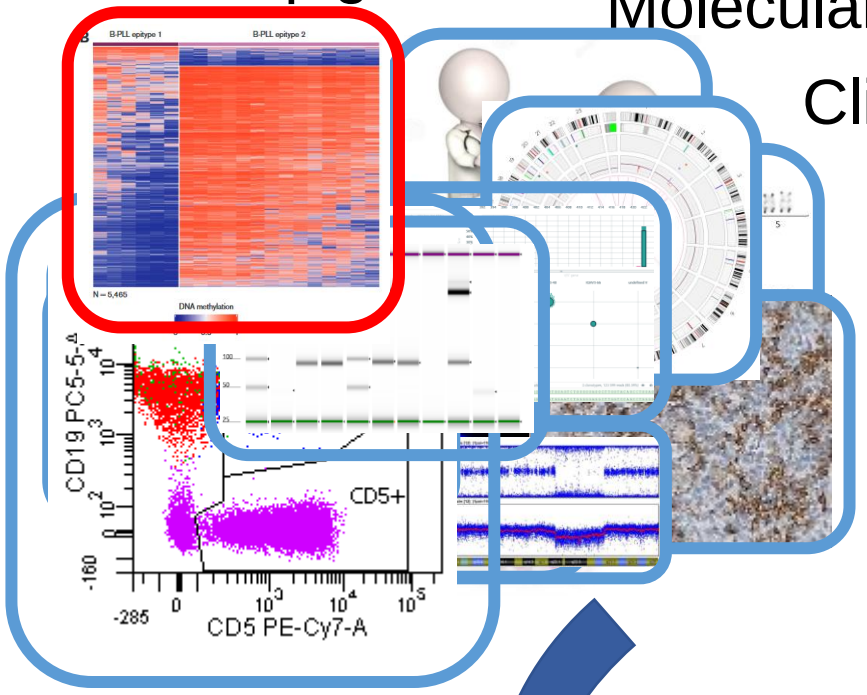
Molecular

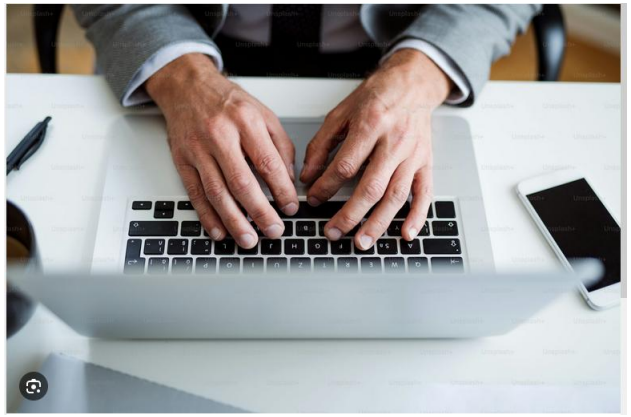
Clinical

Morphology

Immunophenotype

Cytogenetic





Diagnosis
Prognosis
Treatment

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