Pathogenesis of Follicular Lymphoma: Early steps, Genomics and Heterogeneity

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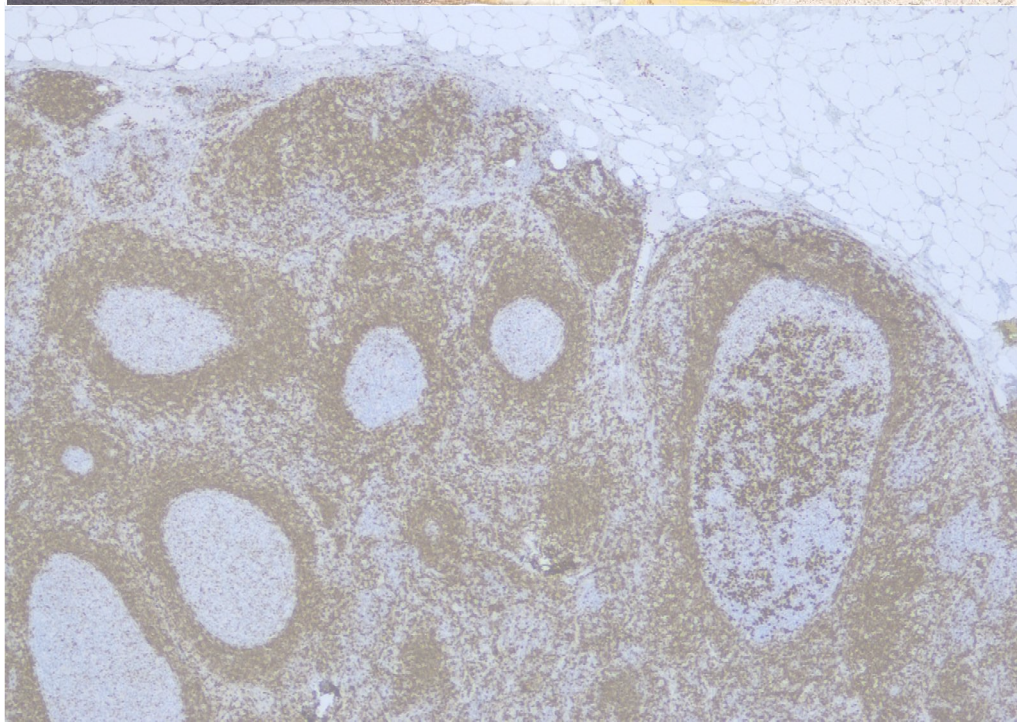
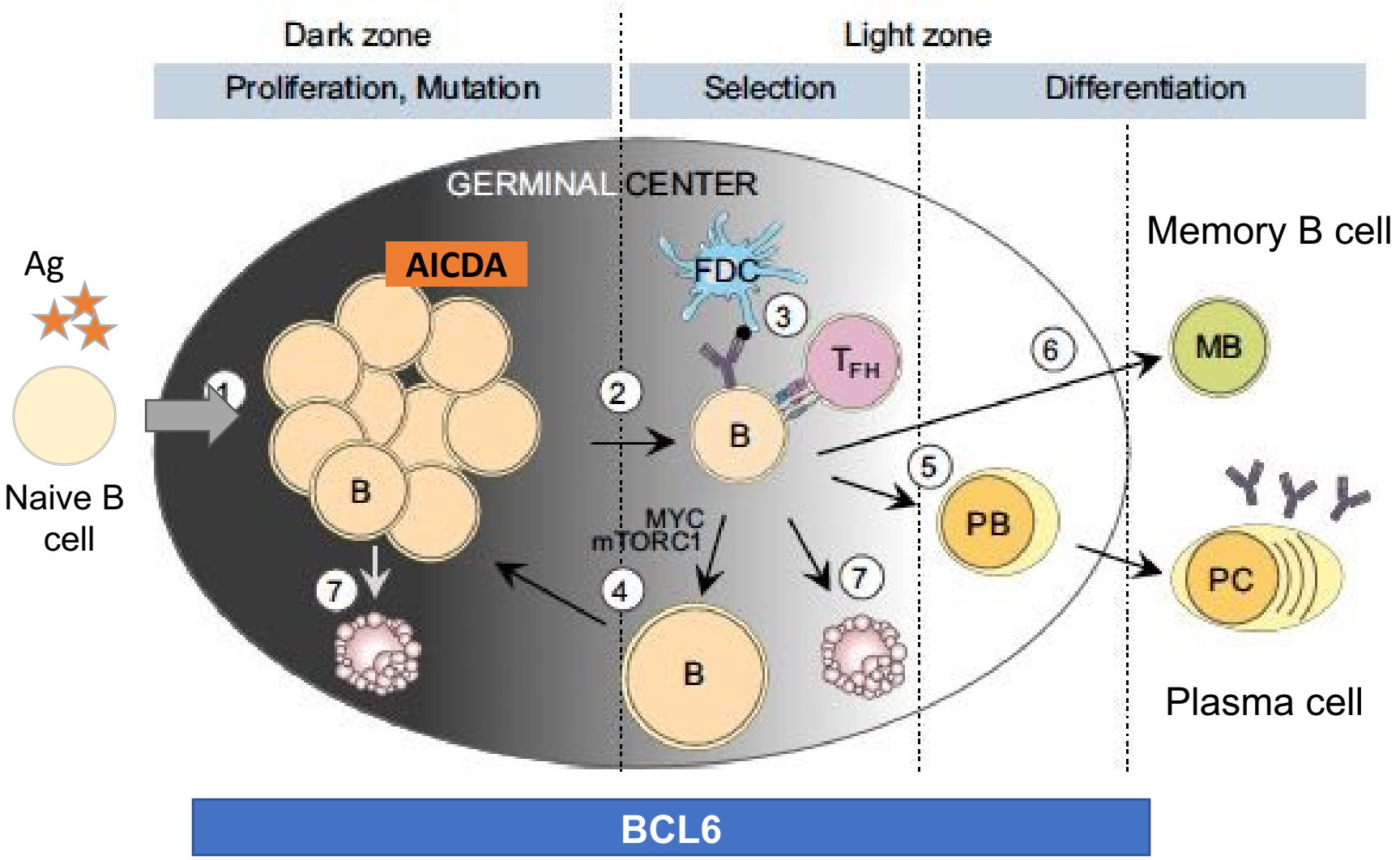
@SRoulland



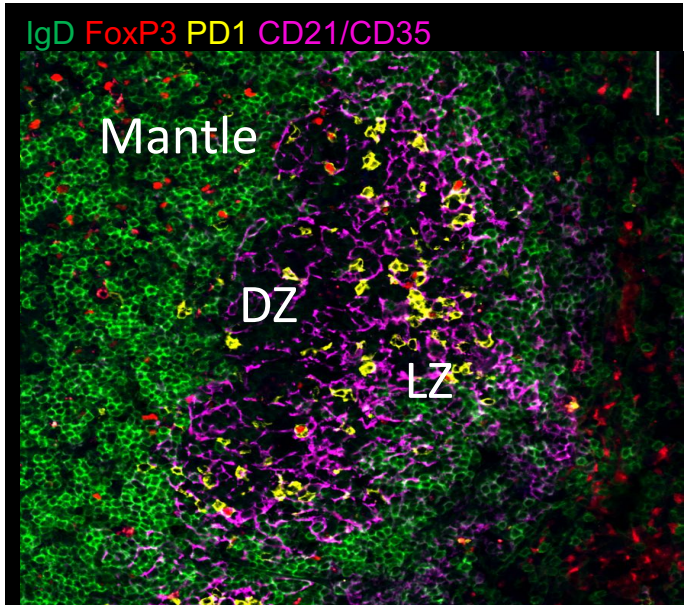
Agenda

- Reaction du Centre Germinatif
- FL : Epidemiologie, Heterogeneity clinique
- Etapes précoces du FL et Modèle de lymphomagénèse actuel
- Génomique des LF et impact sur le CG et le microenvironnement
- Evolution clonale et notion de CPC (Cancer Precuror cells)
- Que sont les CPCs et lesquels vont progresser vers le LF
- Ciblage thérapeutique de la CPC et éradication du LF ?

The Germinal Center (GC) reaction is required for Ig affinity maturation

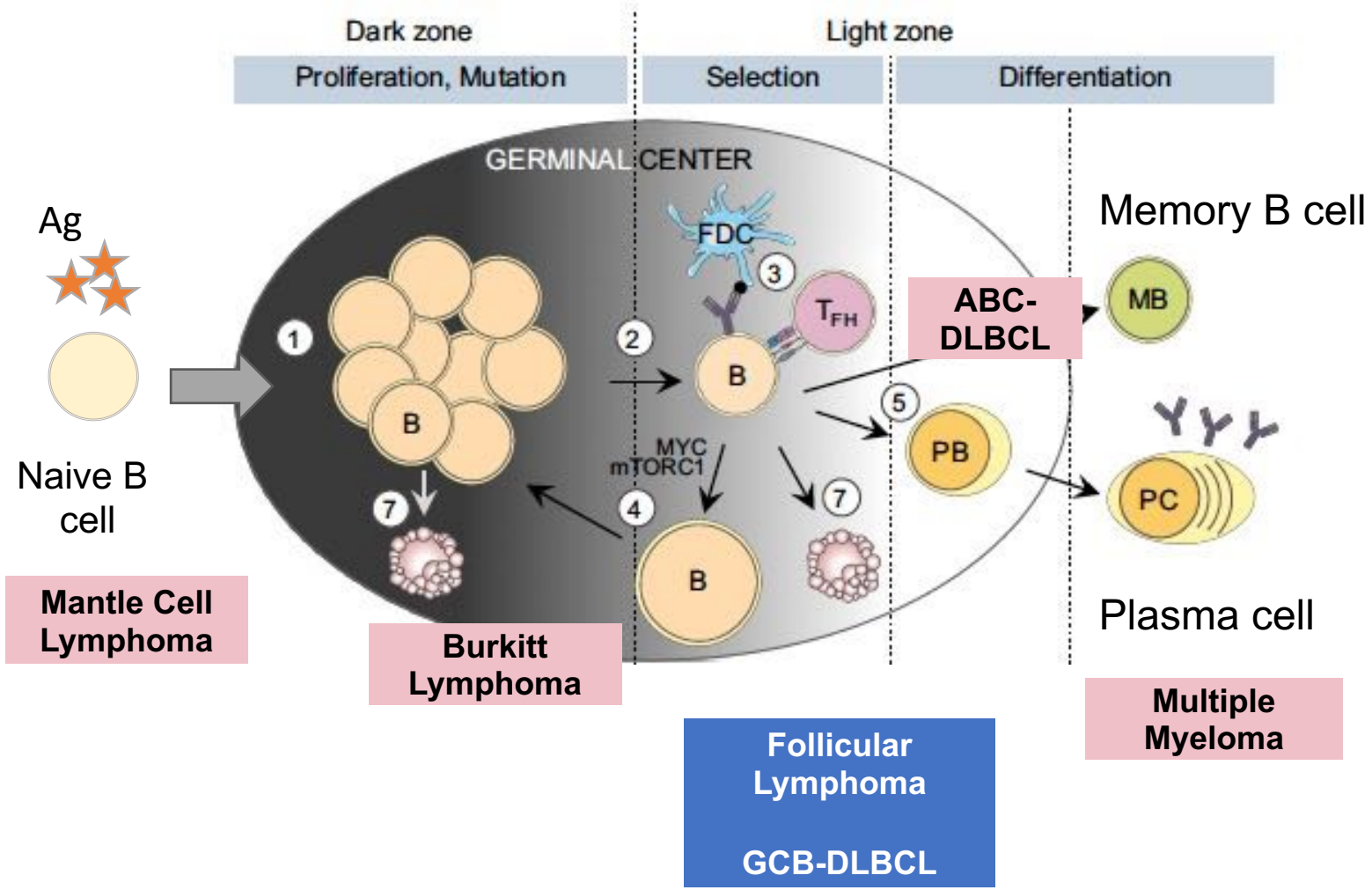


Mouse GC Bcells (Day 10 after 1st antigenic challenge)



Adapted from Hatzi, 2014

B cell lymphoma subtypes reflect the origin from B cells at different stages of B cell differentiation : The Cell of Origin (COO)

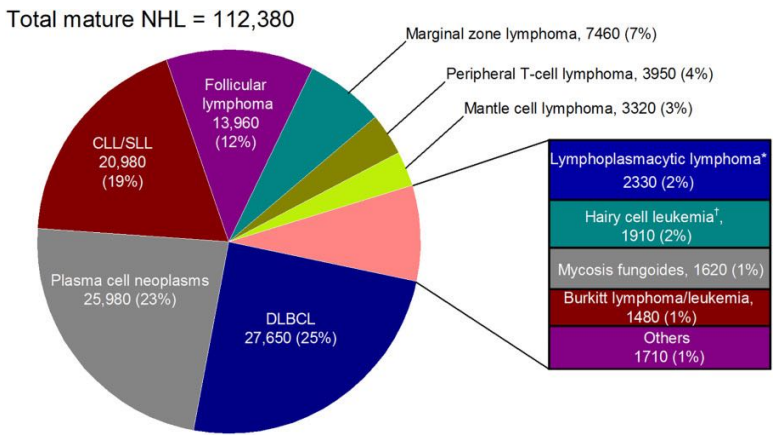


'FL is the malignant counterpart of frozen GC LZ cells '

DLBCL : Diffuse Large B Cell Lymphoma

Follicular Lymphoma: Epidemiology

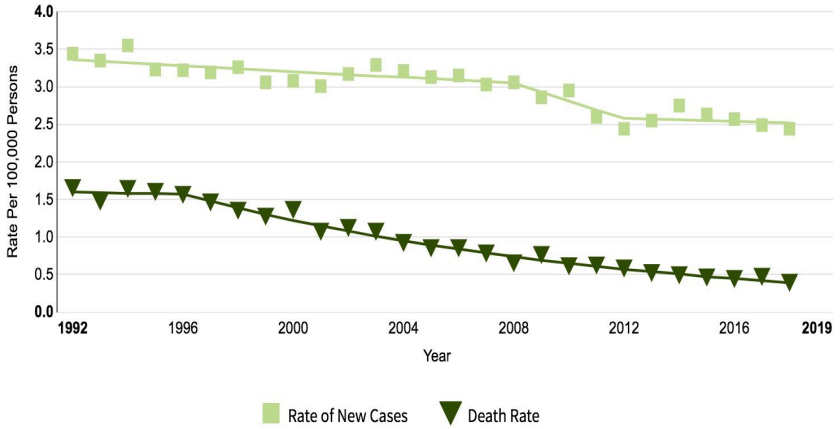
2nd most common B-NHL



Teras *et al.* CA Cancer J Clin 2016 – US registries

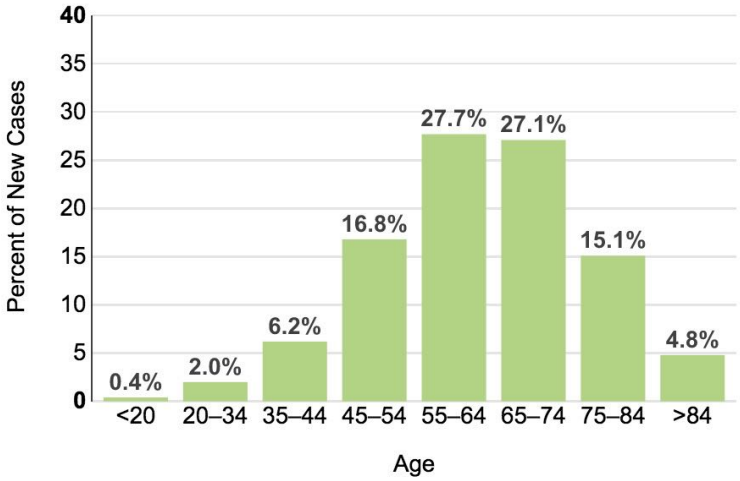
3066 cases in France (2018)

FL incidence rates (US)



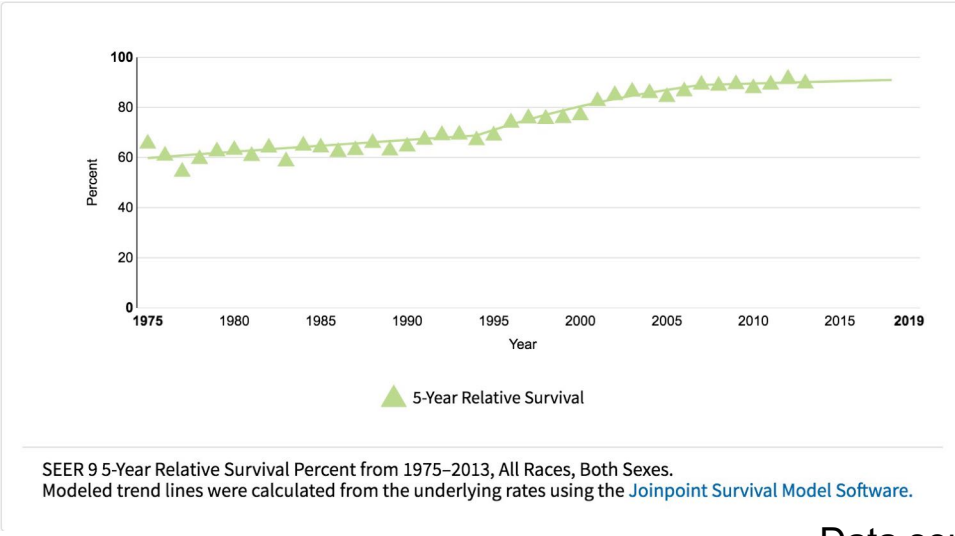
2,9 /100 000 (M)
2,0 / 100 000 (F)

Incidence rates with age (US)



Median age: env. 65 years

5-year survival rate (US)

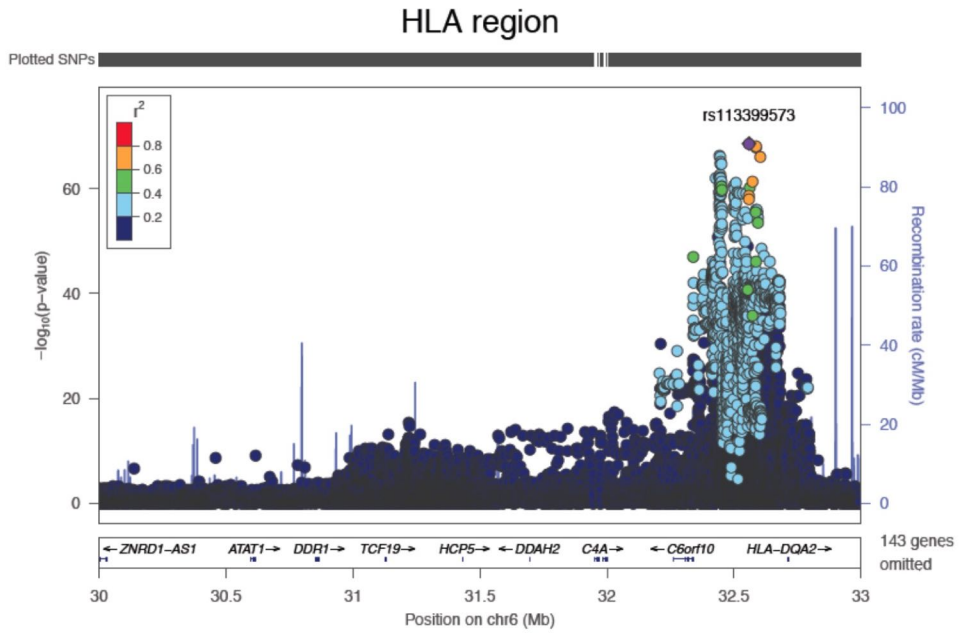


Data source, SEER, 2021

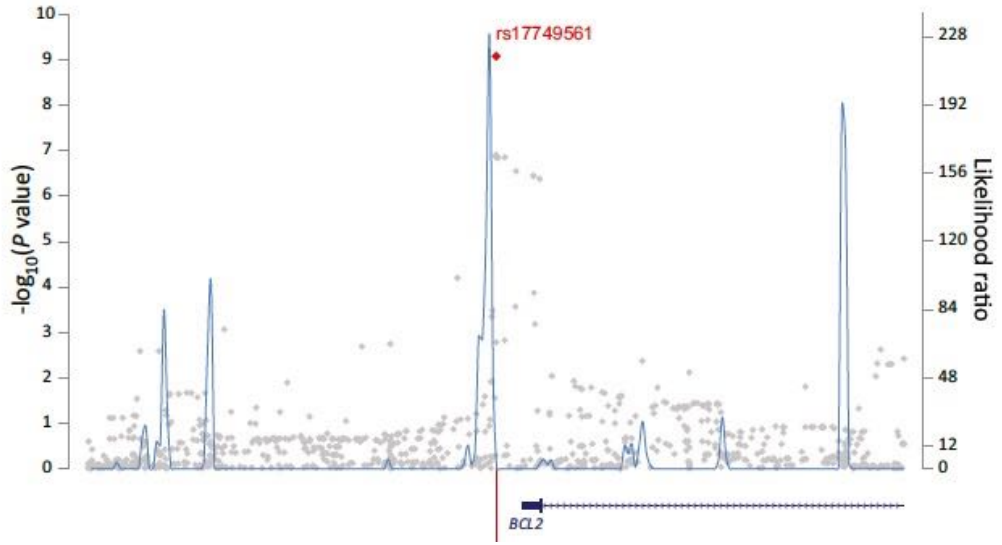
Follicular Lymphoma: Genetic Susceptibility

GWAS Study - Consortium Interlymph
4 523 cases FL & 13 344 controls

Regional plot of the HLA locus 6p21.31-33



Regional plot of the locus rs17749561 in 18q21 (near Bcl2)



At risk SNPs
HLA locus + 5 non-HLA loci (CXCR5, ETS1, LPP, PVT1, BCL2)

Skibola CF, Am J Hum Genet., 2014
Skibola CF, Nat Genet, 2010

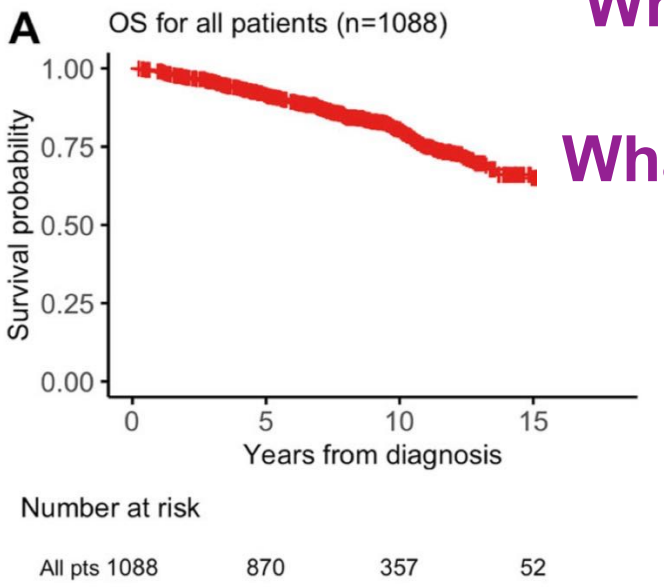
Follicular Lymphoma : Clinical Heterogeneity & Challenges

ARTICLE Open Access

Follicular lymphoma in the modern era: survival, treatment outcomes, and identification of high-risk subgroups

Connie L. Batlevi[1], Fushen Sha[1], Anna Alperovich[1], Ai Ni[2,3], Katy Smith[1,4], Zhitao Ying[1,5], Jacob D. Soumerai[1,6], Philip C. Caron[1], Lorenzo Falchi[1], Audrey Hamilton[1], Paul A. Hamlin[1], Steven M. Horwitz[1], Erel Joffe[1], Anita Kumar[1], Matthew J. Matasar[1], Alison J. Moskowitz[1], Craig H. Moskowitz[1,7], Ariela Noy[1], Colette Owens[1], Lia M. Palomba[1], David Straus[1], Gottfried von Keudell[1], Andrew D. Zelenetz[1], Venkatraman E. Seshan[2] and Anas Younes[1]

MSKCC newly diagnosed FL patients (2008-2018)

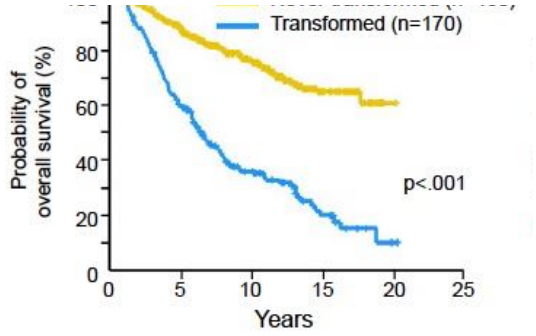


Batlevi C, Blood Cancer J, 2021

- **Mostly indolent clinical course and sensitive to therapies (R-CHOP era)**
- **Disease punctuated by relapse –remitting pattern (80%)**
- **Subset of High risk FL (20%)**

Why patients systematically relapse ?

What are the biological determinants of this clinical heterogeneity ?



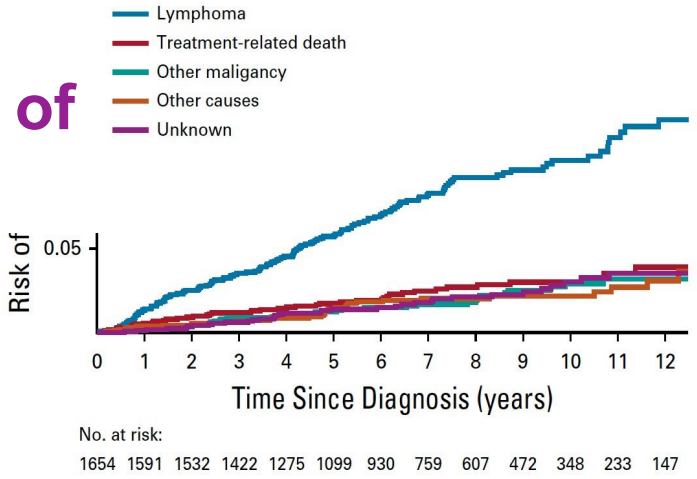
Casulo C, JCO, 2015

original report

Cause of Death in Follicular Lymphoma in the First Decade of the Rituximab Era: A Pooled Analysis of French and US Cohorts

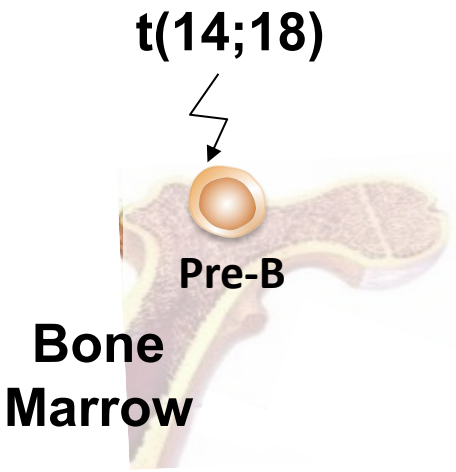
Clémentine Sarkozy, MD[1]; Matthew J. Maurer, MS[2]; Brian K. Link, MD[3]; Hervé Ghesquieres, MD, PhD[1]; Emmanuelle Nicolas, MD[4]; Carrie A. Thompson, MD[2]; Alexandra Traverse-Glehen[1]; Andrew L. Feldman, MD[2]; Cristine Allmer[2]; Susan L. Slager[2]; Stephen M. Ansell, MD, PhD[2]; Thomas M. Habermann, MD[2]; Emmanuel Bachy[1]; James R. Cerhan, MD, PhD[2]; and Gilles Salles, MD, PhD[1]

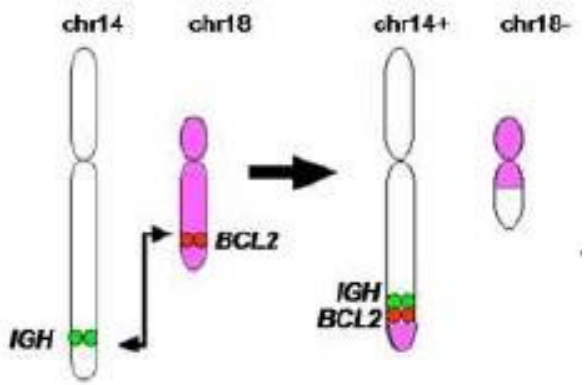
Causes of Death (COD) FL patients (n=1654 pts)



Sarkozy C, J Clin Oncol, 2020

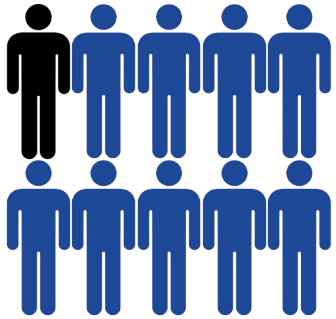
t(14;18) is the primary genetic hit of FL

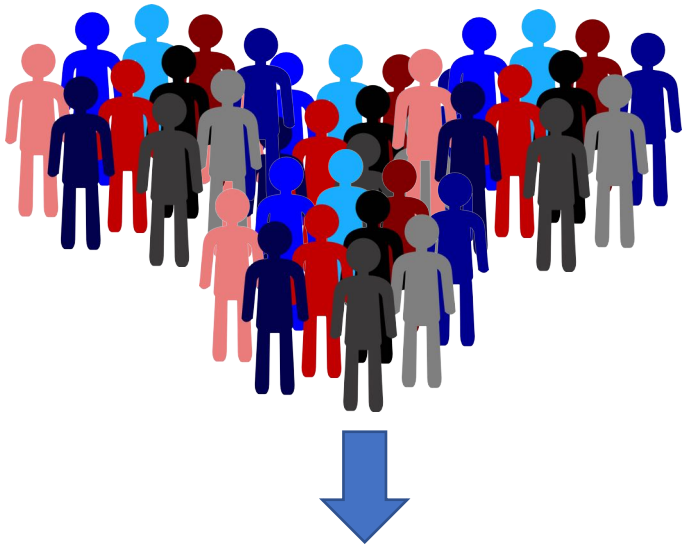




> Ectopic expression of the anti-apoptotic BCL2

90% of FL have the t(14;18)





- **70% of healthy individuals carry circulating t(14;18)$^{+}$ cells at low frequency (1 / 1 000 000)**

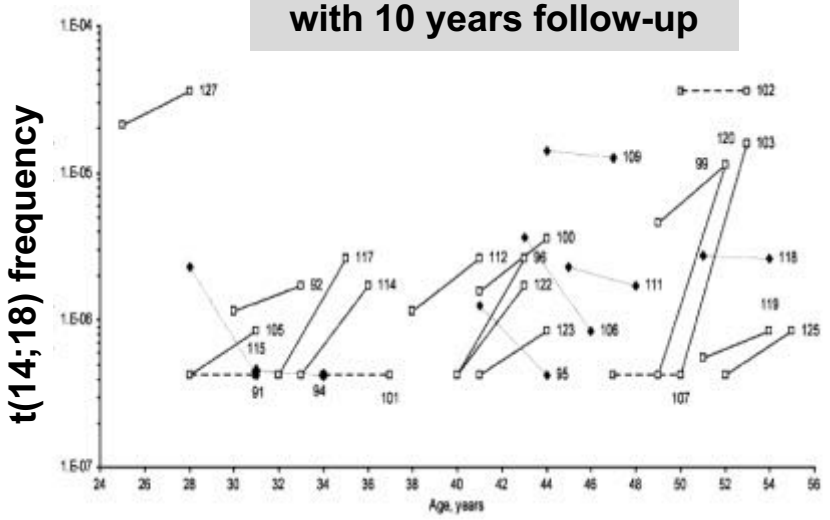
→ BCL2 'weak' driver: not sufficient to trigger malignant progression

→ suggest the notions of asymptomatic carriers

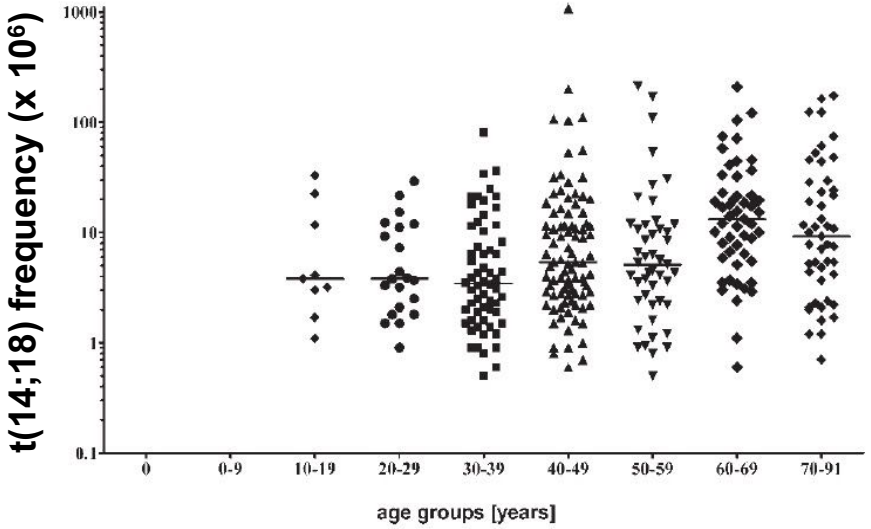
→ Cancer Precursor Cells (CPC)

t(14;18) important but not sufficient for FL development

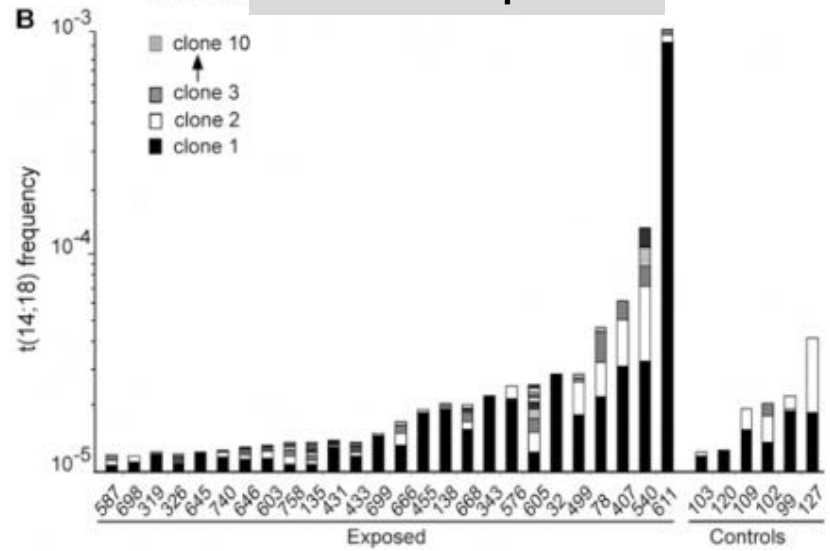
Persistence/increase with 10 years follow-up



Increase with age



Pesticide exposure

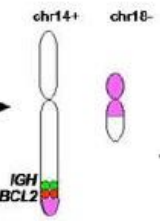


→ **Increase with conditions linked to FL progression (aging, occupational exposure..)**

→ **Long process requiring niches and clonal expansion**

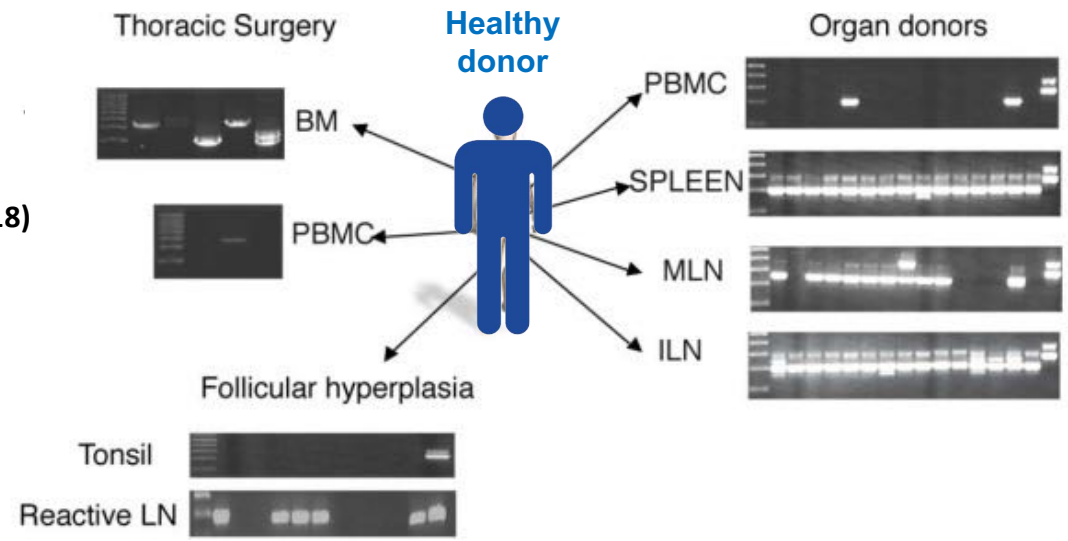
Roulland Int J Cancer 2003
Roulland Leukemia 2006
Schüler Int.J.Cancer 2009
Agopian J Exp Med 2009

Defining t(14;18)+ in healthy individuals : long-living with FL-like features



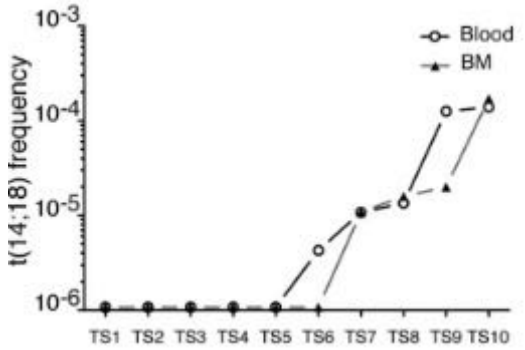
PCR t(14;18)

Early dissemination and early niches

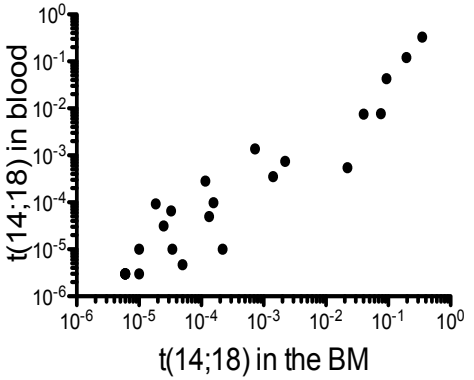


Bone marrow colonization

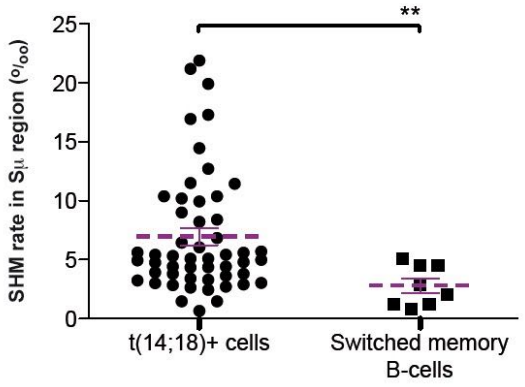
Healthy individuals

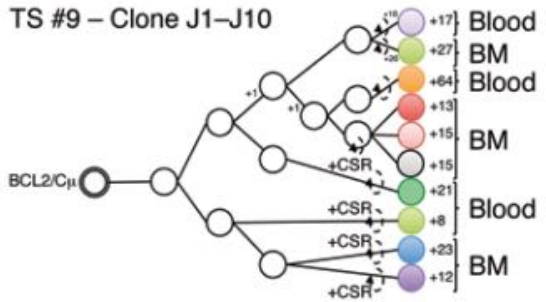


Low-mass FL



Clonal expansion and genomic instability



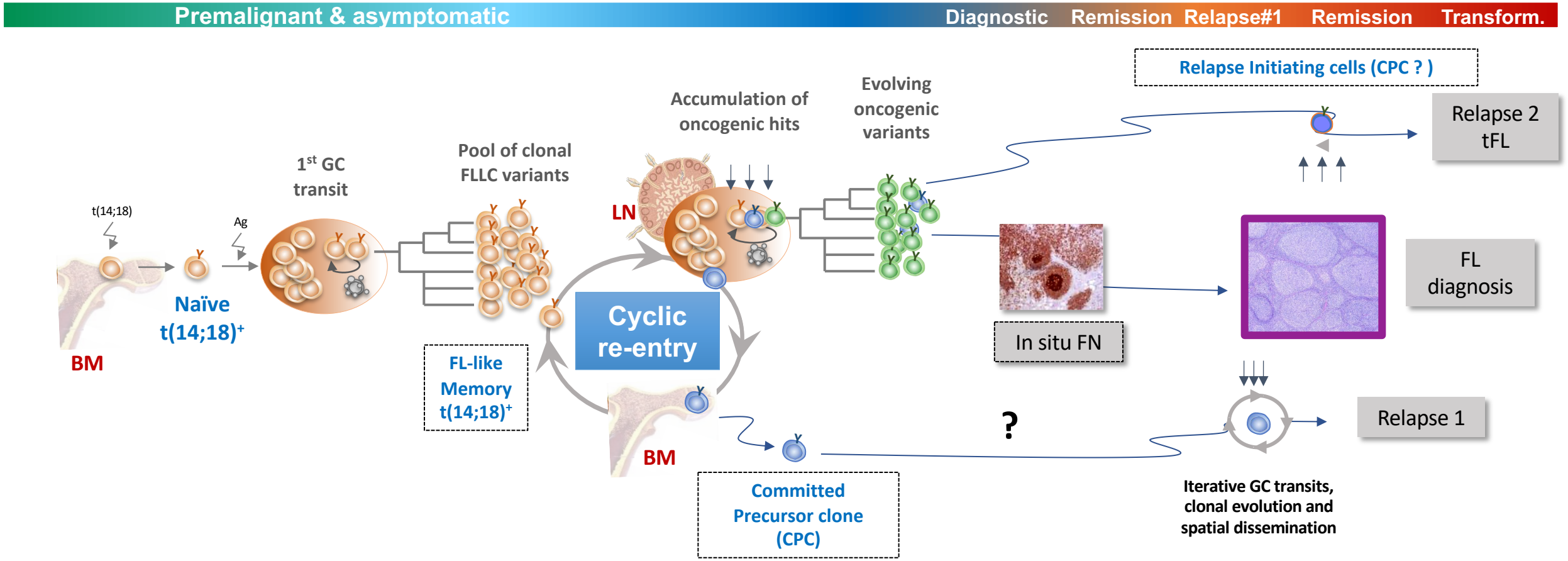


Features of FL-like cells

- ✓ GC/memory-like phenotype : **AID⁺CD10⁺BCL6⁺BCL2⁺ CD27+**
- ✓ Non-proliferating (CXCR4-Ki67-) in line with centrocyte COO
- ✓ Increased genomic instability (more SNVs)
- ✓ Extensive dissemination in remote organs
- ✓ **Bone Marrow niche**

Roulland, J Exp Med, 2006; 2009; Sungalee, J Clin Invest, 2014; Tellier et al. Blood, 2014

A model for how lymphomas arise from the normal immune response over decades of B cell reactivation



Roulland et al. *J Exp Med*, 2006
Agopian et al. *J Exp Med* 2009
Roulland et al. *J Clin Oncol* 2014
Sungalee et al. *J Clin Invest* 2014
Mamessier et al. *Haematologica* 2015
Carbone, Roulland et al. *Nat Rev Primers*, 2020
Milpied et al. *Adv Immunol*, 2021

Open questions

FL lymphomagenesis generates complex intra-tumoral heterogeneity which may represent an obstacle to patient stratification and cure

Besides t(14;18), what are the additional driver genetic events that lead to the emergence of the FL dominant clones ?

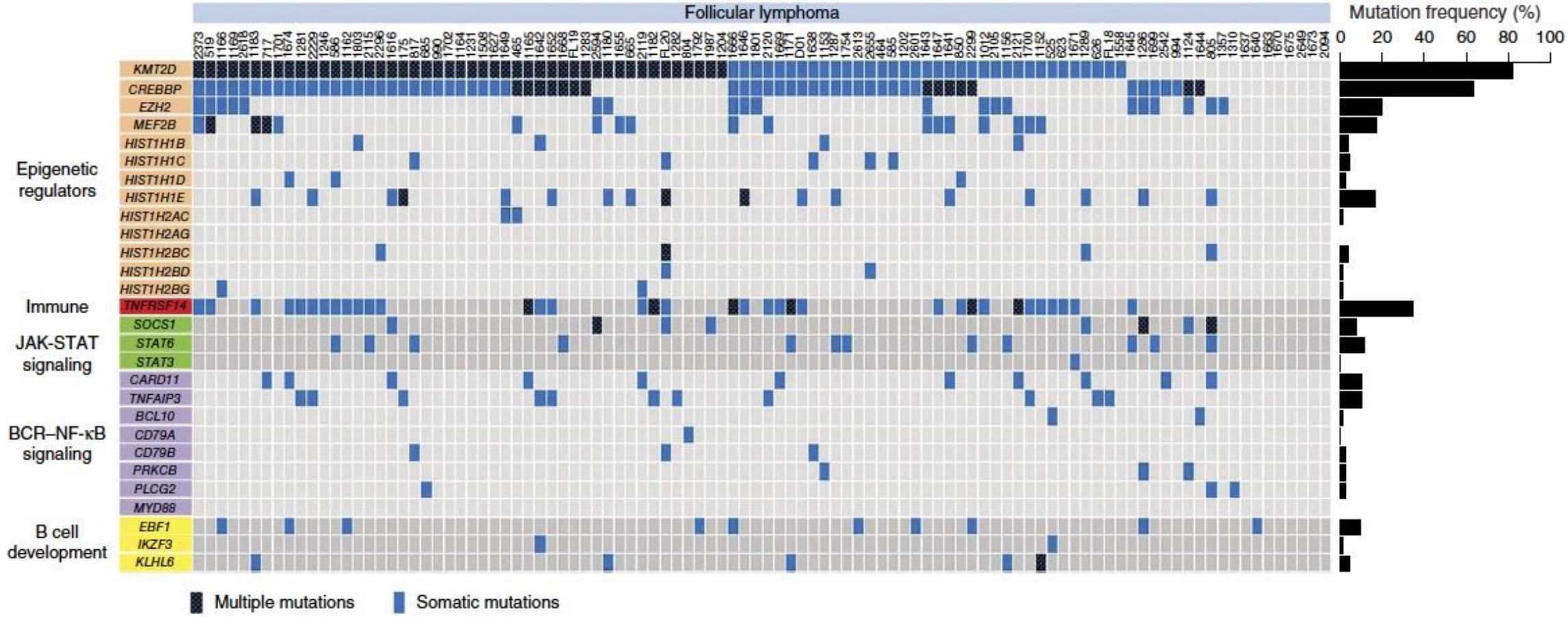
What is the functional impact of genetic alterations on the dynamics of germinal center B cells, immune response and surrounding environment ?

What can we learn from t(14;18) + precursors , what are the different CPC flavors and which one give rise to follicular lymphomas and subsequent relapses ?

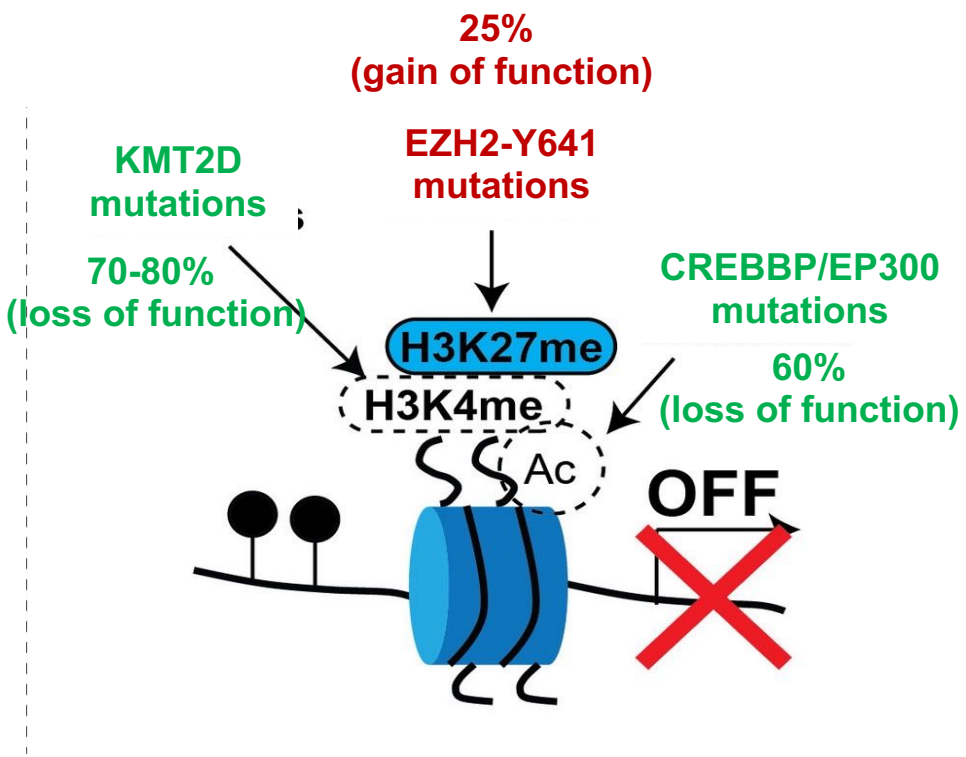
Can we use these CPC molecular insights to propose novel therapeutics to target FL?

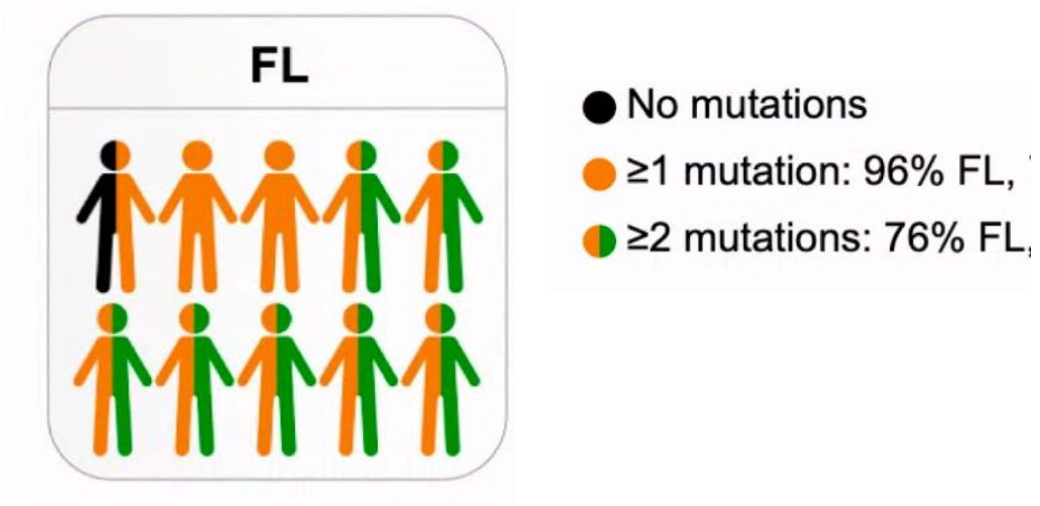
The mutational landscape of FL dominated by epigenetic mutations

> 1000 patients with WES (> 100 mutations/patient)



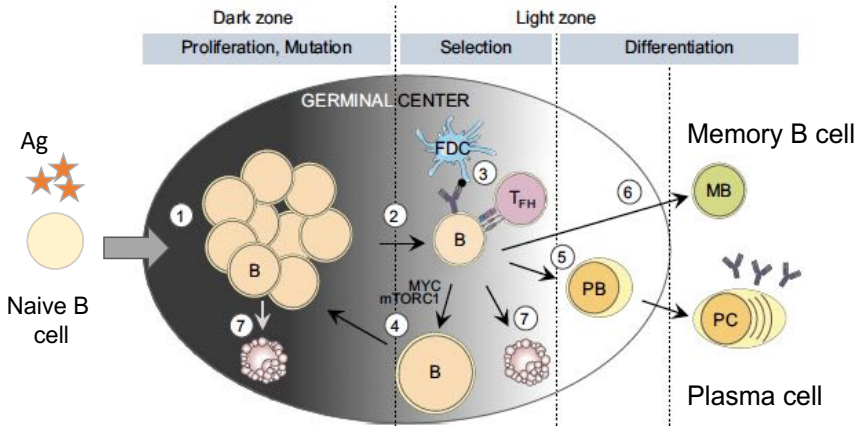
Epigenetic regulators are virtually mutated in all FL patients





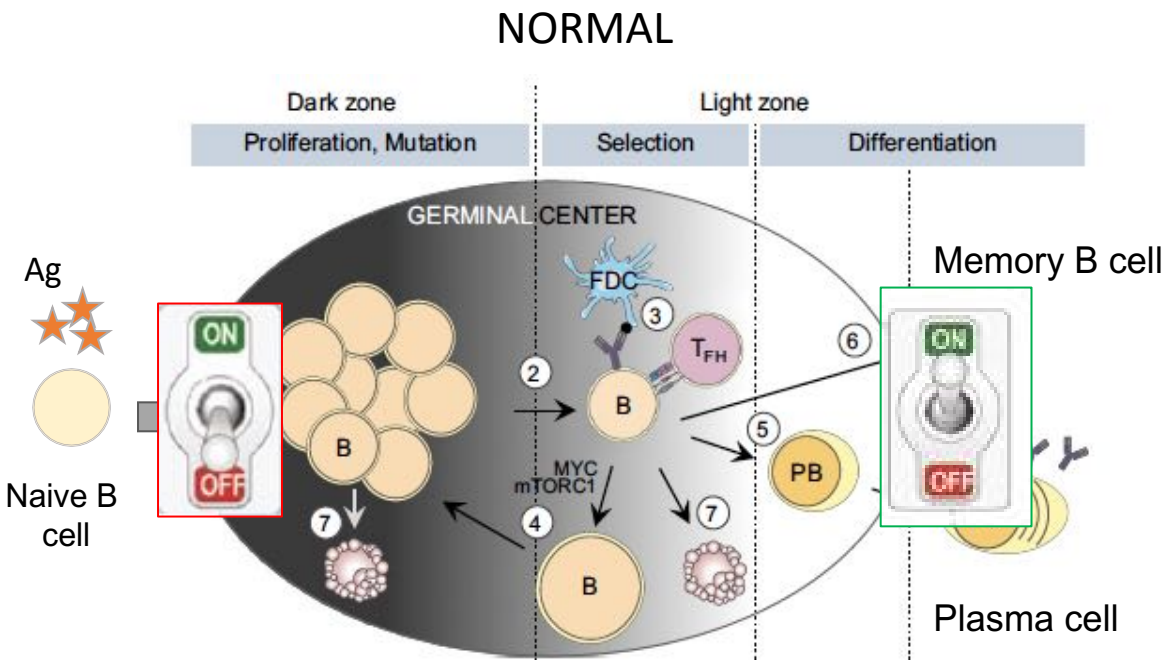
The main players :
KMT2D: H3K4 Methyl transferase (activating marks et enh.)
CREBBP : H3K27 Acetyl transferase (activating marks at enh.)
EZH2 : H3K27 methyl transferase (Repressive marks at prom.)

GC need to reprogram their epigenome during the immune response



Morin et al. Nature 2011
Pasqualucci et al. Nature 2011

GERMINAL CENTER NEED TO REPROGRAM THEIR EPIGENOME



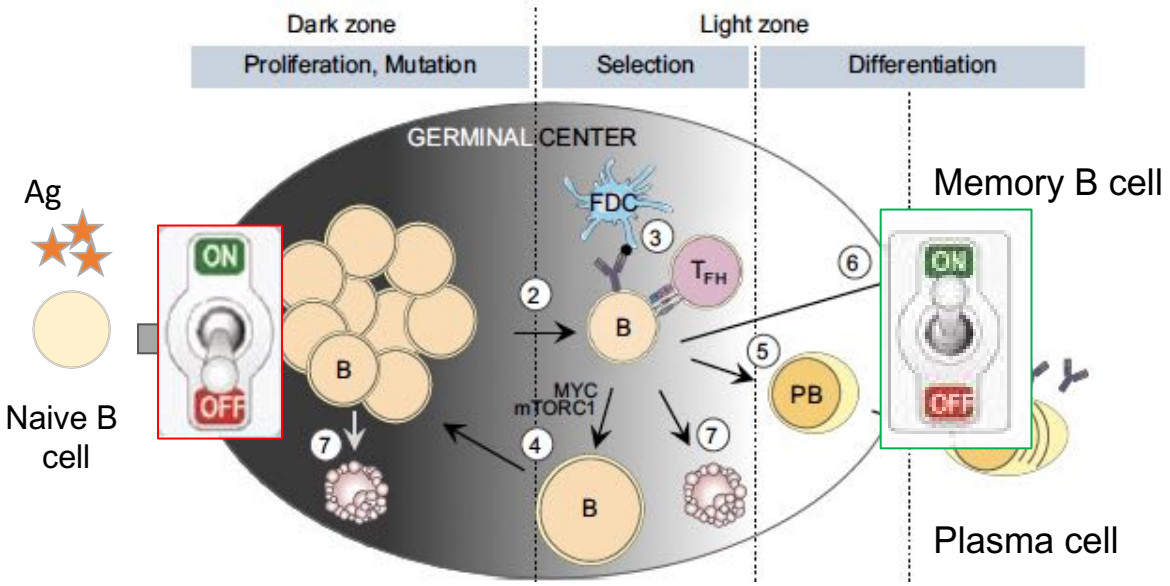
Epigenetic regulator genes: epigenetic switch-off of hundreds target genes to allow the GC phenotype to emerge

BCL6 orchestrates

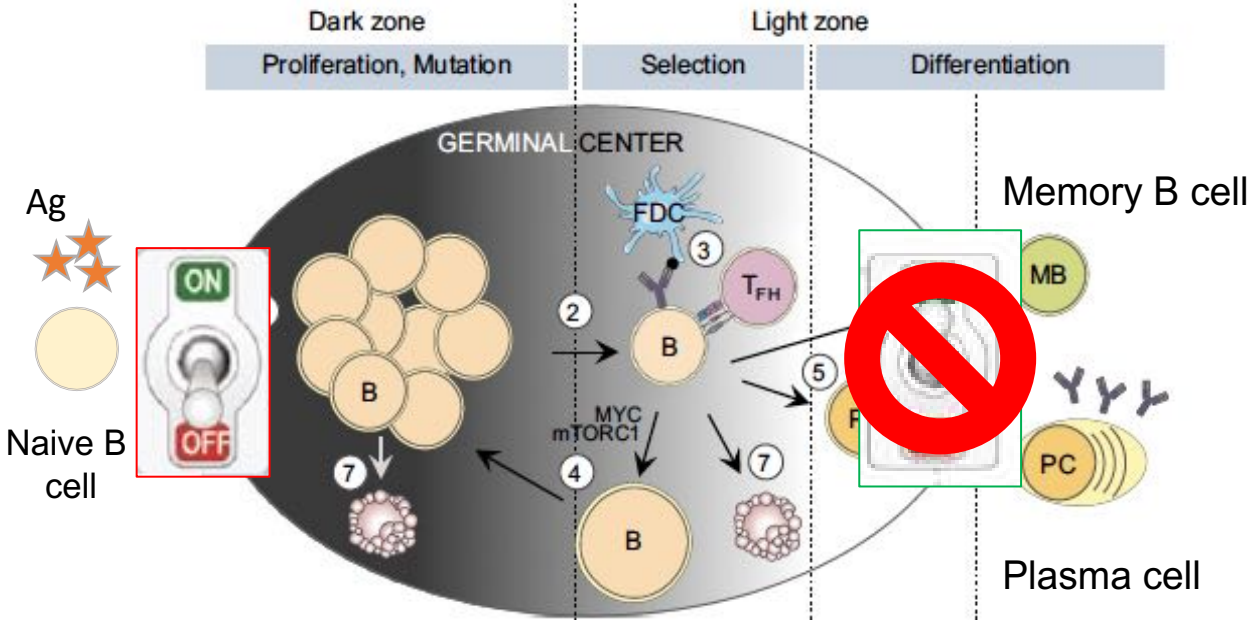
- Cell cycle checkpoints
- DNA damage response (ATR, TP53..)
- Immune signaling and surveillance (MHCII, CIITA, CD40..)
- GC exit genes
- PC differentiation (IRF4, NFKB, PRDM1 ..)

Epigenetic regulator mutations maintain B cells in the GC phenotype

NORMAL



MALIGNANT



Epigenetic regulator genes: epigenetic switch-off of hundreds target genes to allow the GC phenotype to emerge

Epigenetic regulator mutations = Failure to reactivate GC promoter and enhancers of GC exit and diff.

BCL6 orchestrates
- Cell cycle checkpoints
- DNA damage response (ATR, TP53..)
- Immune signaling and surveillance (MHCII, CIITA, CD40..)
- GC exit genes
- PC differentiation (IRF4, NFKB, PRDM1 ..)

Increased GC formation

Altered immune signaling (BCR)

Antigen presentation

Proliferation

Differentiation

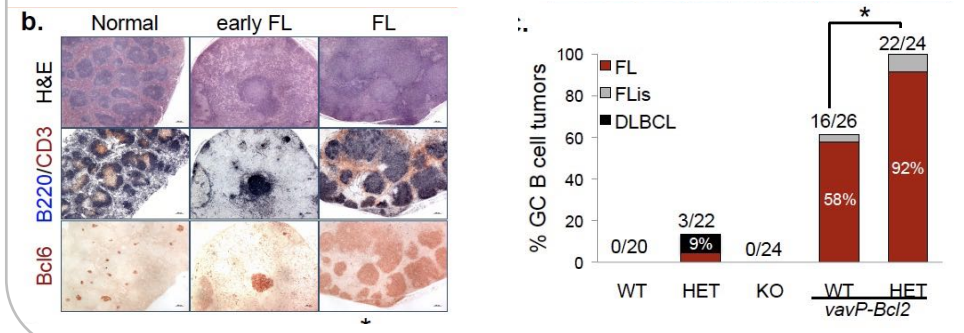
Immune escape

Mlynarczyk *et al.* Immuno Rev 2019

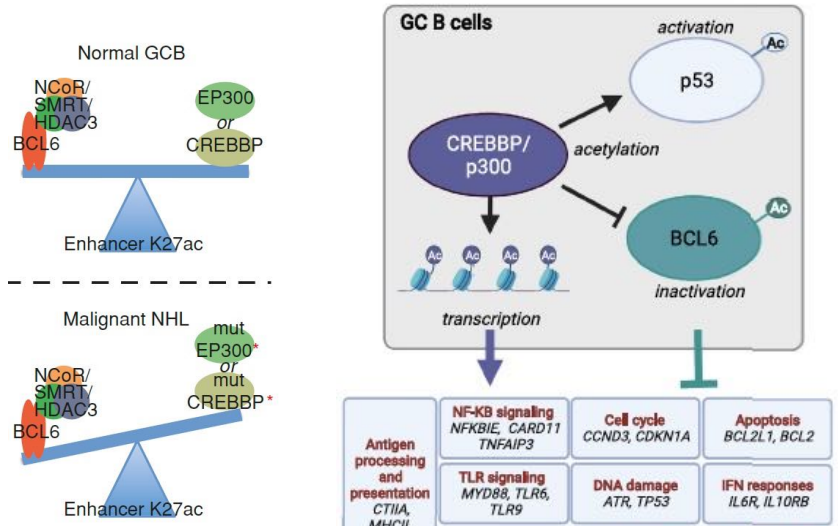
How do (epi)genetic mutations + BCL2 perturb the GC immune response to push premalignant entities into FL ?

CREBBP loss accelerates lymphomagenesis of Bcl2-driven lymphomas and favors immune escape

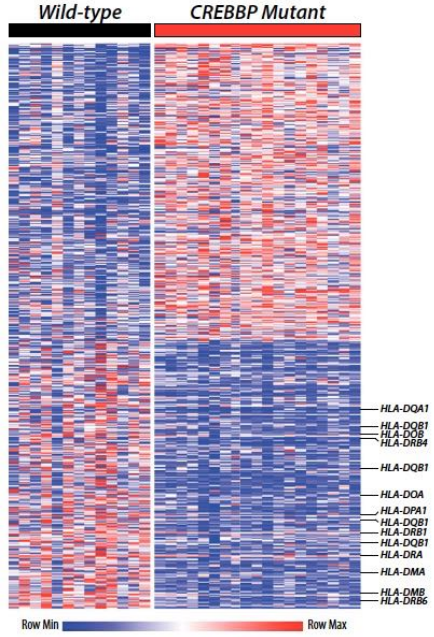
Lymphoma onset in combination with BCL2



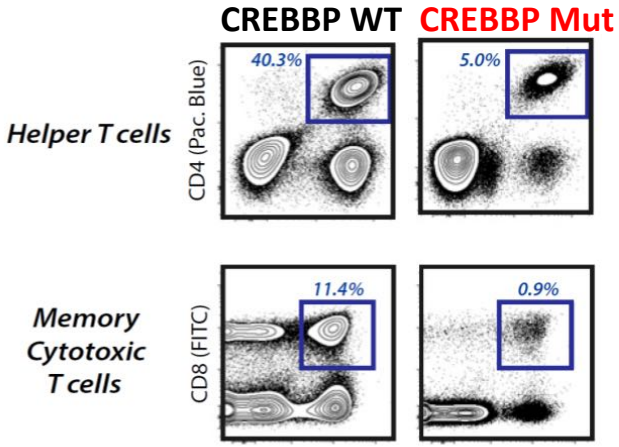
Biological programs modulated by CREBBP



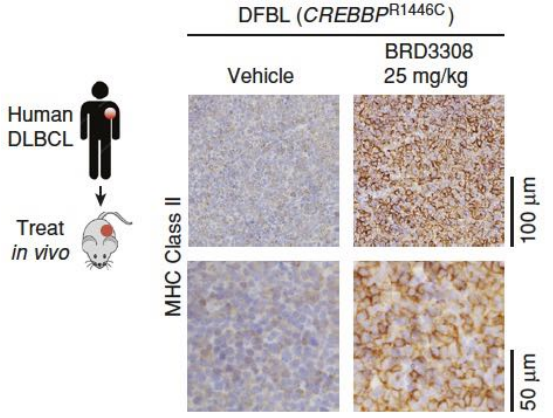
Downregulation of Ag processing and presentation



Decreased T-cell infiltration



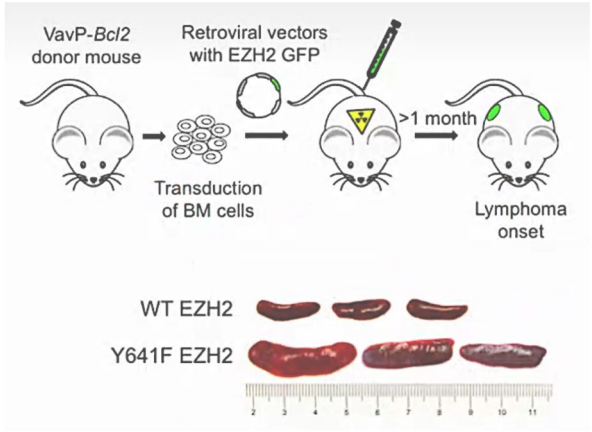
Phenotype reverted by HDAC3 inhibitors



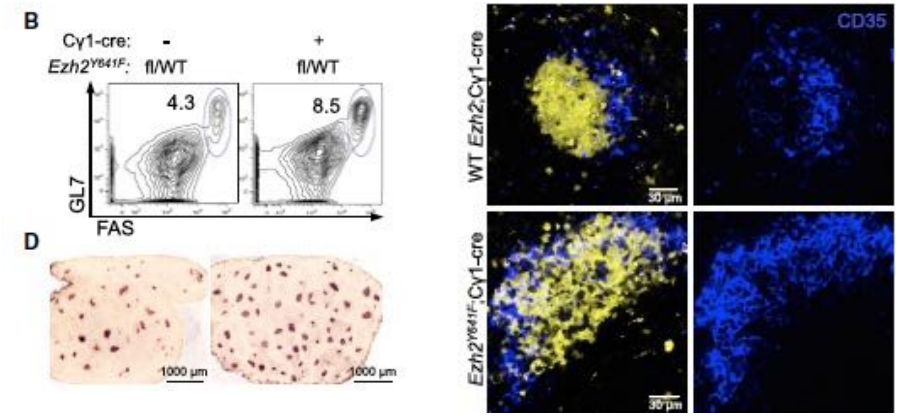
Green *et al.* PNAS 2015
Jiang et al., Nat Med, 2017
Zhang et al., Nat Med, 2017
Mondello *et al*. Cancer Discovery 2020

EZH2 mutation induces a premalignant lymphoma niche by causing a preneoplastic GC hyperplasia and reprogramming the immune synapse

Lymphoma onset in combination with BCL2

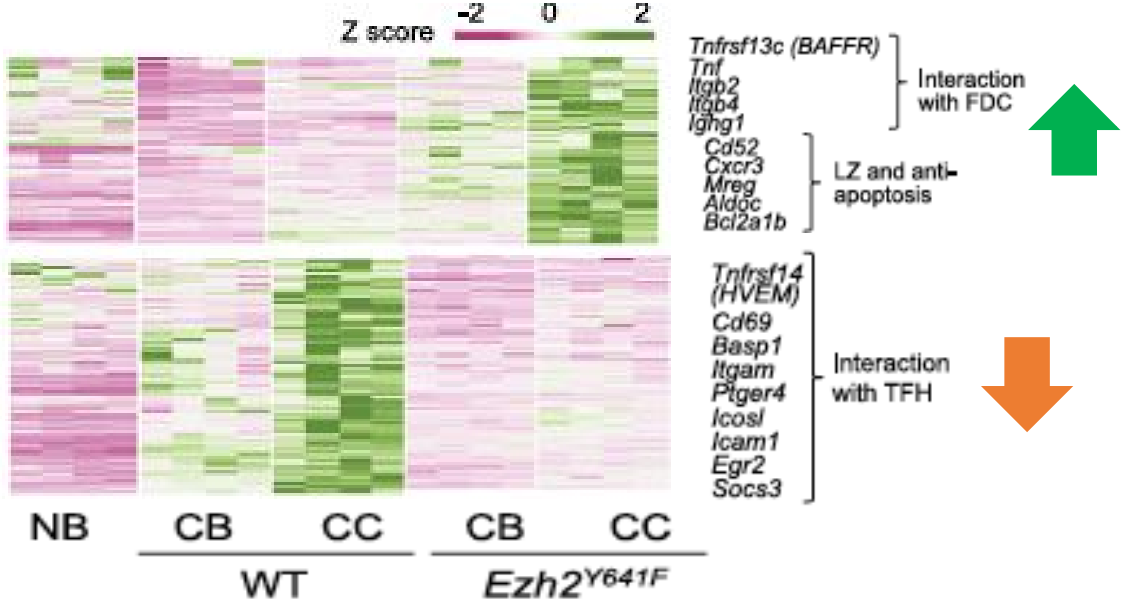


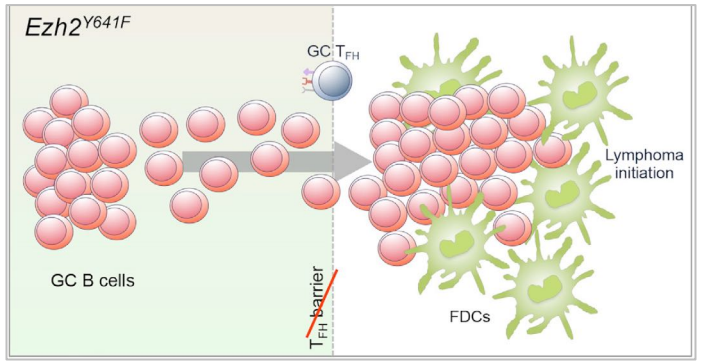
Increased GC formation and LZ expansion



B cells FDC

Premalignant remodeling of the GC niche



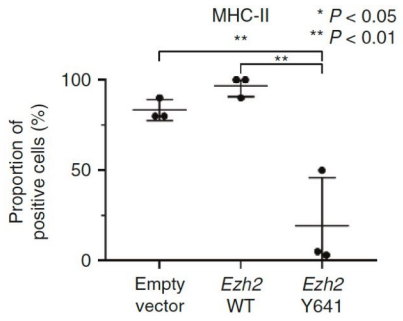


- **Decreased requirement for T cell help**
- **More dependency to FDC**

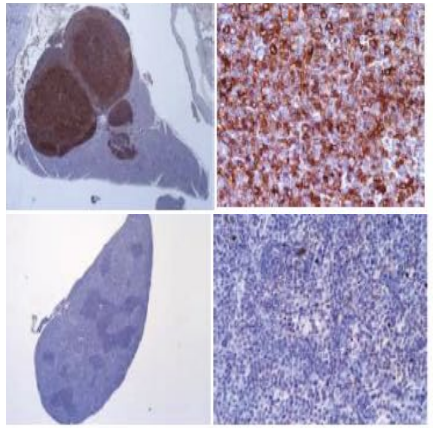
Béguelin, Cancer cell, 2013, 2016 2020

EZH2 mutations favor immune escape and EZH2 inhibitors can restore immune recognition

EZH2^{m} linked to loss of MHC expression and reduced T-cell infiltrates



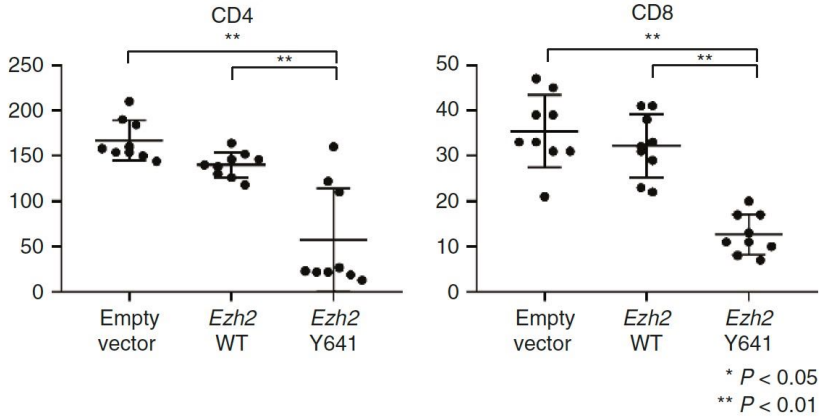
MHC-II immunostaining



WT

EZH2 Y641

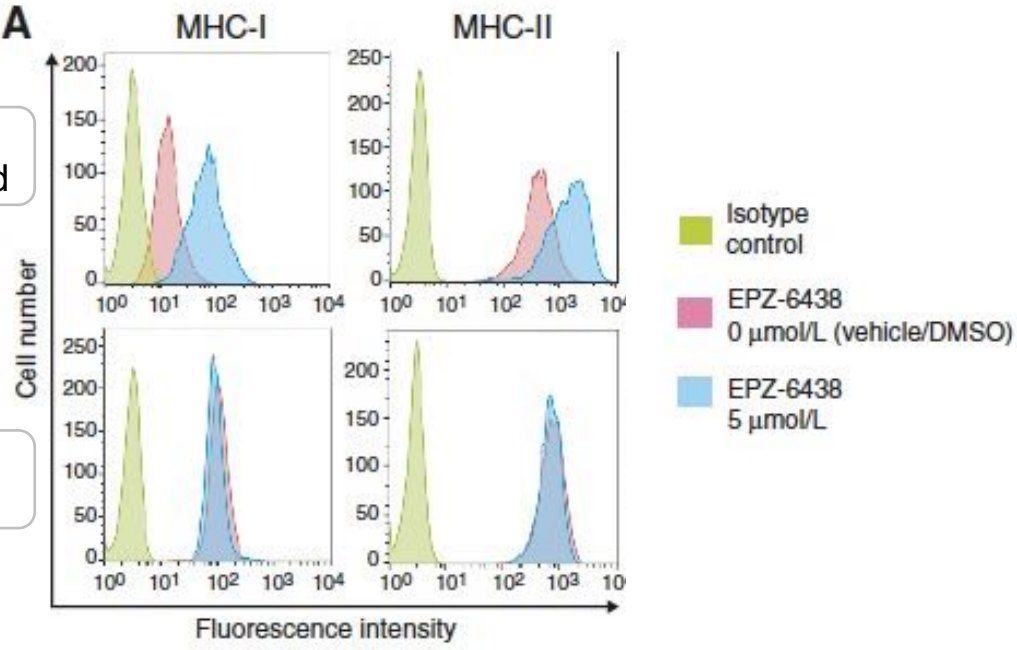
T cell infiltration in mouse tumors



MHC-I and MHC-II restoration with EZH2 inhibitor (EPZ-6438) in FL/DLBCL human cell lines

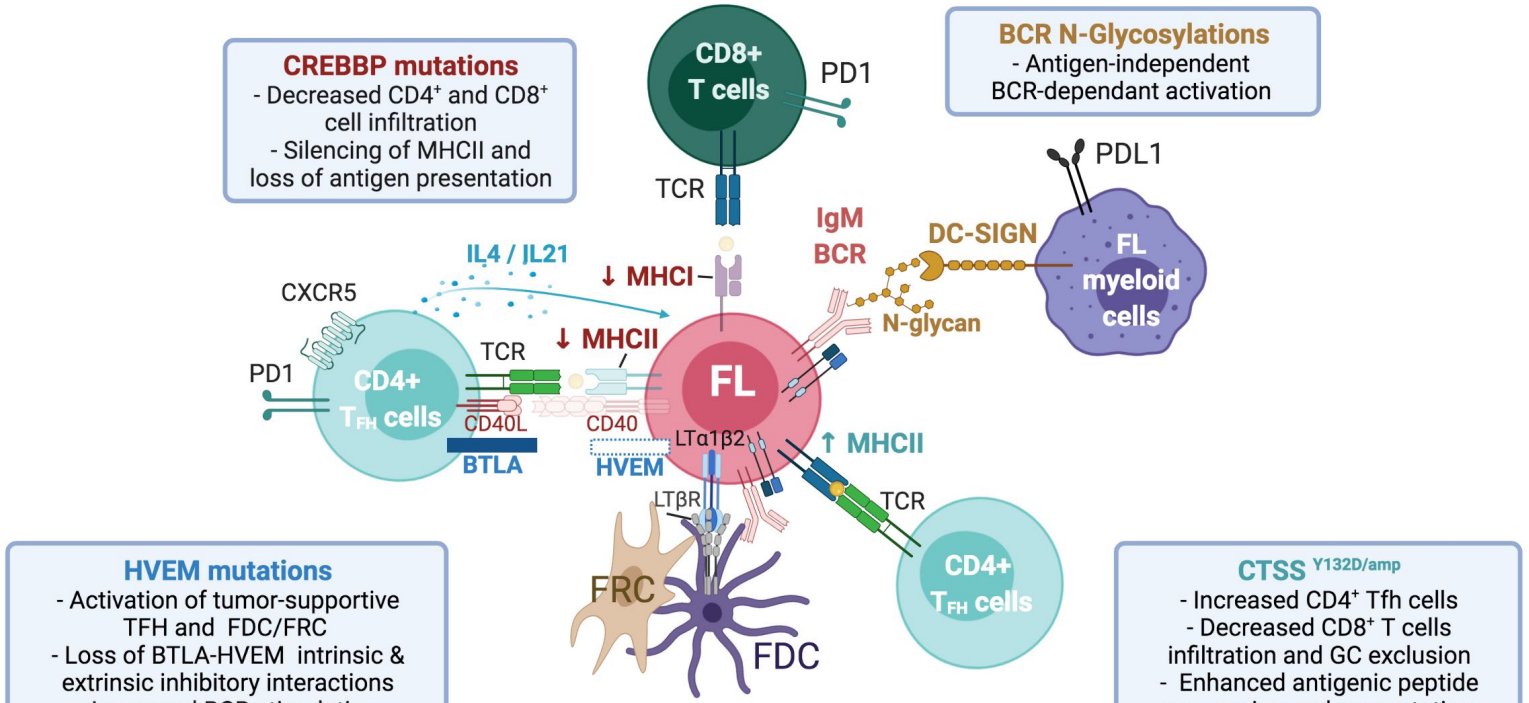
SUDHL4
EZH2 mutated

SUDHL8
EZH2 WT



Ennishi Cancer Discovery 2019

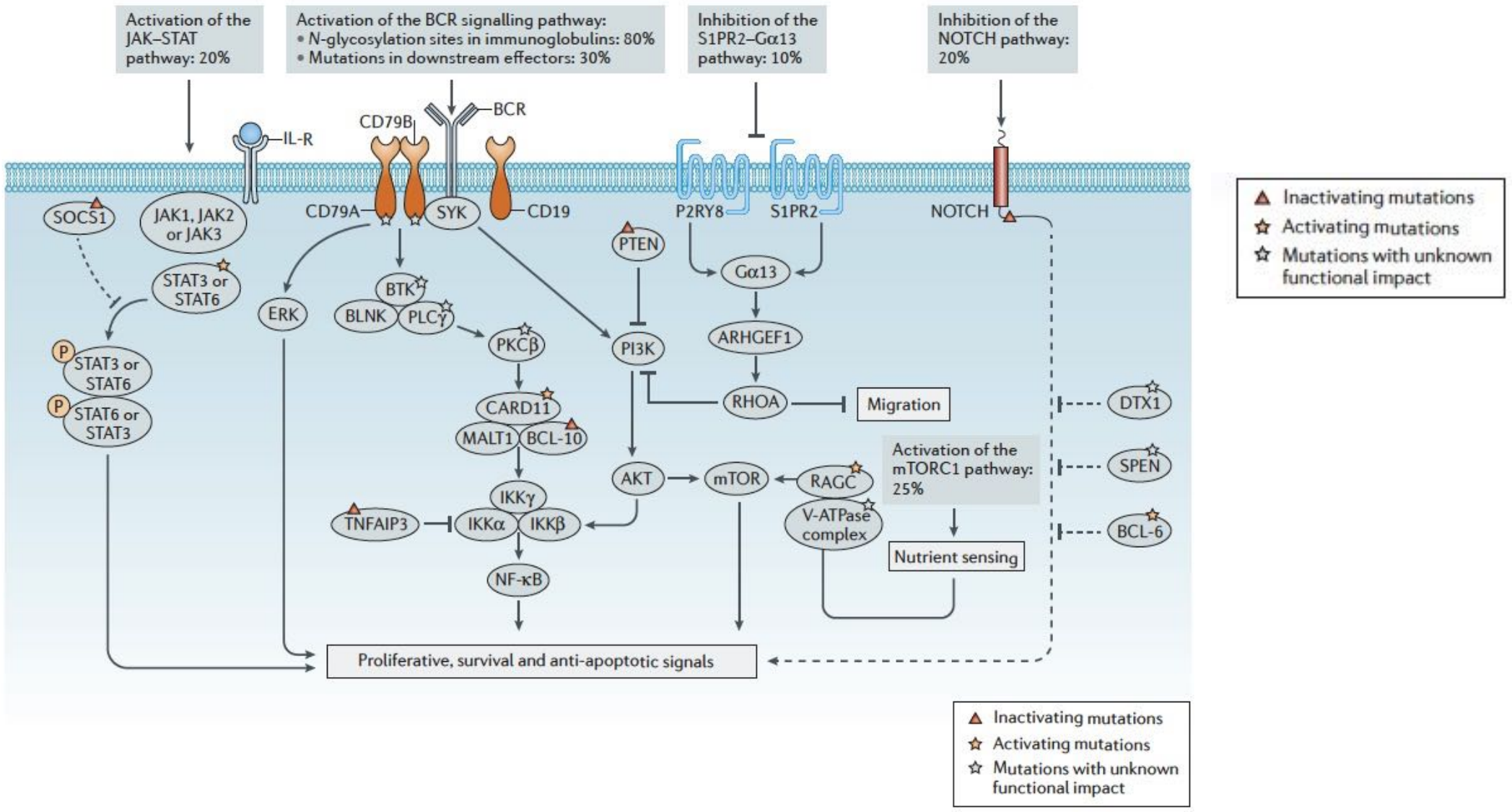
FL mutational landscape and immune microenvironment interplay



Adapted from Milpied et al. Adv Immunol, 2021

Green et al. PNAS 2015
Amin et al. Blood 2015
Linley et al. Blood 2015
Boice et al. Cell 2016
Ortega-Molina et al. Nat Metabol 2019
Mondelli et al. Cancer Disc, 2020
Béguelin et al. Cancer Cell 2020
Bararia et al., Cell Rep 2020
Dheilly et al., Cancer Cell 2020

Other pathways and signals driving proliferation and survival of FL cells



Huet et al. Nat Rev Cancer, 2018

Can we learn something from these t(14;18) clones?

Among intermediates identified as « precursors » or « CPC » : what are the different CPC flavors and which one could give rise to follicular ***lymphomas*** *?*

What are the evolutionary pathways along disease progression ?

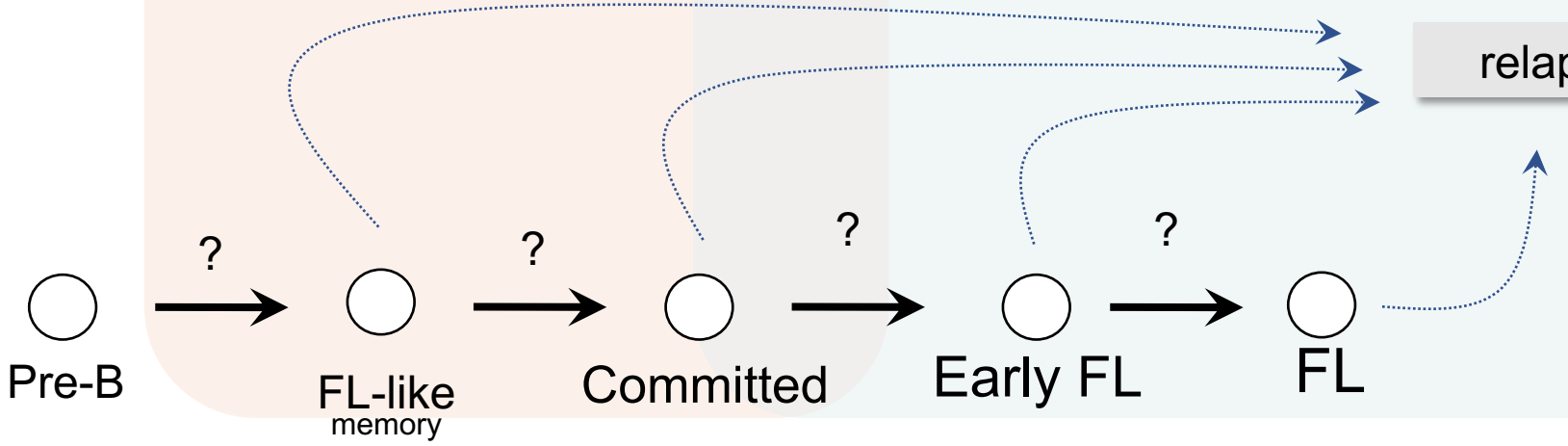
How to map CPCs?

Deep-seq in:
t(14;18)$^{+}$ healthy individuals

NGS in
patient biopsies

Healthy adults:

- Prevalence:
  50% t(14;18)$^{+}$

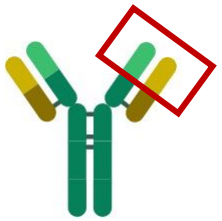


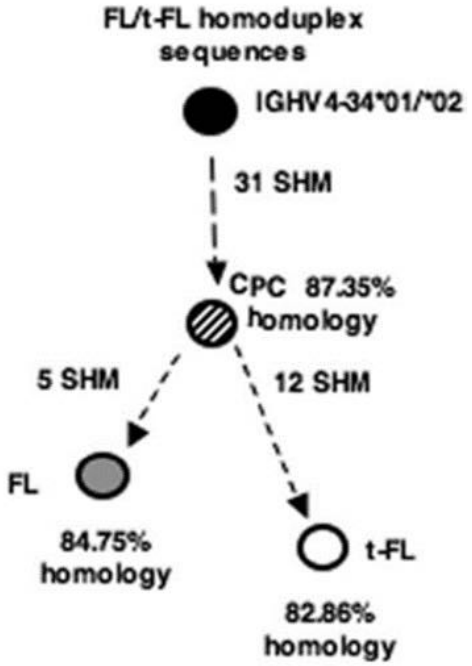
FL patients:

- Prevalence: **0,03%**
  (**0,5%** > 65yrs)

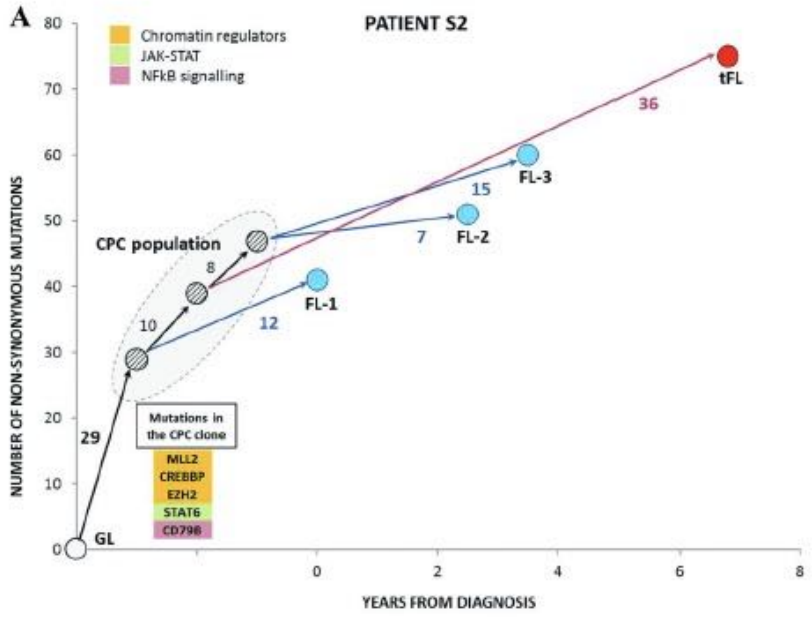
Frequency in 'healthy' blood or lymphoid organs

Longitudinal analysis in paired FL biopsies and NGS inferred the existence of a reservoir population of Common Precursor Cells (CPC)

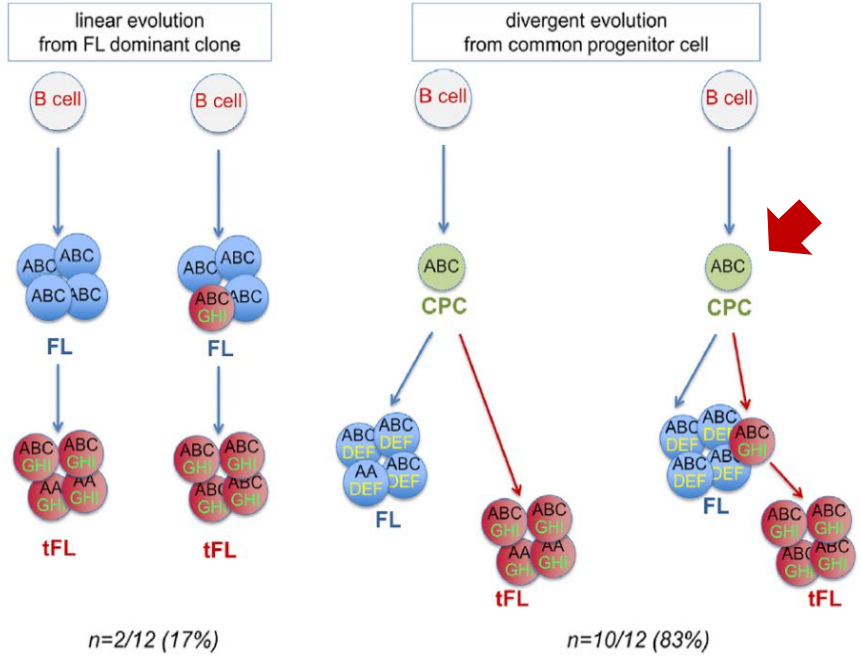




Carlotti *et al. Blood* 2009



Okosun *et al. Nat Gen* 2014

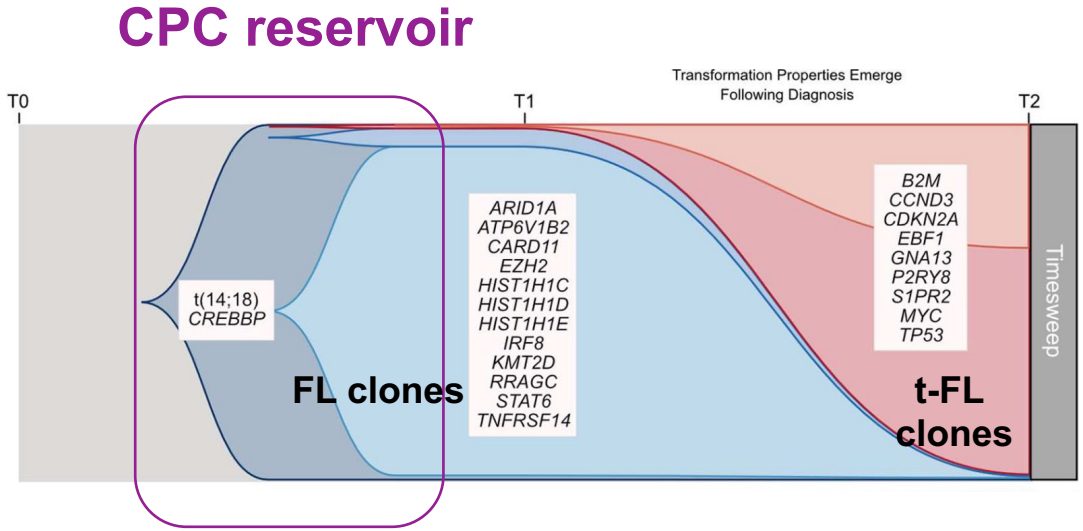


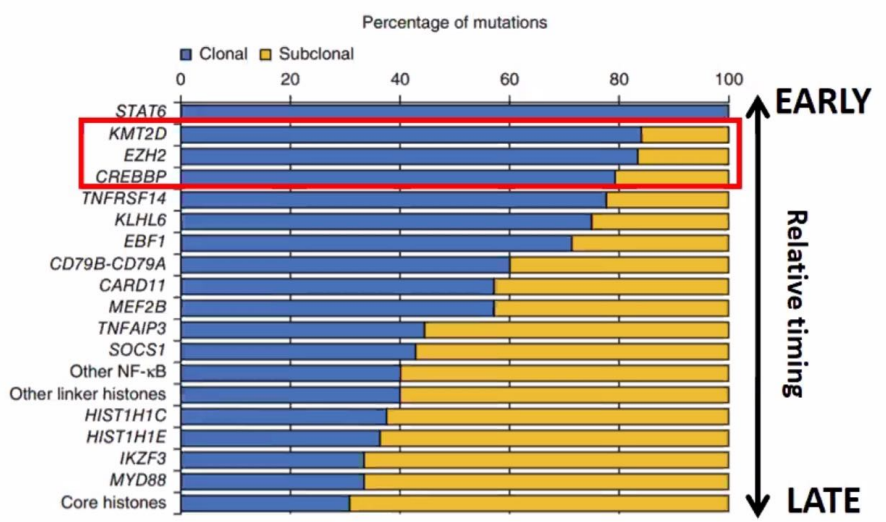
Pasqualucci *et al. Cell Rep* 2014

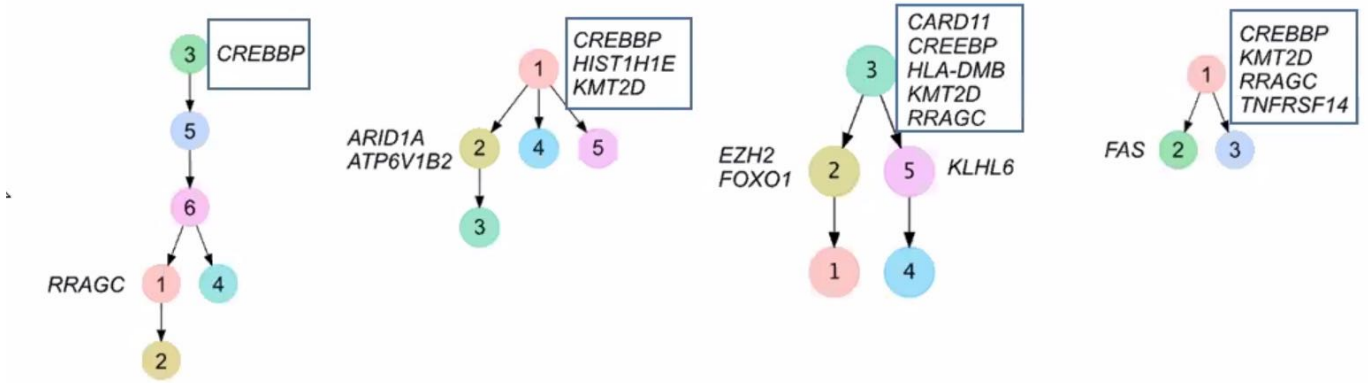
→ Existence of a reservoir population (CPC)
→ Progression occurs via divergent evolution in > 80% cases

and also
Green et al. PNAS 2015
Kridel et al. Plos Med 2016

Epigenetic regulator mutations are early initiating events acquired by a CPC



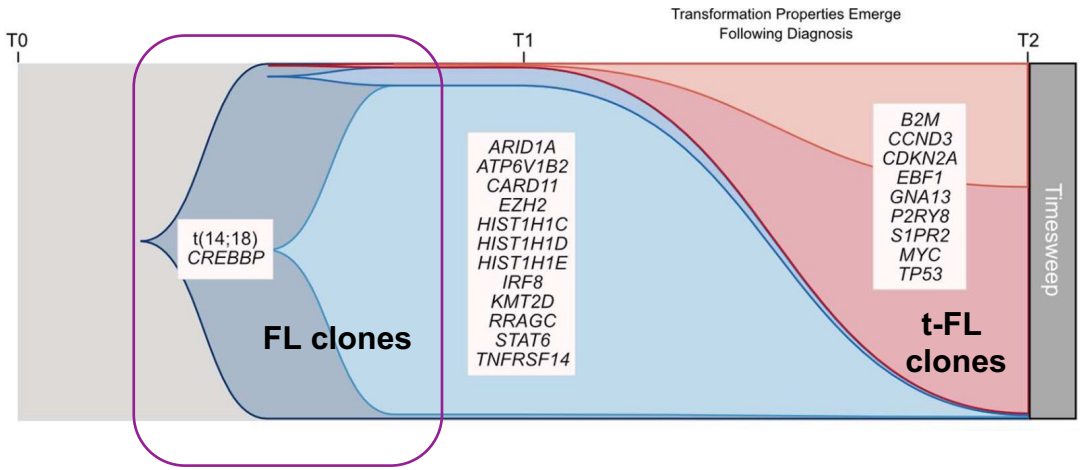


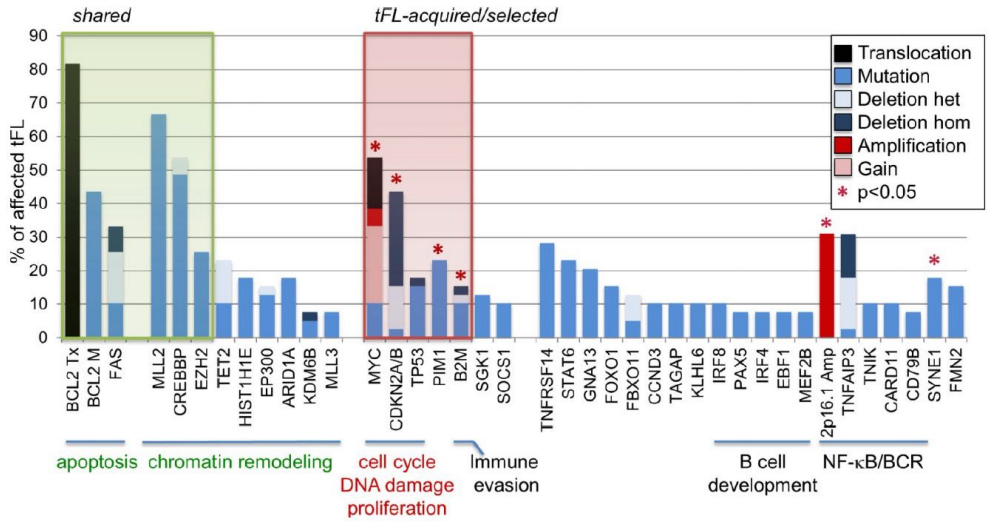


Okosun et al. Nat Genet 2014
Pasqualucci et al. Cell Rep 2014
Green et al. PNAS 2015
Kridel et al. Plos Med 2016

Acquired genetic lesions associated with transformation

CPC reservoir





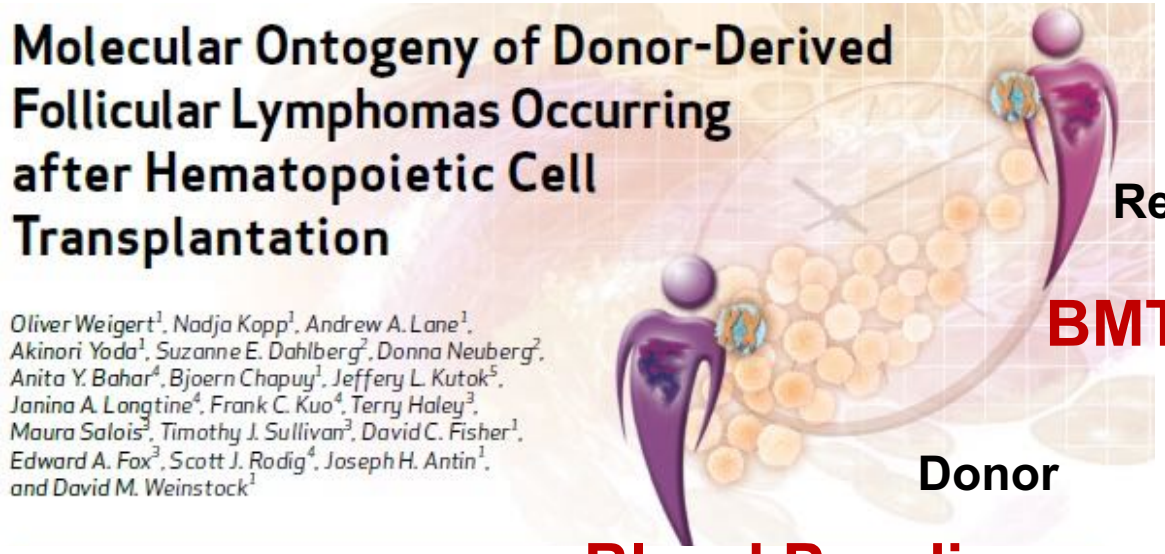
- Recurrent alterations: mutations, CNA, LOH
  - Cell cycle: *CDKN2A/B, MYC*
  - NF-kB: *MYD88, TNFAIP3*, *EBF1*
  - Immune evasion: *B2M, CD58*
  - Apoptosis: *TP53, FAS*
  - Acquired *BCL6* translocation
- Some similar to GCB-DLBCL

- Subclone variants /CPC often undetectable in bulk diagnosis sample

→ 'Late' druggable pathway @ relapse hard to predict from diagnosis sample

Okosun et al. Nat Genet 2014
Pasqualucci et al. Cell Rep 2014
Green et al. PNAS 2015
Kridel et al. Plos Med 2016

Direct evidence for a CPC reservoir in ‘healthy’ individuals years before diagnosis



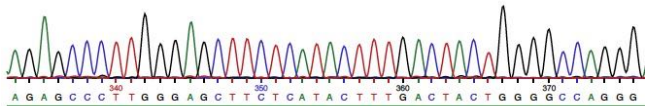
Recipient → FL_R

BMT → 9 years

Donor → FL_D

Blood Pre-diag

t(14;18) ~5.10^{-4}

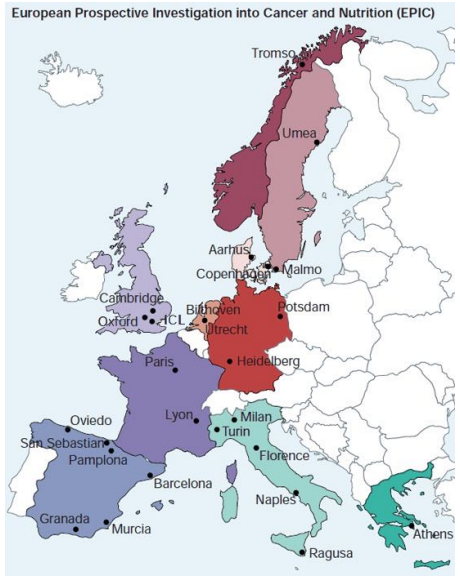


- ✓ Identical t(14;18)
- ✓ Shared IGH VDJ rearrangements
- ✓ Shared oncogenic mutations (EP300, KLHL6..)
- ✓ **FL commitment already in place 9 years before diagnosis**
- ✓ **BM may represent a niche for CPC**

Carlotti et al. *Blood* 2009
Weigert et al. *Cancer Discovery* 2012

t(14;18) screening in blood from healthy individuals who later developed FL

EPIC cohort



>½ Million

« healthy » volunteers at enrollment

>350,000 blood samples

« pre-diagnostic » blood DNA

20 years follow-up: registration of cancer incidence

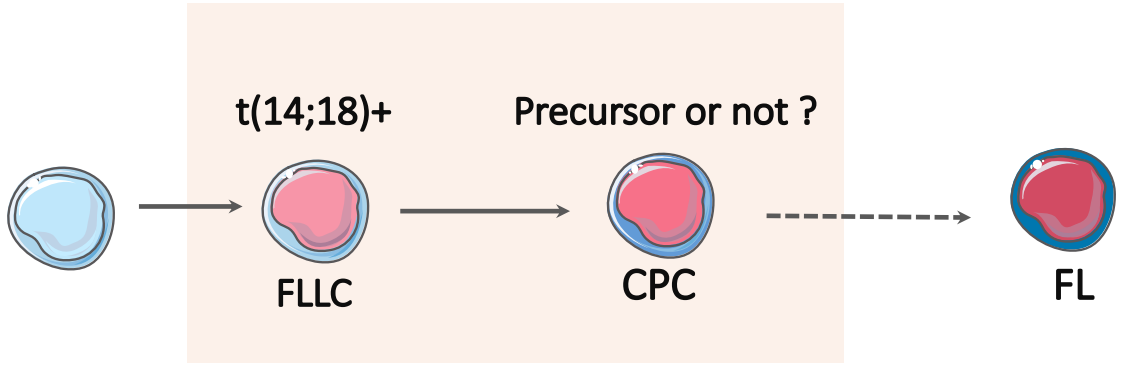
Incident FL

Matched controls

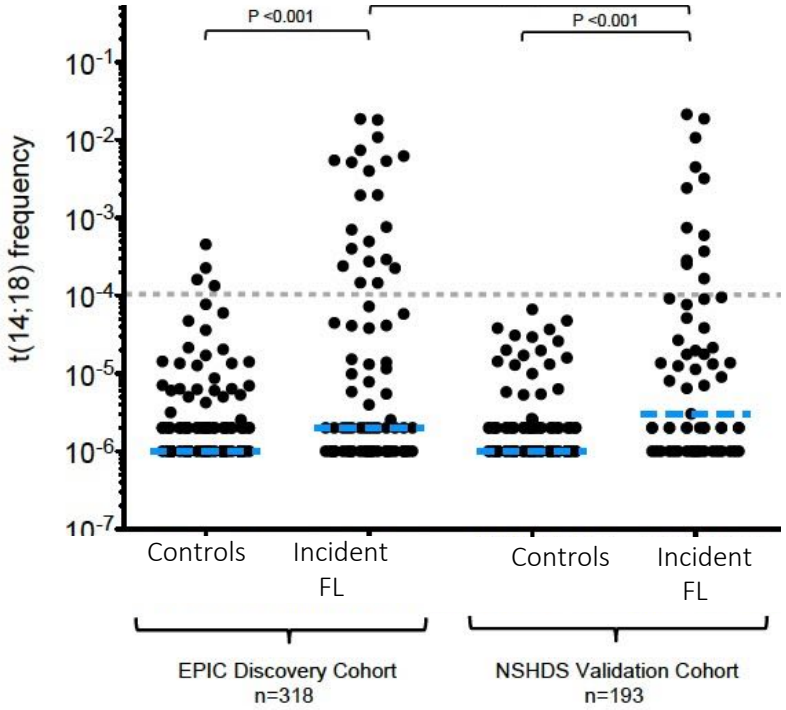
Collaboration P. Vineis , R Kelly (Imperial College London- IARC)

Roulland et al. J Clin Oncol 2014

Progression from FL committed precursors >10 years before diagnosis

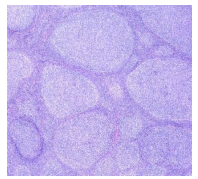


Increased t(14;18) frequencies above 10^{-4} (25% of cases)

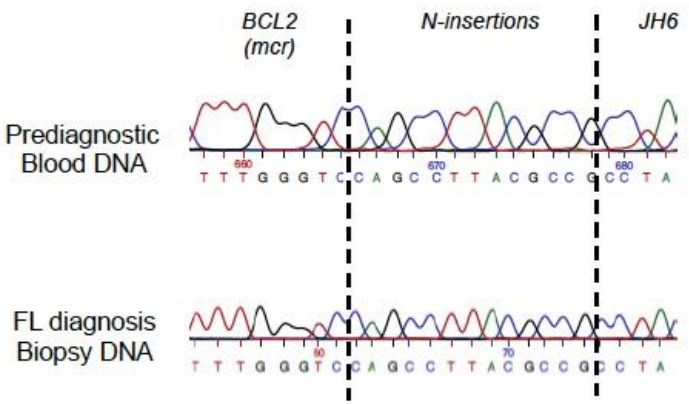


Same clone in FL biopsy and prediagnostic blood

years



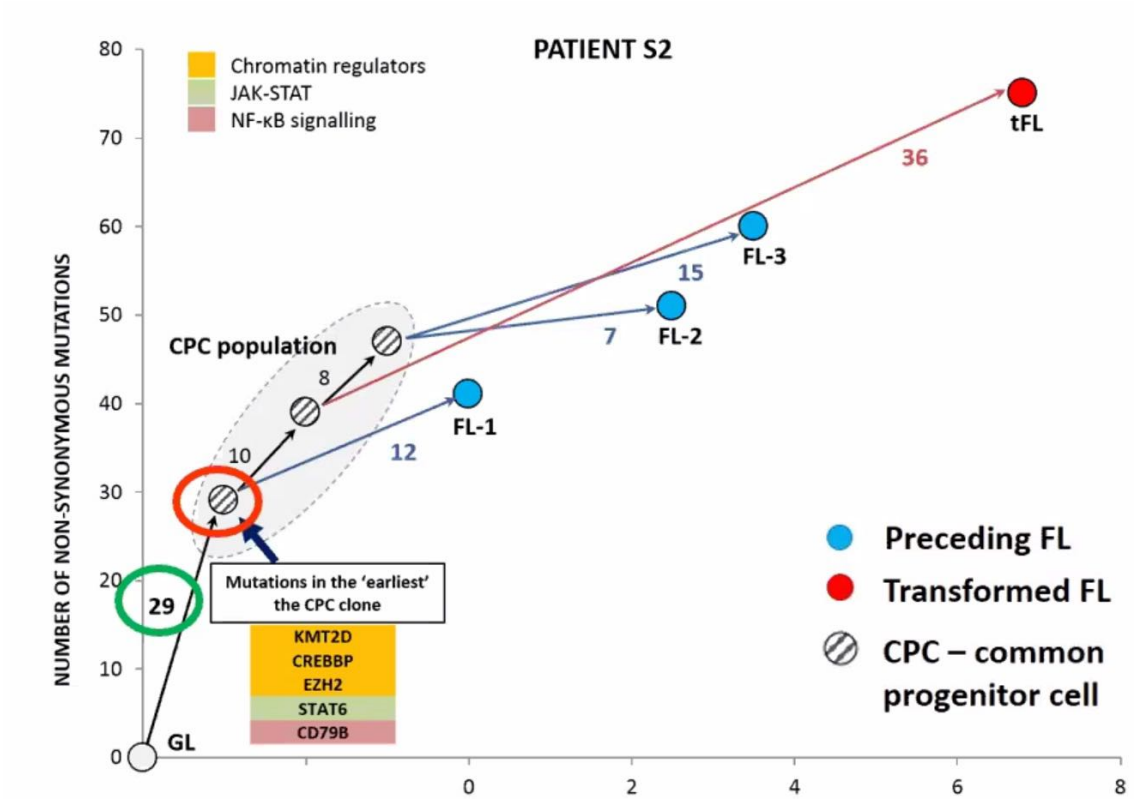
Case # 03-12089 **BCL2 /J_H6 translocation**



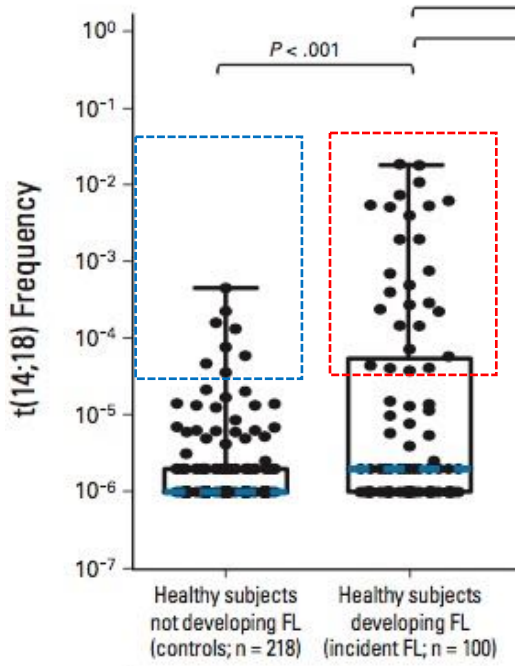
Roulland et al. J Clin Oncol 2014

Defining genomic landscape of FL precursors/CPC

Chromatin modifier mutations are hallmarks of FL and likely early events in FL genesis



Selected pre-FL patients and controls with **elevated t(14;18) levels**

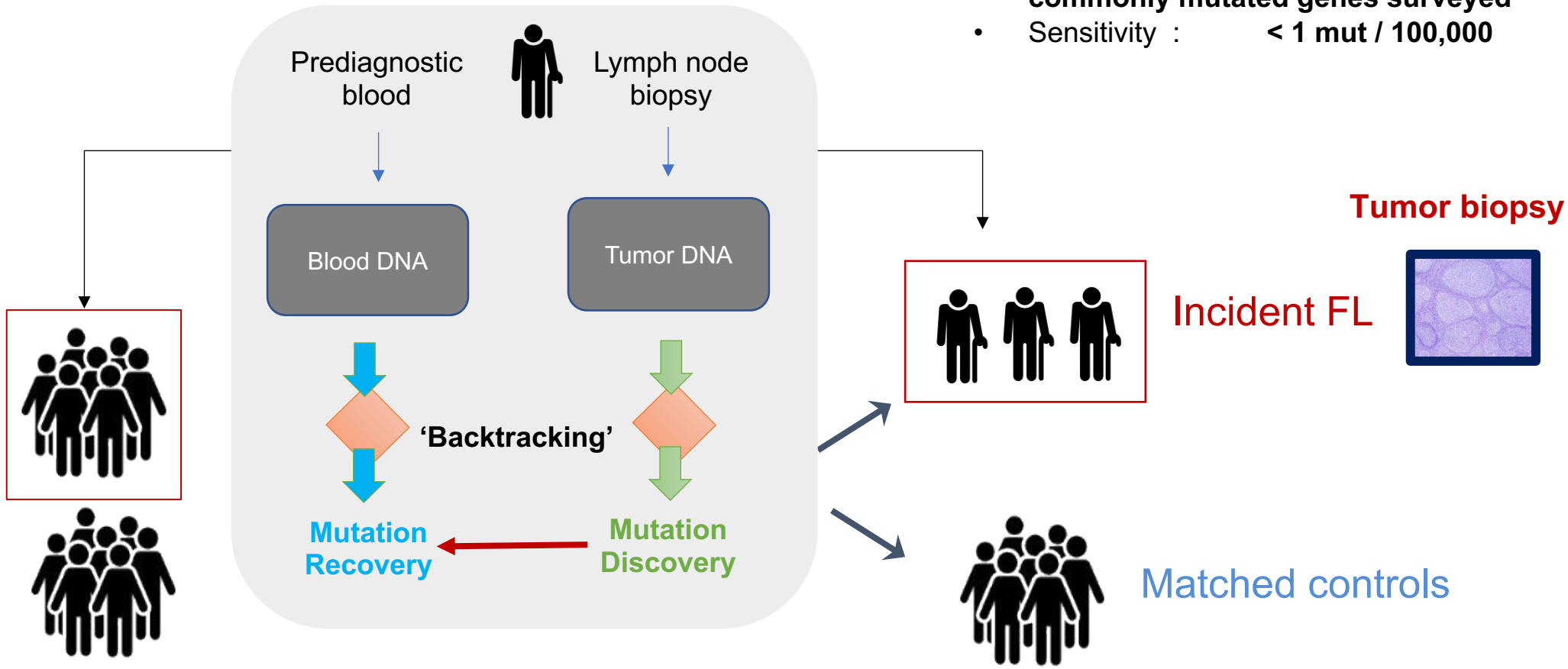


CAPP-seq to characterize paired Blood/tumor FL biopsies

- Ultrasensitive detection of circulating tumor DNA by CAPP-seq* : **334 commonly mutated genes surveyed**
- Sensitivity : **< 1 mut / 100,000**

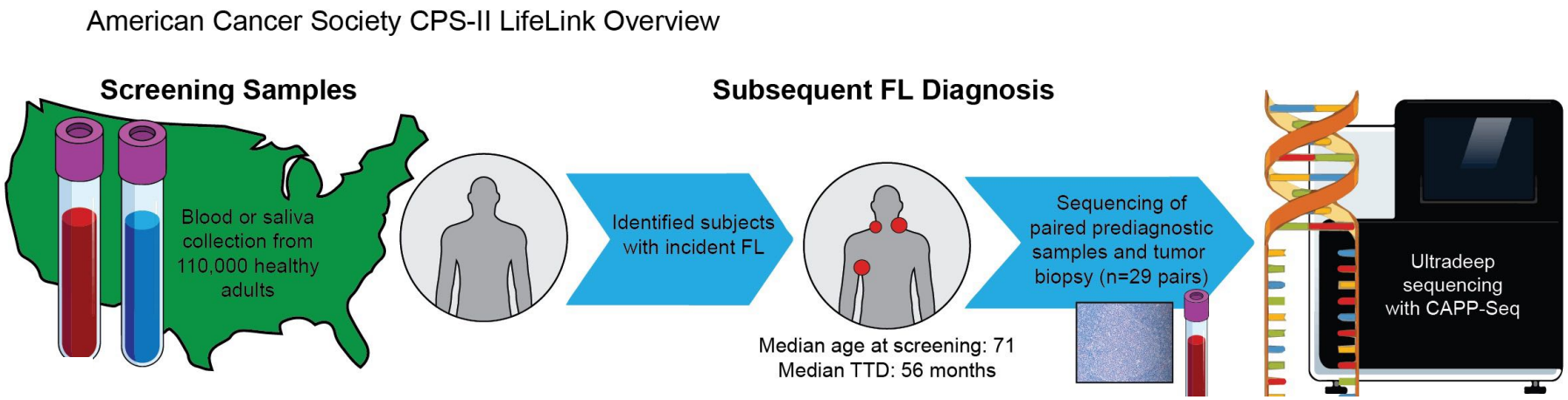
American Cancer Society CPS II cohort

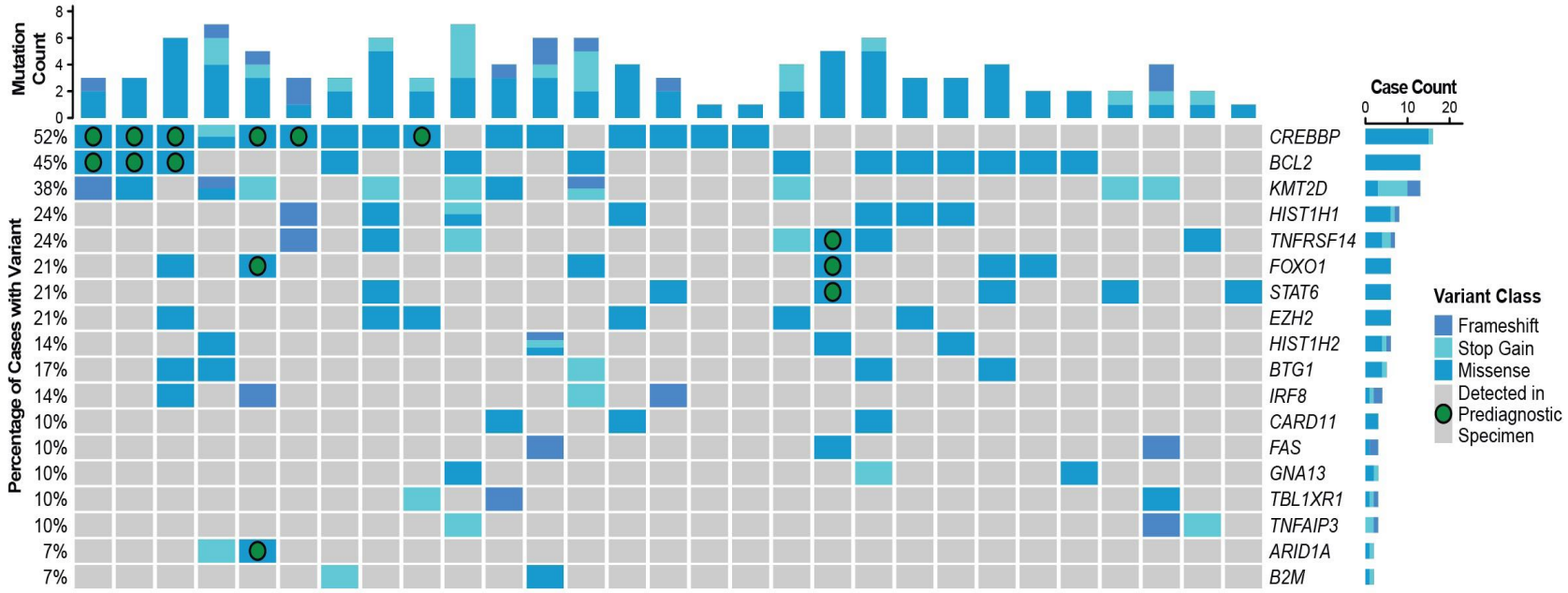
> 100,000
« healthy » volunteers at enrollment

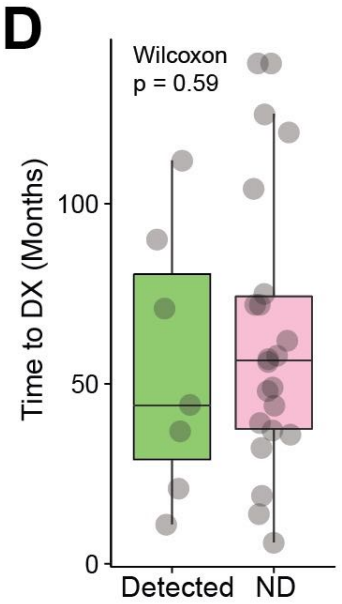


Collaboration L. Teras, J. Song (ACS)

Tumor-confirmed FL mutations are present years prior clinical diagnosis



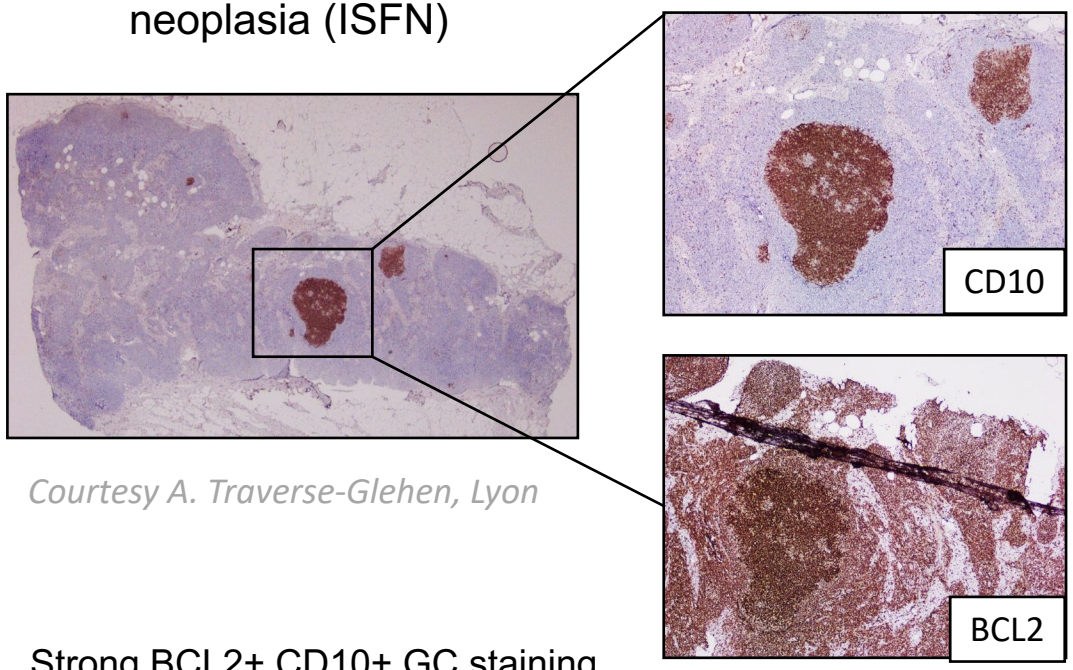




Collaboration AA. Alizadeh (Stanford), L. Teras (ACS)

What about mutations in pathological tissue precursor conditions ?

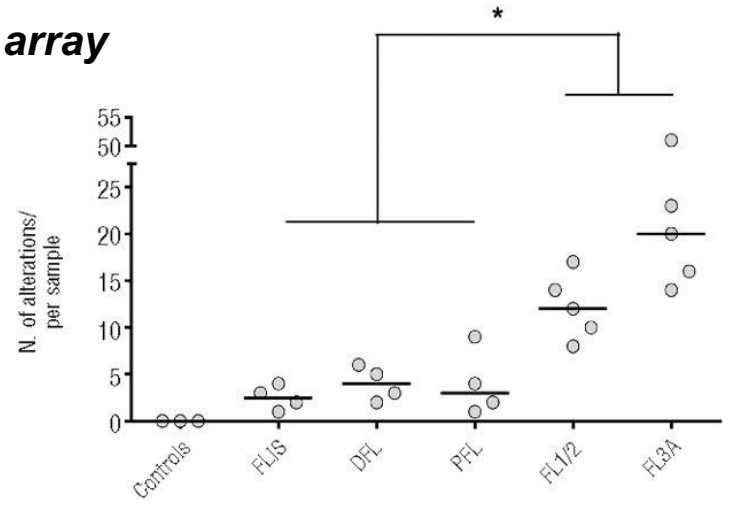
In situ follicular neoplasia (ISFN)



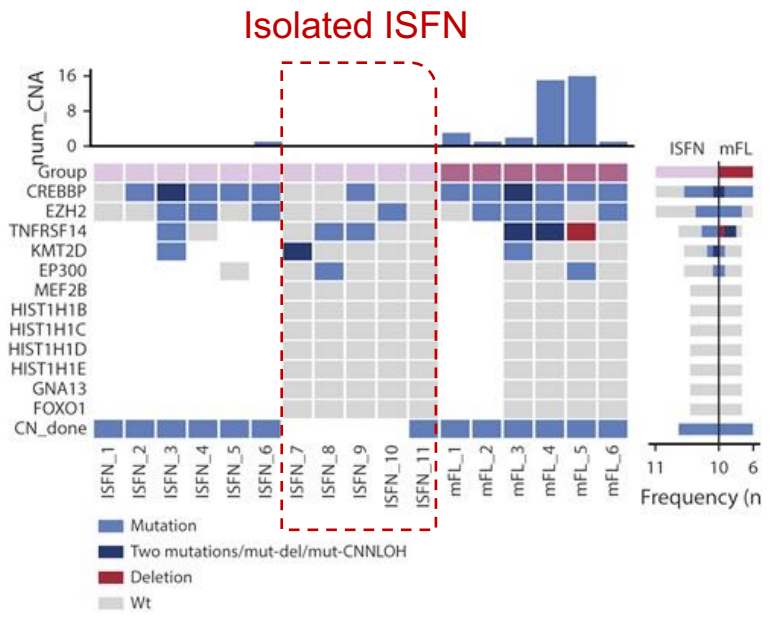
Courtesy A. Traverse-Glehen, Lyon

- Strong BCL2+ CD10+ GC staining
- Low number of SNVs and mutations
- Low risk of progression (5-10%)

CGH array



NGS

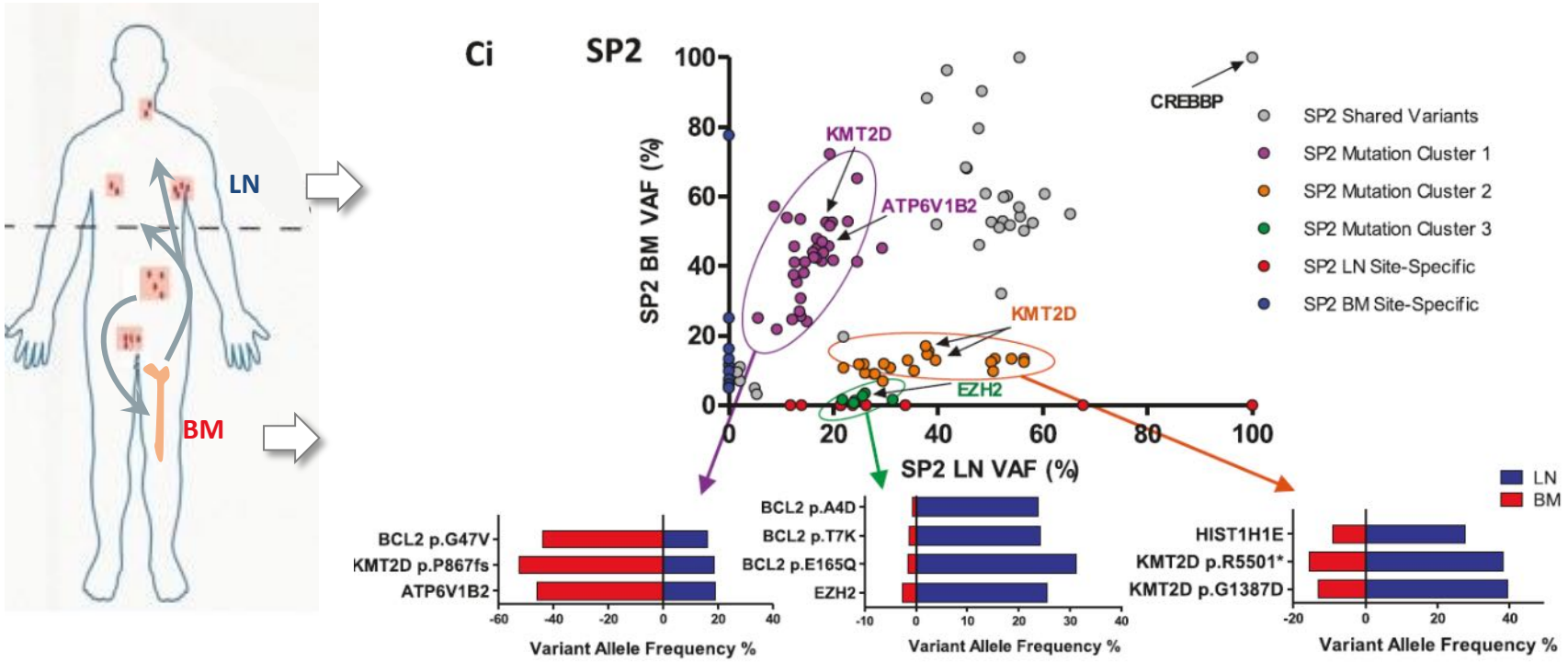


Cong et al. Blood, 2002
Mamessier et al. Haematologica, 2014
Schmidt et al. Blood , 2018

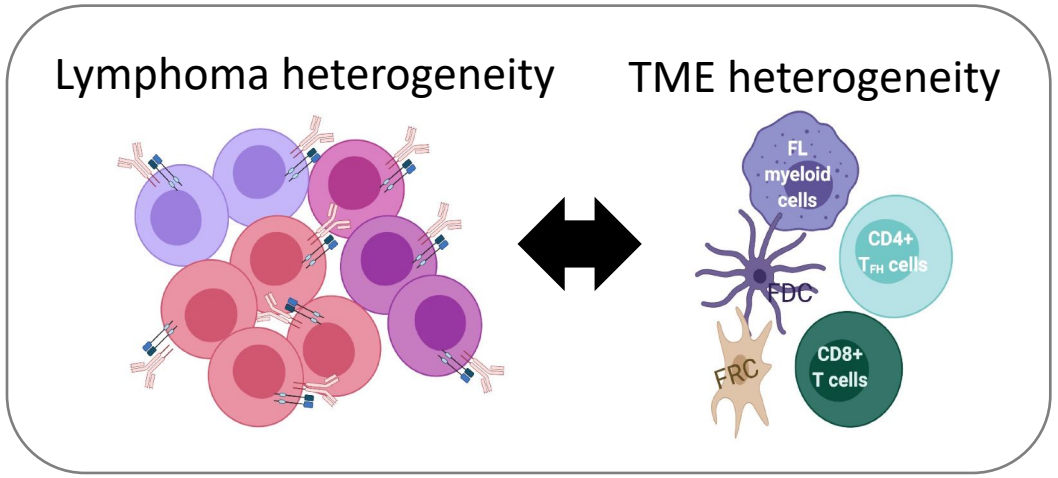
Large subclonal genetic intra-tumoral heterogeneity in FL

Genomic profiling reveals spatial intra-tumor heterogeneity in follicular lymphoma

Shamzah Araf[1,2] · Jun Wang[3] · Koorosh Korfi[1] · Celine Pangault[4] · Eleni Kotsiou[1] · Ana Rio-Machin[1] · Tahrima Rahim[1] · James Heward[1] · Andrew Clear[1] · Sameena Iqbal[1] · Jeff K. Davies[1] · Peter Johnson[5] · Maria Calaminici[1] · Silvia Montoto[1] · Rebecca Auer[1] · Claude Chelala[3] · John G. Gribben[1] · Trevor A. Graham[6] · Thierry Fest[4] · Jude Fitzgibbon[1] · Jessica Okosun[1]

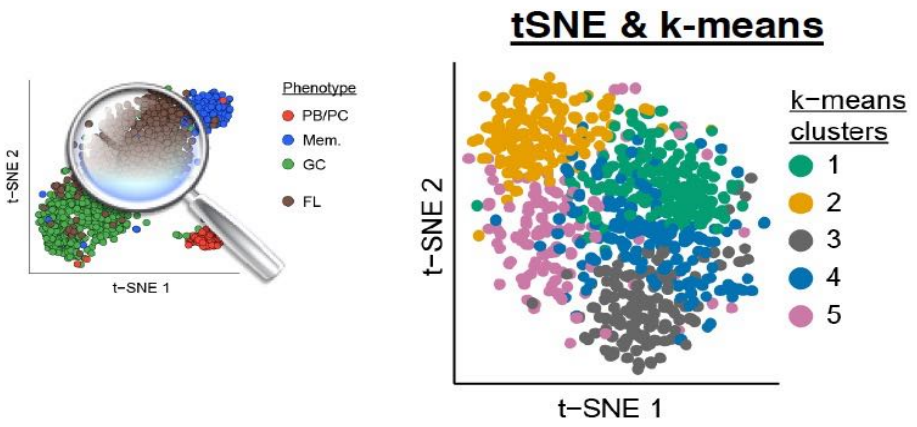


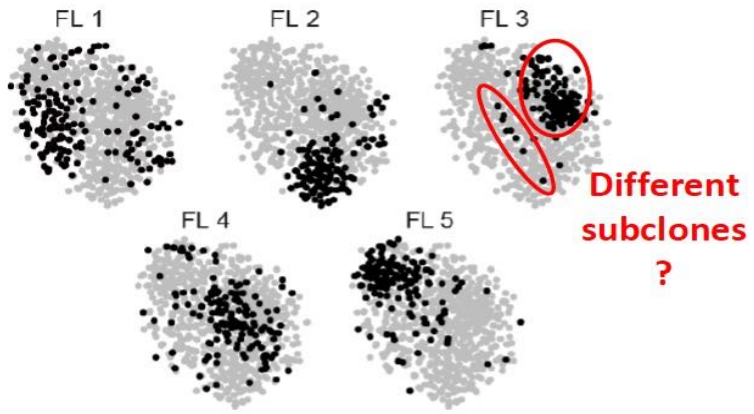
Follicular lymphoma dynamics and heterogeneity at the single cell era



Can we charaterize the CPC ?
Can we identify biomarkers of progression/transformation
Can we identify signature of resistance ?

INTRATUMOR HETEROGENEITY



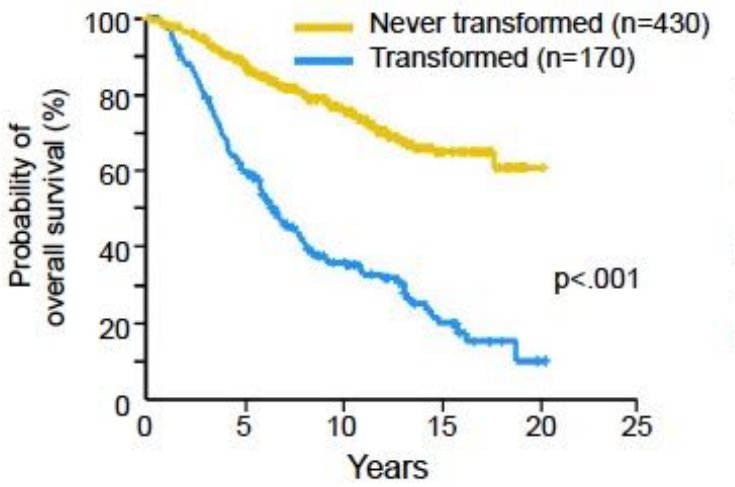


Milpied et al. Nat Immunol 2018

NOT FROZEN, NOT BLOCKED AT A GIVEN GC STAGE !!!

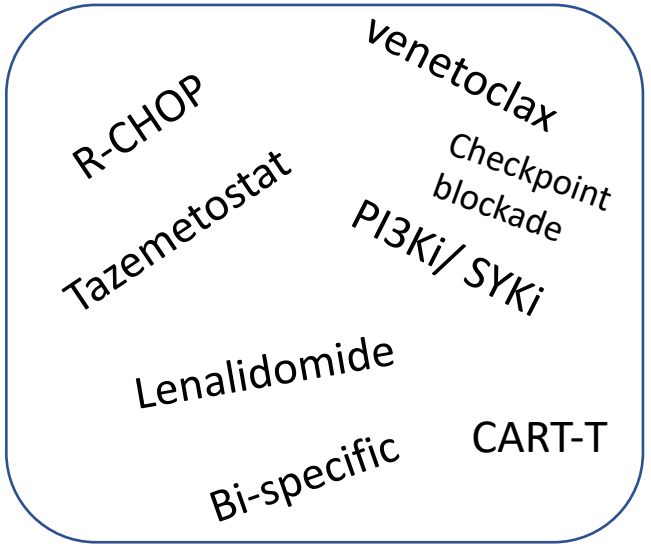
HOW TO USE THIS MOLECULAR INFORMATION ?

Response to treatment



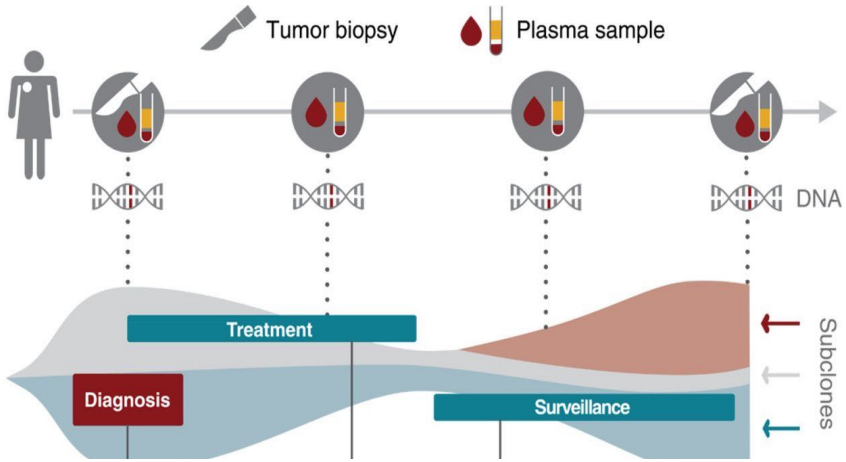
Prognostic biomarker

Targeted Therapy



Predictive biomarker

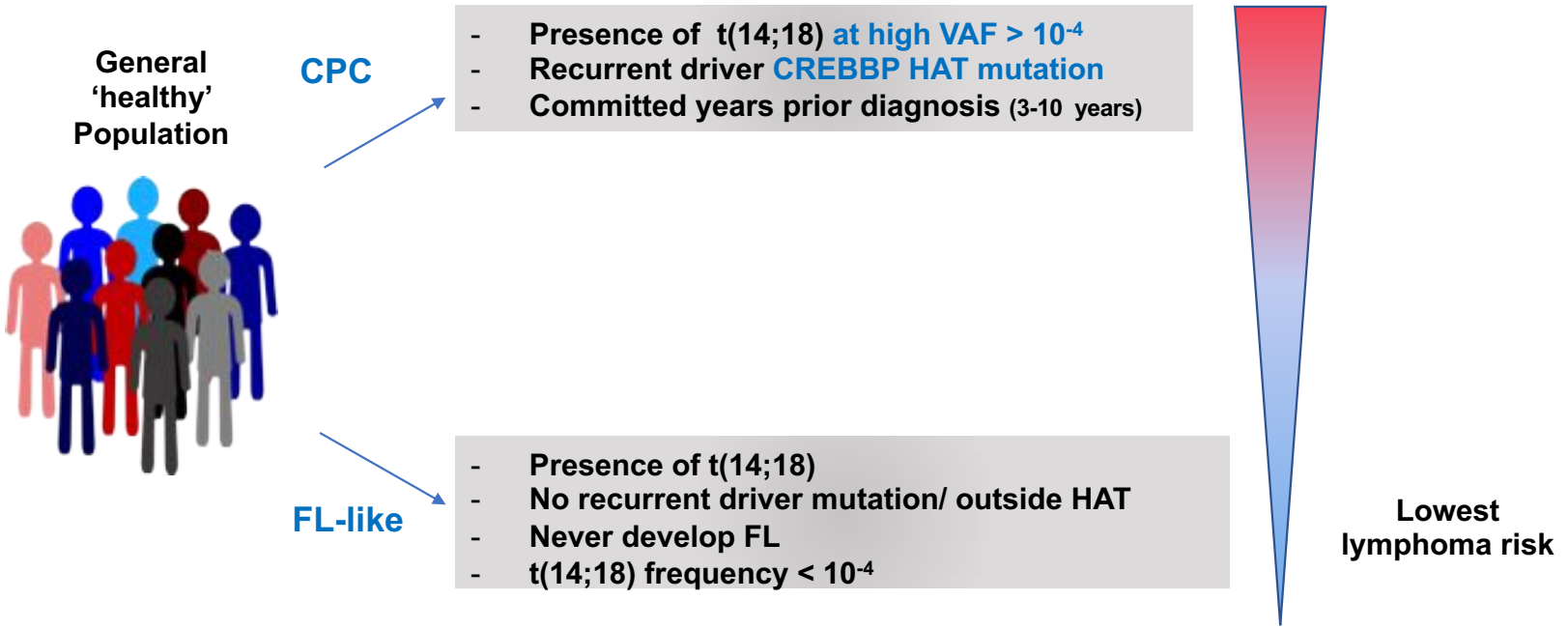
Real time monitoring (CtDNA)



Dynamic follow-up

- Detection of resistance mutations
- Relapse/transformation prediction
- Relapse/transformation detection

How to use this molecular information in the ‘preclinical’ context ?

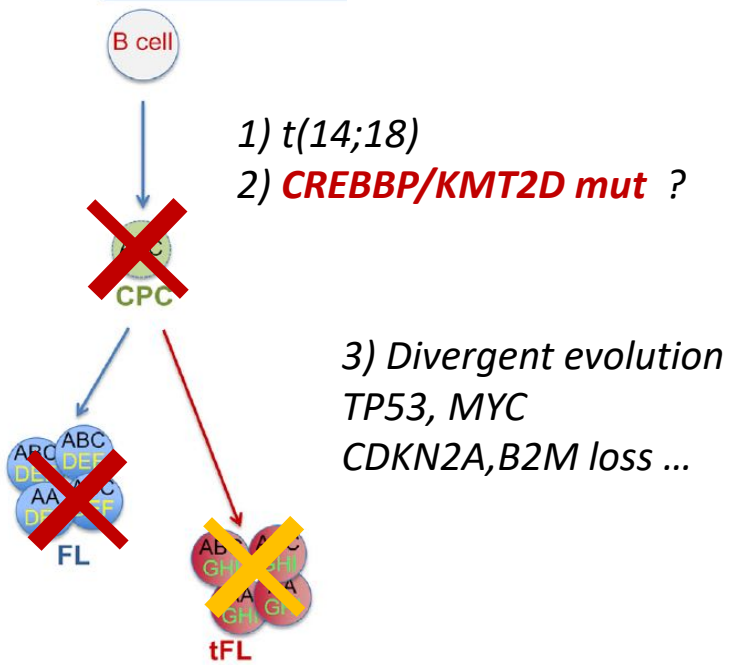


Q1 :Could we envision prevention studies / precursor mutation screening approaches in liquid biopsies from at-risk populations ?

- **The Cons**
- Watch & Wait still a SOC in indolent FL …
- **The Pros**
- Improve the predictive power of our biomarker by including more parameters: t(14;18) frequency; CREBBP KAT and at risk SNP ?
- Could help in stratifying individuals at elevated risk of clinical malignancy (ISFN ..)

Implications of CPC characterization in the clinical context

→ Q2 : If we are to target CPCs to delay/eradicate relapses, how to proceed prospectively?



Could we envision Precursor Targeted Therapies to cure FL ?

- Targeting vulnerabilities in BCL2 + KMT2D/CREBBP mutant cells could provide therapeutic benefits by eradicating the reservoir of CPCs
- **Epigenetic therapies** (HDAC3/KDM5i/EZH2) could be an important therapy to curtail the repopulating potential of this CPC population
- Opportunities for **early combination with immunotherapies**

MERCI POUR VOTRE ATTENTION

Conclusions

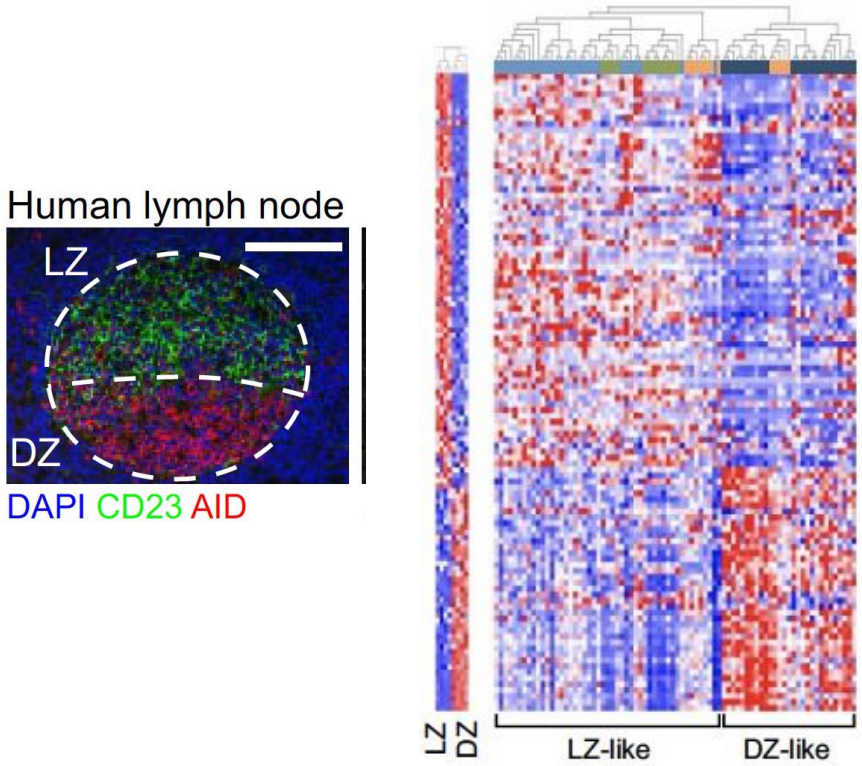
Starting to unravel discrete steps of FL tumorigenesis

- Overt FL preceded by an insidious phase of asymptomatic growth
- Path to transformation is a complex multi-hit process
- Starting from the hallmark t(14;18) & constitutive BCL2 expression
- Escalates along successive
  - derailments of B-cell receptor diversification mechanisms (cell-intrinsic hits)
  - subversion of specific immunological properties of B-cells (cell-extrinsic hits)

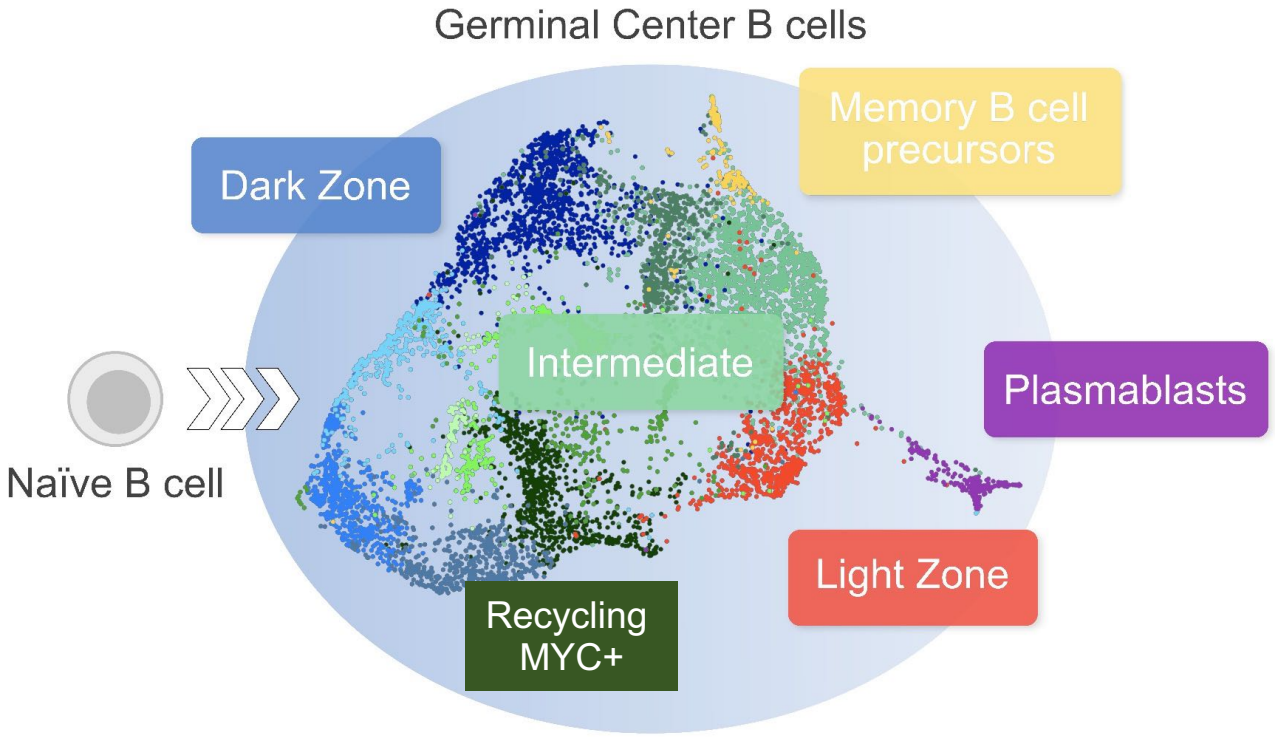
- **FL clonal dynamics is shaped very early on, long before *bona fide* malignant transformation**
- A long process requiring niches and clonal expansion
- **Primary FL emerges from committed precursor clones (CPCs) evolving & disseminating over decades, and which might participate to subsequent relapses**
- **Role of associated "early" microenvironment is probably crucial but remains to date virtually unknown** (therapeutic perspectives^{++})
- **CPC oncogenic landscape remains to be precisely defined** (therapeutic perspectives^{++})

Tumors (Lymphomas) are complex ecosystems and we are just starting to explore intra-tumor heterogeneity at single cell resolution

GC was defined in two fonctionnal zones (LZ and DZ)

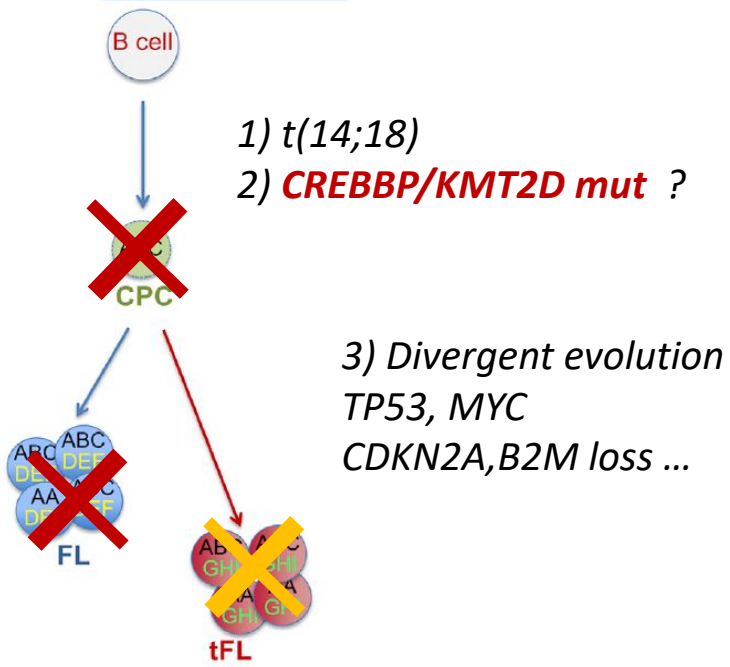


As many as 13 distinct normal GC transcriptional states revealed by single cell gene expression profiling (GEP)



> GC Bulk measurements mask cellular heterogeneity

Milpied et al. Nat Immunol 2018
Holmes et al. J Exp Med 2020



1) t(14;18)
*2) **CREBBP/KMT2D mut** ?*

3) Divergent evolution
TP53, MYC
CDKN2A,B2M loss …

Could we envision Precursor Targeted Therapies to cure FL ?

- Targeting vulnerabilities in BCL2 + KMT2D/CREBBP mutant cells could provide therapeutic benefits by eradicating the reservoir of CPCs
- **Epigenetic therapies** (HDAC3/KDM5i) could be an important buffer to reduce gene expression heterogeneity and curtail the repopulating potential of this CPC population
- Opportunities for **early combination with immunotherapies**

Can we find and destroy lymphoma precursors? Research directions and open questions

→ **We need to isolate and further characterize them (**phenotypic, spatial and functional level) before disease onset, and/or in sequential patient biopsies at diagnosis or after therapy (MRD ?)

→ We need to **integrate the study of 'early' microenvironmental factors (immune and stromal) promoting CPC genesis,**

- Spatial and kinetic heterogeneity of (pre)tumor niches (BM ? LN ? ..) – Single-cell omics
- Innovative *in vivo* preclinical mouse models

Implications of CPC characterization in the 'preclinical'/ clinical context ?

→ **Could we envision Prevention studies/ Precursor Mutation screening approaches in blood from at-risk populations?**

→ **Could we envision Precursor Targeted Therapies?**

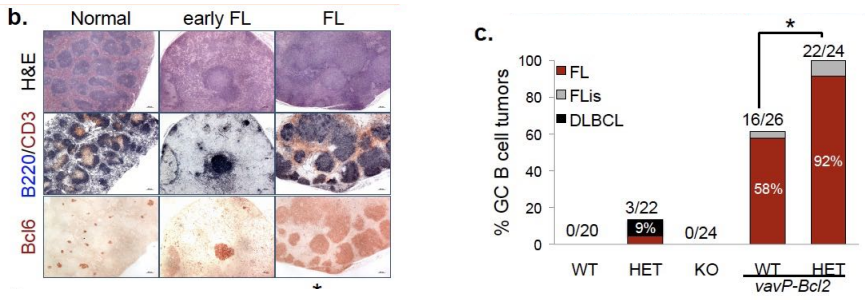
- Are CPC mutations linked to specific oncogenic dependencies ? Epigenetic therapies (HDAC3/KDM5 i) + BCL2 i
- *Epigenetic therapies could conceivably be an important buffer to reduce gene expression heterogeneity and curtail the repopulating potential of this B cell pool (Jude Fitzgibbon, Nat Rev Dis Primers, 2020)*

CHALLENGE : Dependencies could be heterogeneous considering intra-(pre)tumor heterogeneity

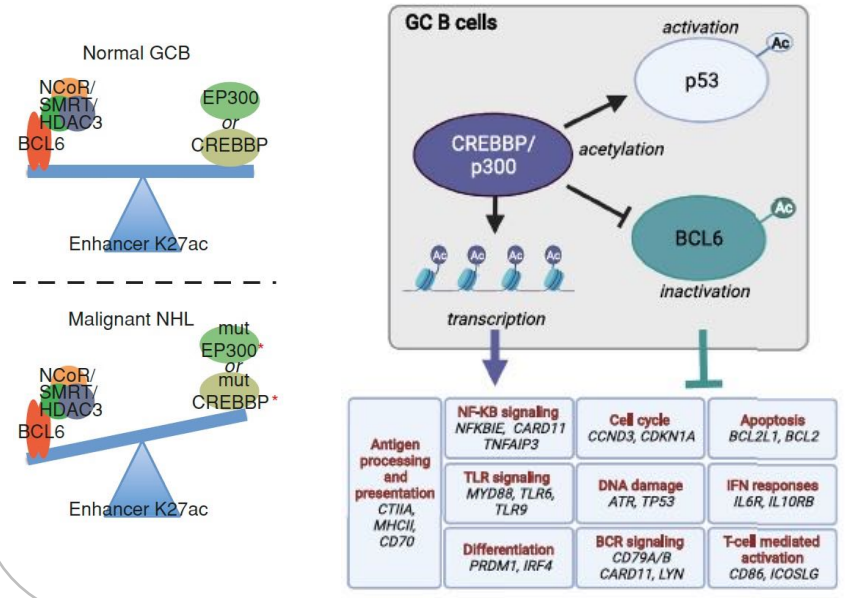
How do (epi)genetic mutations + BCL2 perturb the GC immune response to push premalignant entities into FL ?

CREBBP loss accelerates lymphomagenesis of Bcl2-driven lymphomas and favors immune escape

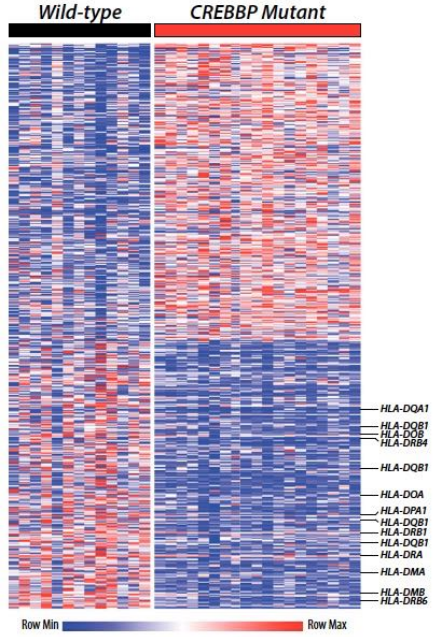
Lymphoma onset in combination with BCL2



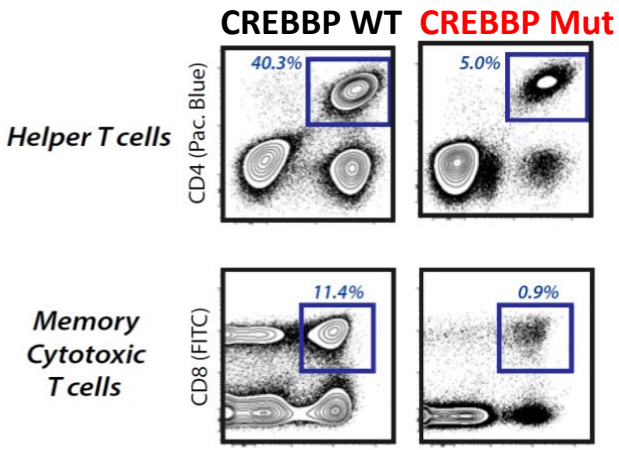
Biological programs modulated by CREBBP



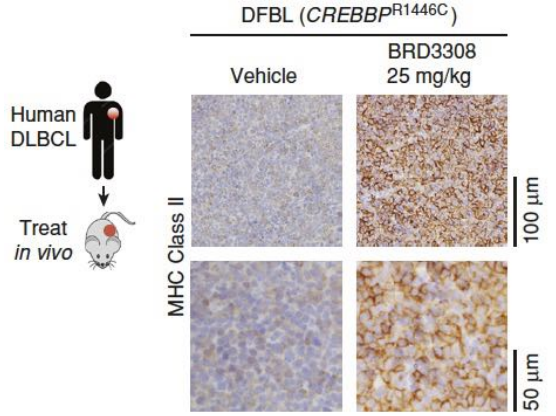
Downregulation of Ag processing and presentation



Decreased T-cell infiltration



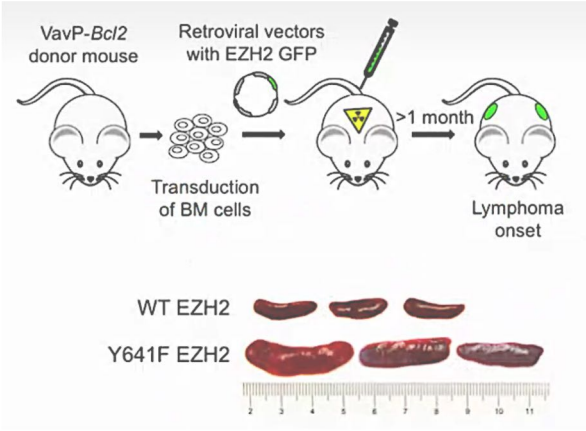
Phenotype reverted by HDAC3 inhibitors



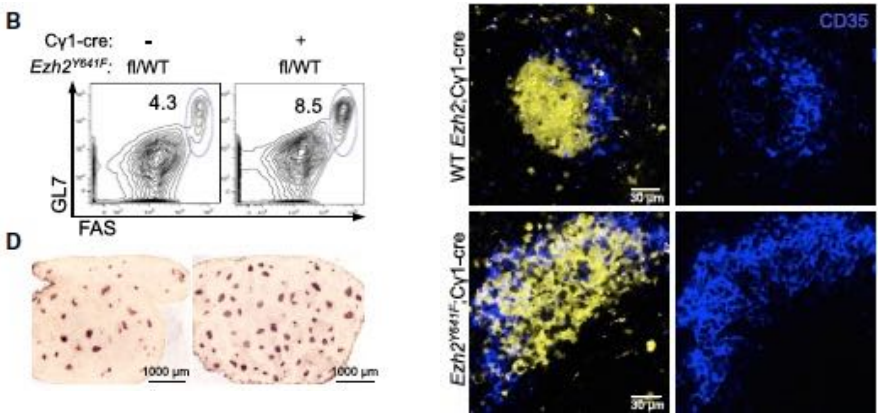
Green *et al.* PNAS 2015
Jiang et al., Nat Med, 2017
Zhang et al., Nat Med, 2017
Mondello *et al*. Cancer Discovery 2020

EZH2 mutation induces a premalignant lymphoma niche by causing a preneoplastic GC hyperplasia and reprogramming the immune synapse

Lymphoma onset in combination with BCL2

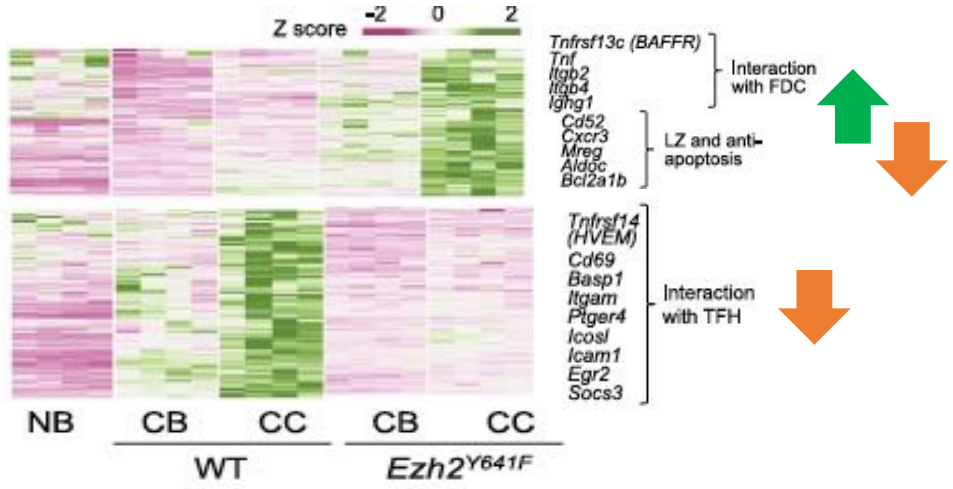


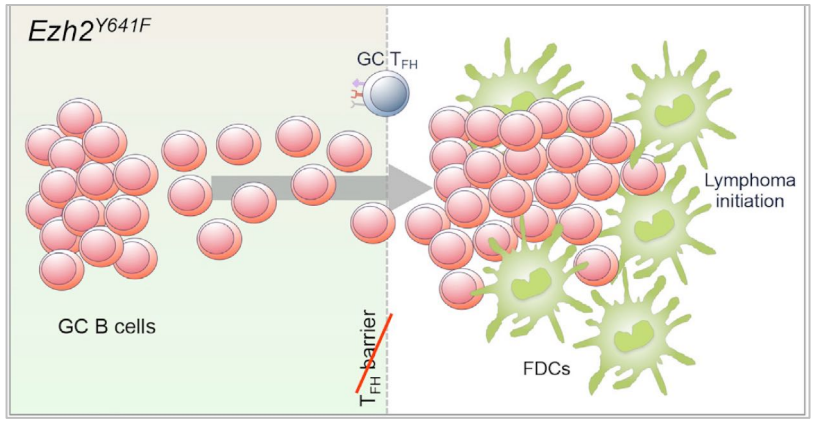
Increased GC formation and LZ expansion



B cells FDC

Premalignant remodeling of the GC niche



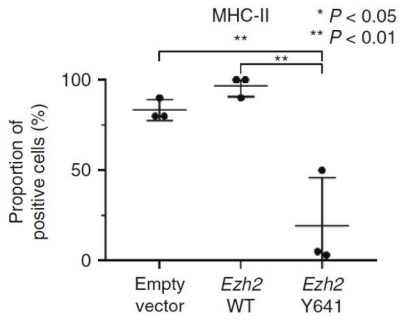


- EZH2 mutated B-cells have decreased requirement for T cell help
- EZH2 mutated B-cells have more dependency to FDC

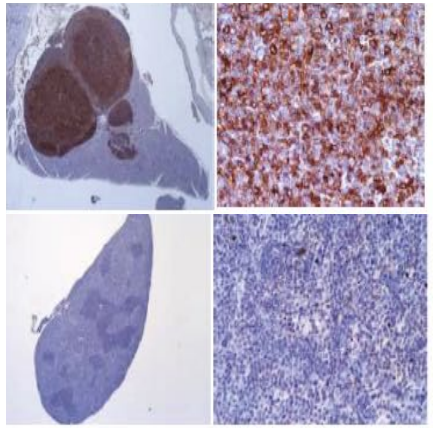
Béguelin, Cancer cell, 2013, 2016 2020

EZH2 mutations favor immune escape and EZH2 inhibitors can restore immune recognition

EZH2^{m} linked to loss of MHC expression and reduced T-cell infiltrates



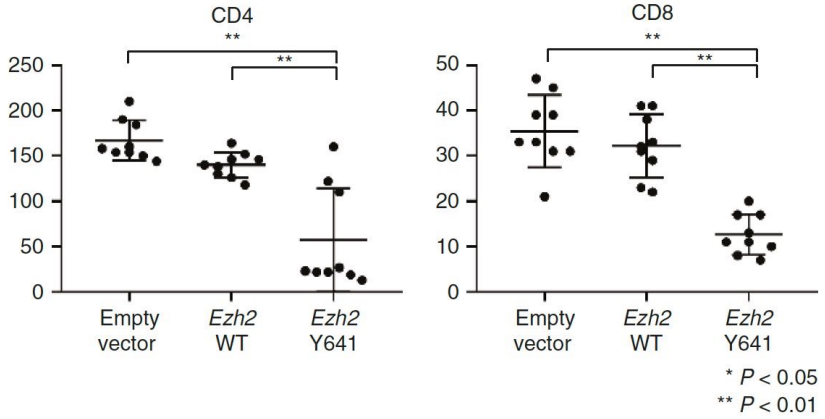
MHC-II immunostaining



WT

EZH2 Y641

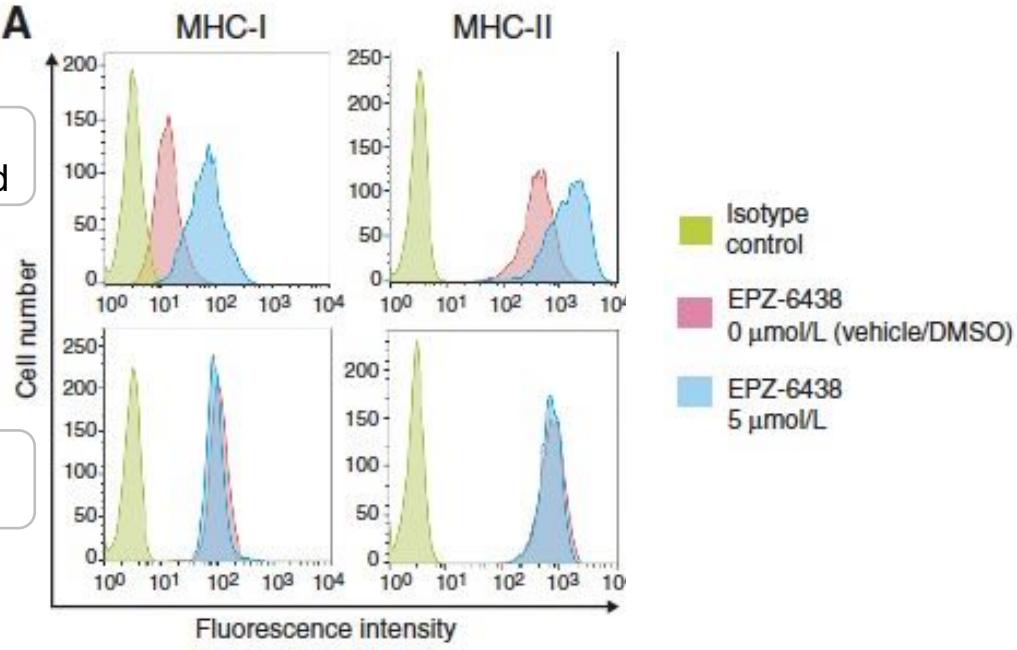
T cell infiltration in mouse tumors



MHC-I and MHC-II restoration with EZH2 inhibitor (EPZ-6438) in FL/DLBCL human cell lines

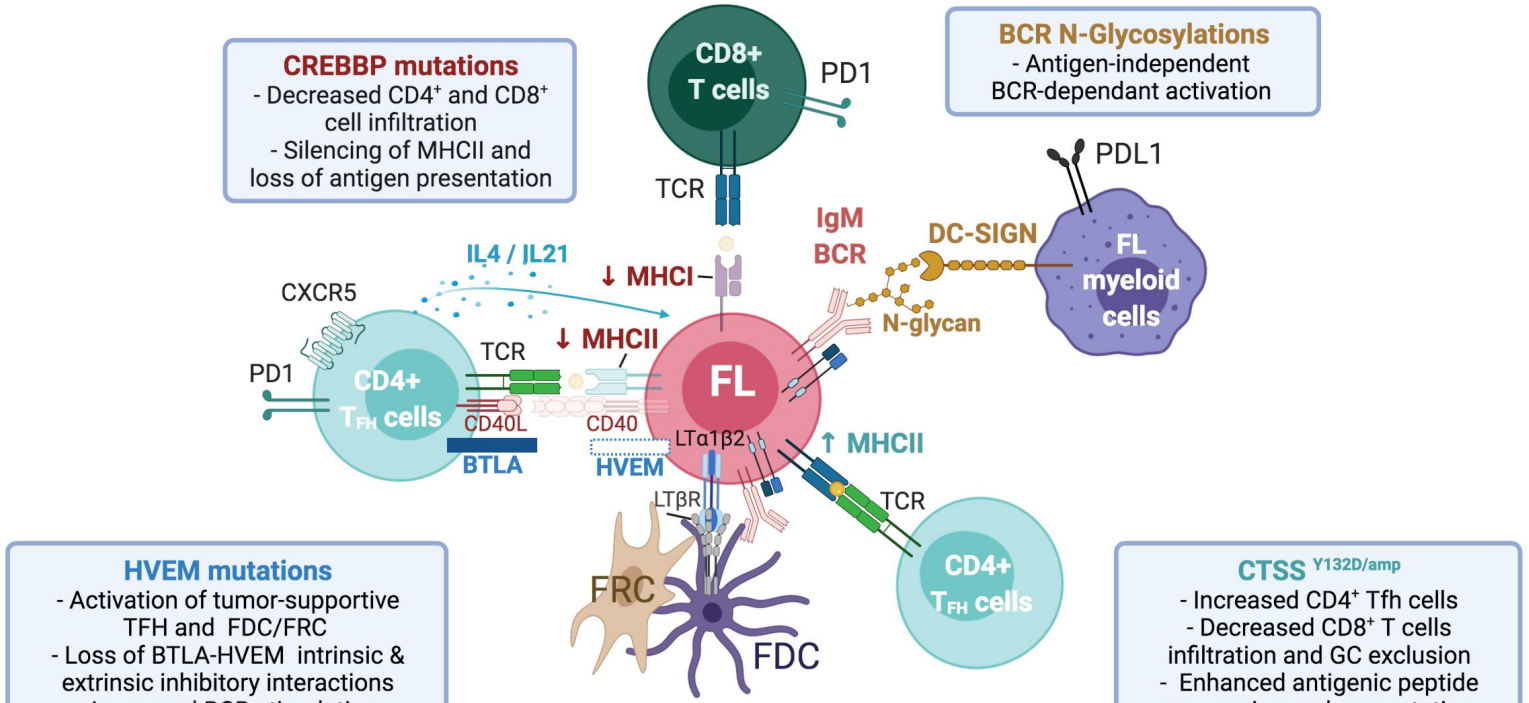
SUDHL4
EZH2 mutated

SUDHL8
EZH2 WT



Ennishi Cancer Discovery 2019

FL mutational landscape and immune microenvironment interplay

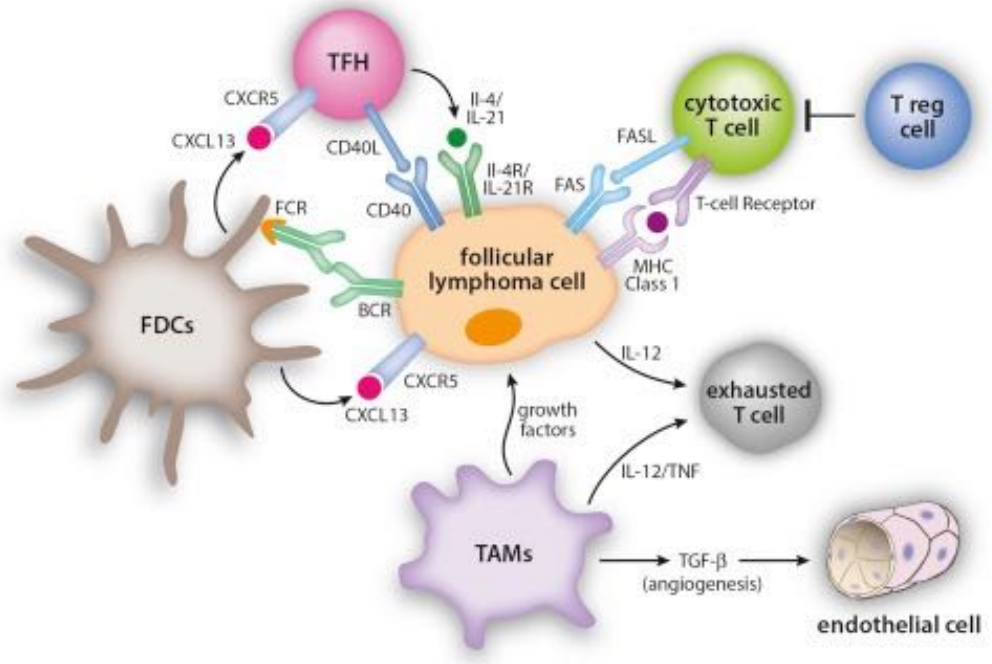


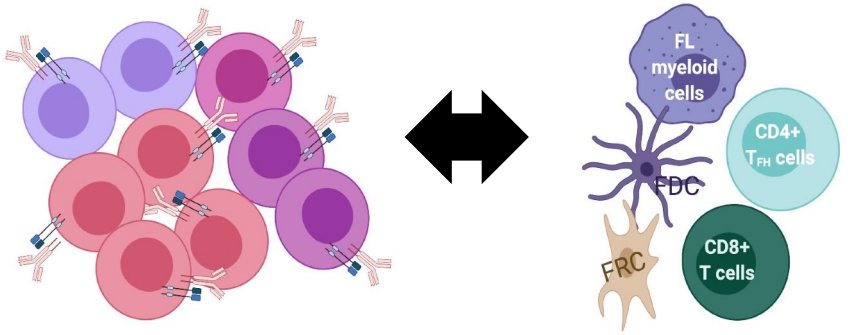
Adapted from Milpied et al. Adv Immunol, 2021

Green et al. PNAS 2015
Amin et al. Blood 2015
Linley et al. Blood 2015
Boice et al. Cell 2016
Ortega-Molina et al. Nat Metabol 2019
Mondelli et al. Cancer Disc, 2020
Béguelin et al. Cancer Cell 2020
Bararia et al., Cell Rep 2020
Dheilly et al., Cancer Cell 2020

Follicular lymphoma dynamics and heterogeneity at the single cell era

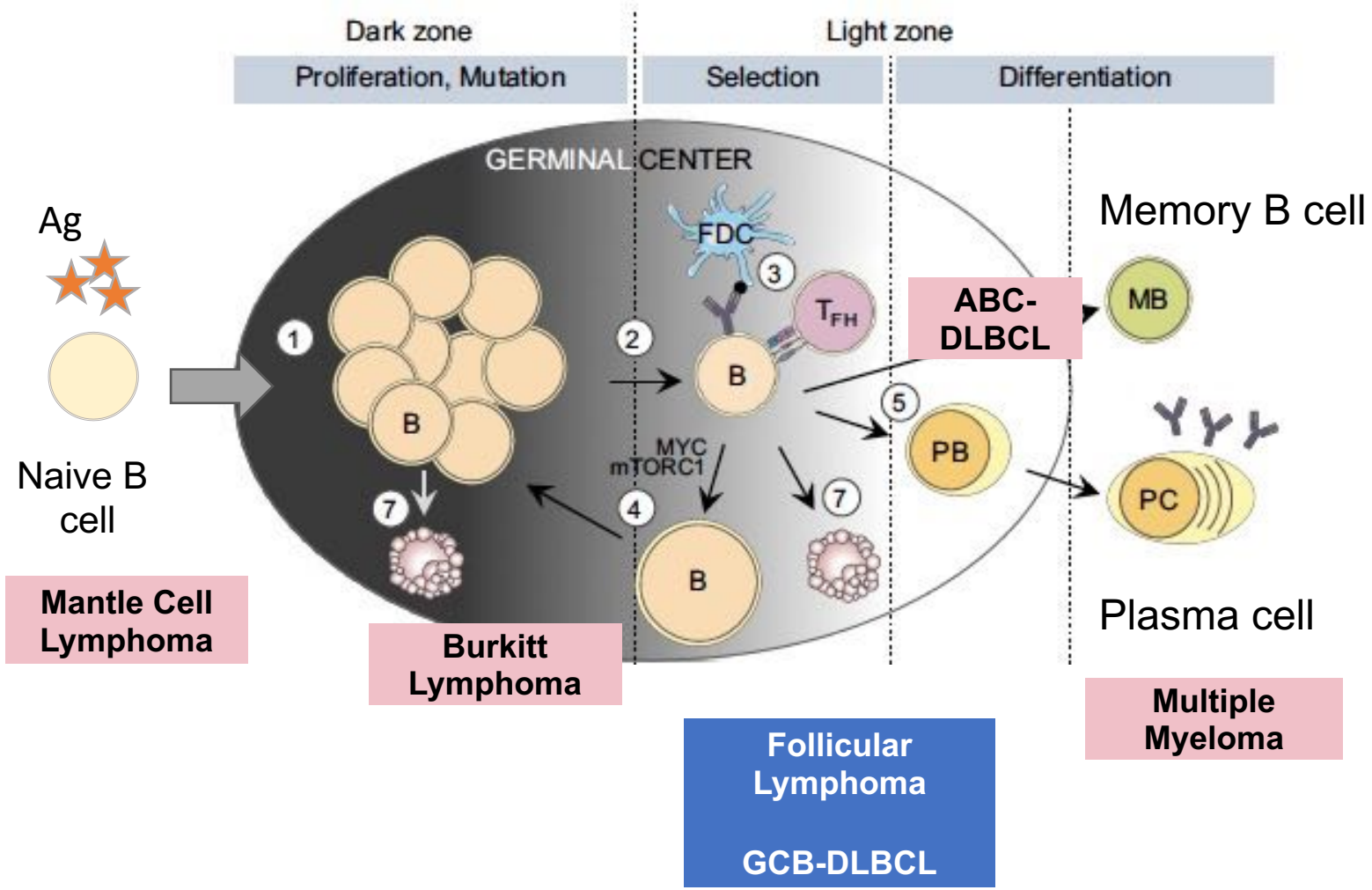
Why single cell approaches?





- Capture and identify the heterogeneity of cells
- Evaluate their functionality and interactions
- Identify common/different gene regulatory networks

B cell lymphoma subtypes reflect the origin from B cells at different stages of B cell differentiation : The Cell of Origin (COO)



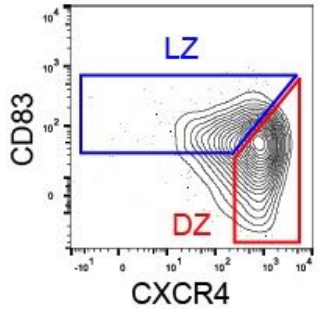
'FL is the malignant counterpart of frozen GC LZ cells '

DLBCL : Diffuse Large B Cell Lymphoma

Follicular lymphoma using bulk transcriptomic approaches

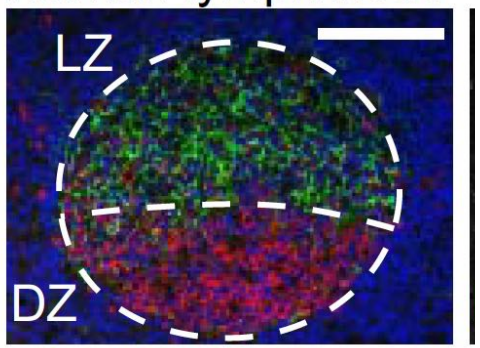
FL share a LZ-related gene expression signature → FL COO

Germinal Center (GC) B cells

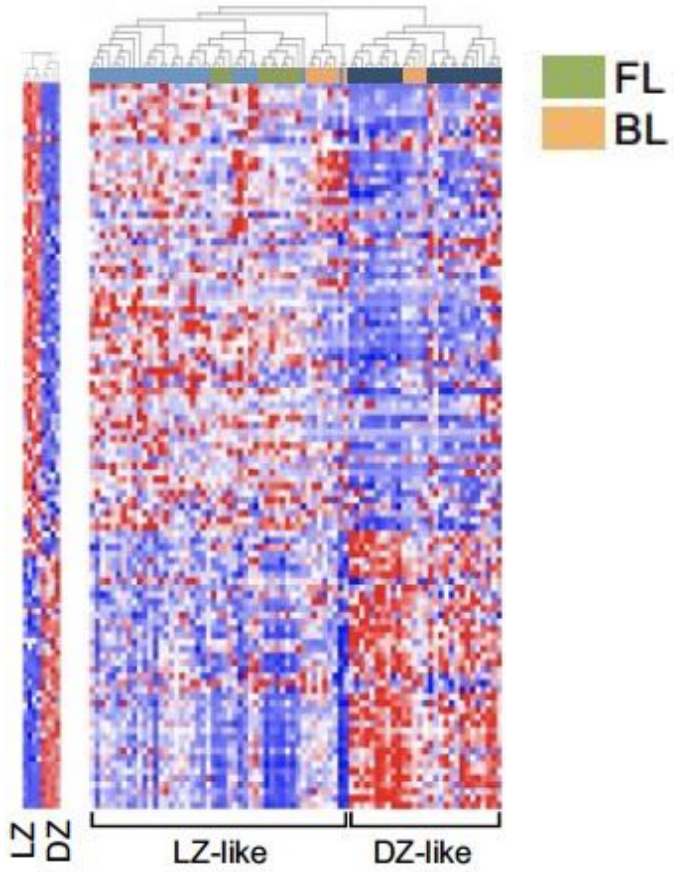


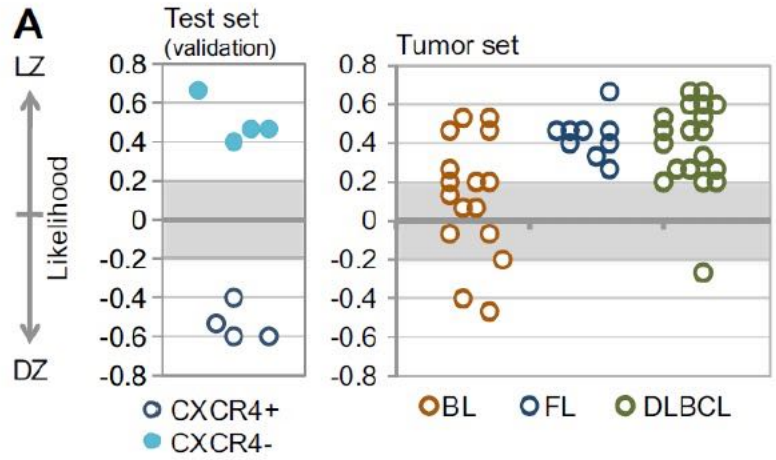
FACS sorting

Human lymph node



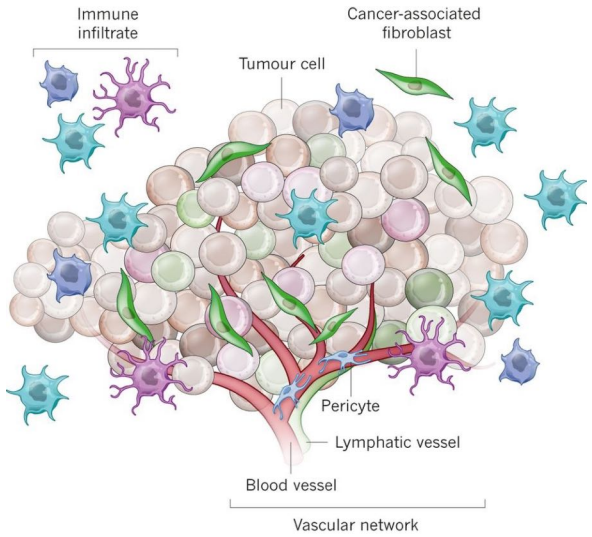
DAPI CD23 AID





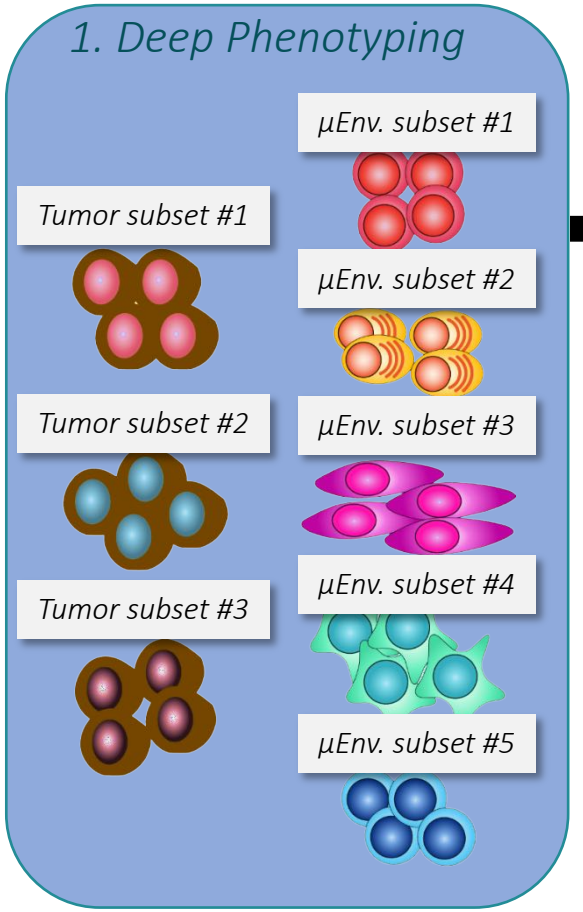
Alizadeh et al. *Nature* 2000
Victora et al. *Blood* 2012

Tumors (Lymphomas) are complex ecosystems



Tumor infiltrating leukocytes (TILs)
Malignant B cells
Stromal compartment

High throughput scRNAseq of live suspension tumor biopsy



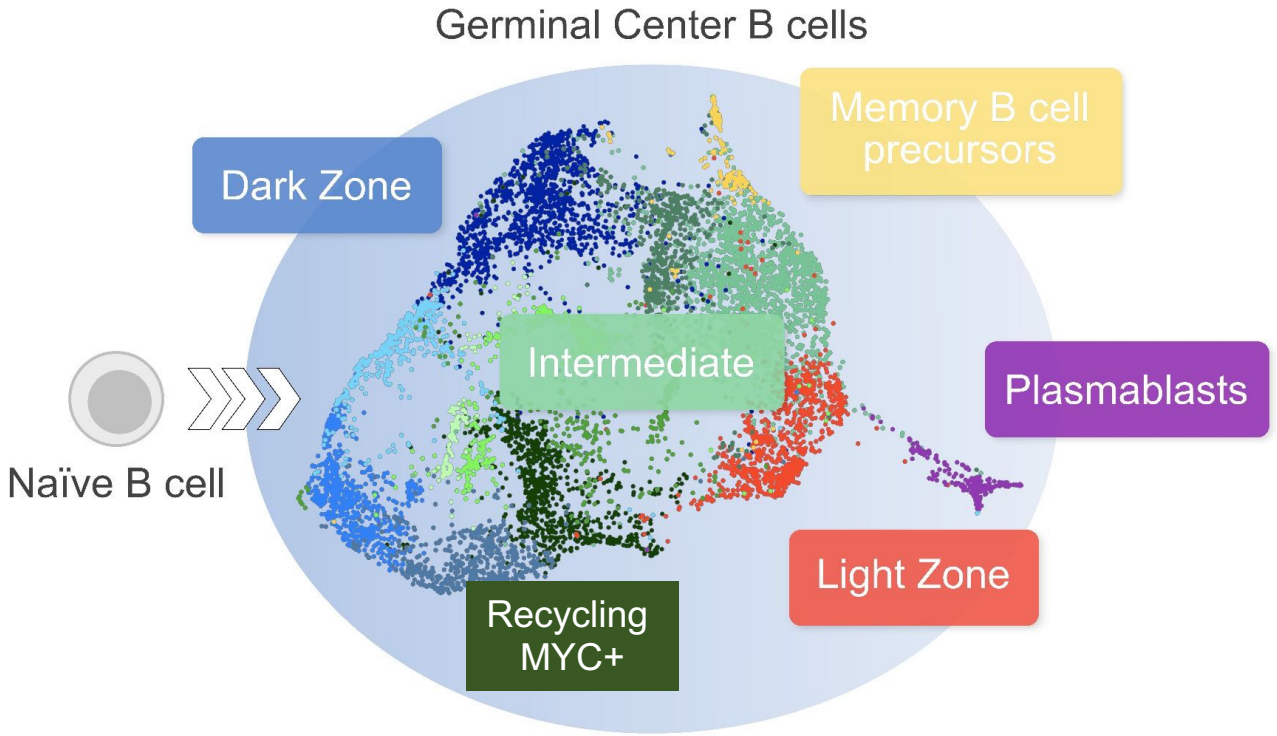
2. GEP on subsets

- ▶Cell identity
- ▶Cell function
- ▶Active biological pathways
- ▶Gene regulatory networks

- ▶Prognostic impact
- ▶New Tx targets
- ▶Refine classification

Follicular lymphoma dynamics and heterogeneity at the single cell era

As many as 13 distinct normal GC transcriptional states revealed by single cell gene expression profiling (GEP)

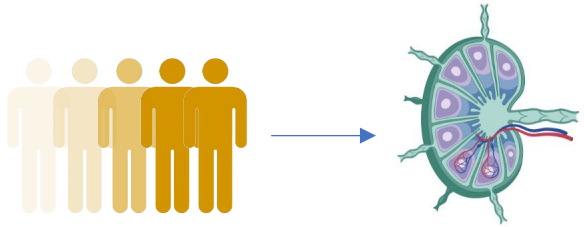


Milpied et al. Nat Immunol 2018
Holmes et al. J Exp Med 2020

GC Bulk measurements mask cellular heterogeneity

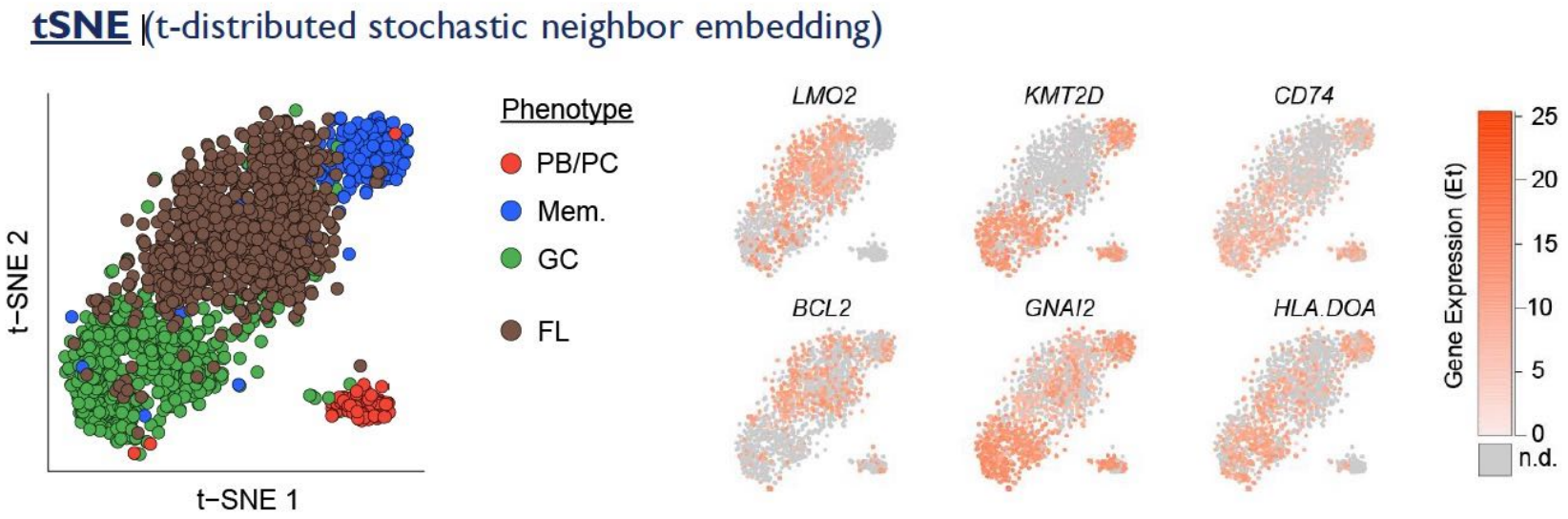
Can we better specify where FL cells are ‘frozen’ and if it differs between patients ?

Follicular lymphoma dynamics and heterogeneity at the single cell era



N=5 FL patients

LN (viable cells)

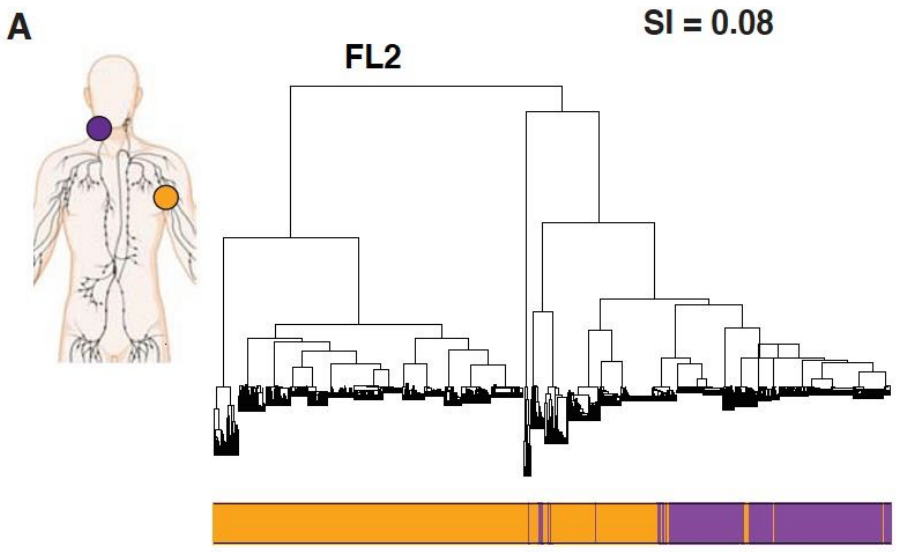


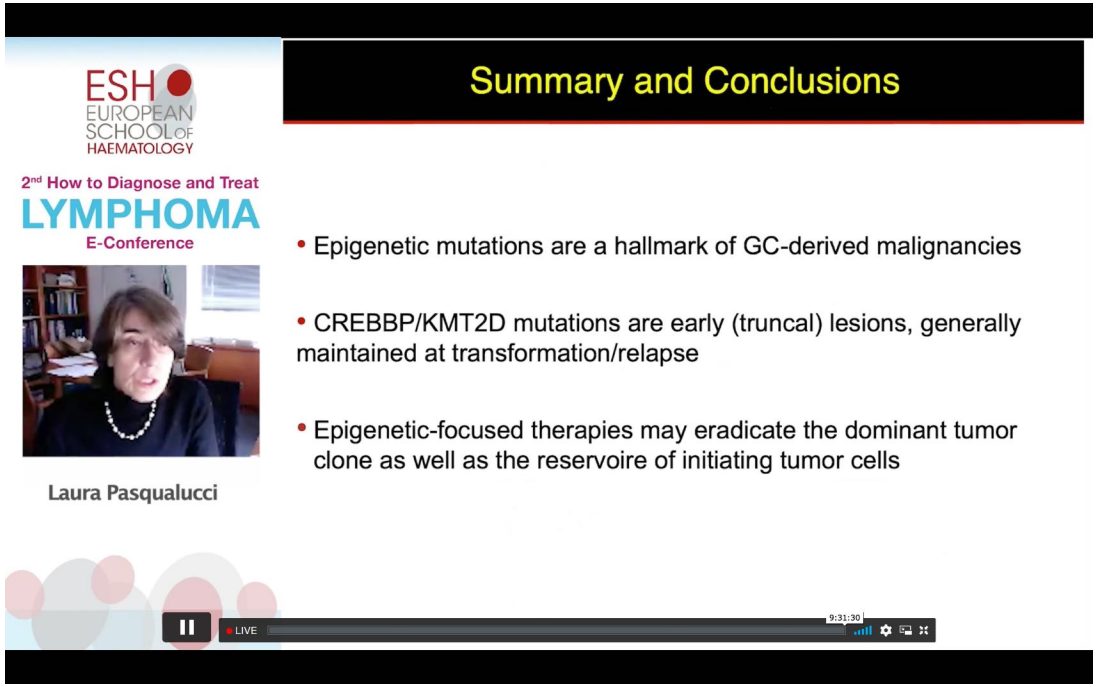
► FL B-cells are a distinct cell type (different from GC B-cells)

► FL-specific gene expression signatures & altered functions (MHC-II presentation, migration)

➢ **Strong common transcriptional signature → defines FL B cells as a distinct cell type from their putative GC B cell COO**

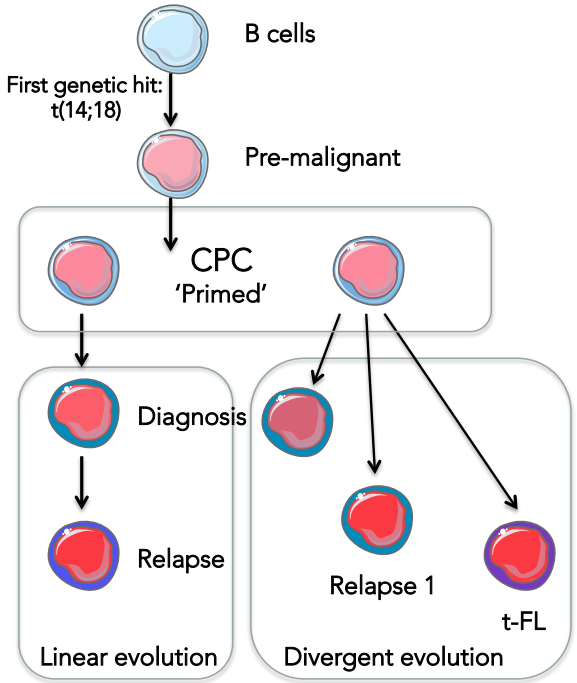
Intra-patient transcriptional diversity of FL cells





Why FL is a good place to start talking about precursors ?

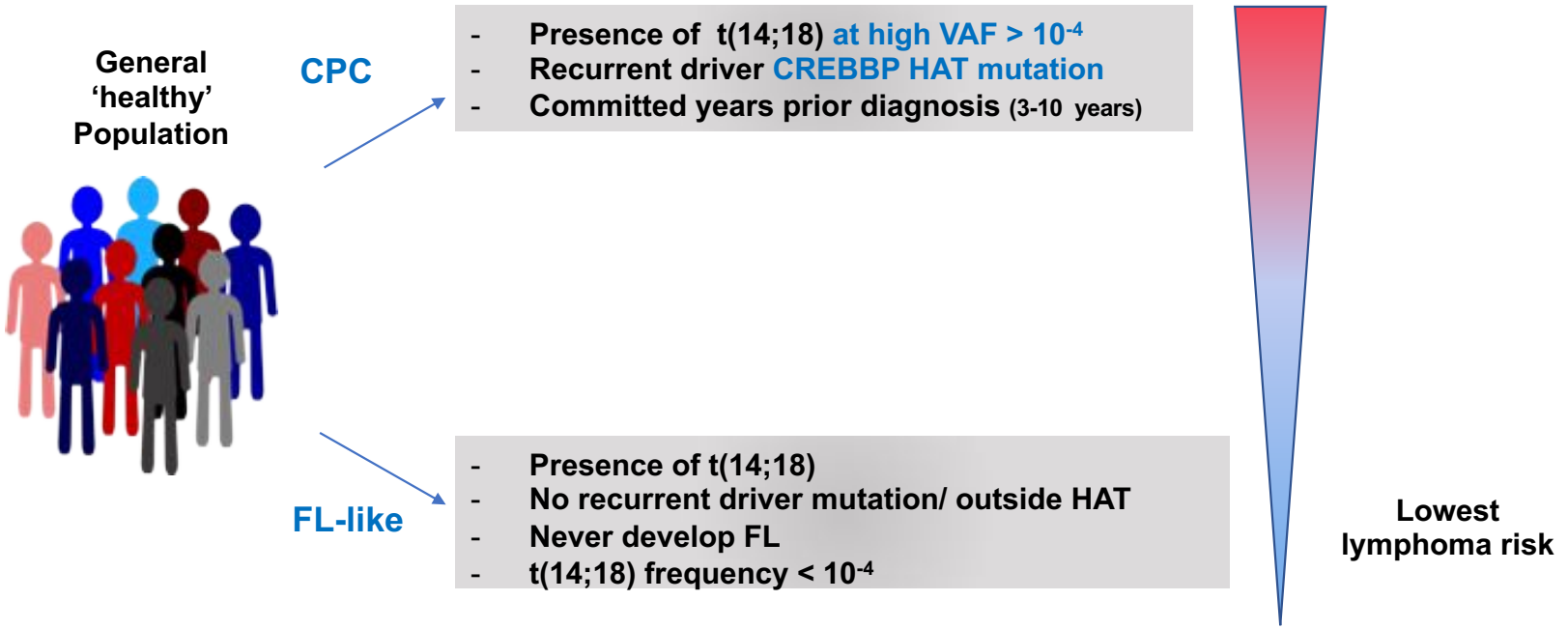
- Most frequent indolent B cell Lymphoma (median OS :15 years)
- Very effective therapies (anti-CD20 immunotherapy +/- chemo) but FL remains incurable
- Clinical challenges → Contrasting clinical behaviours
  - Clinical course punctuated by relapse/remitting periods becoming refractory to therapy
  - **20% of cases : Early progressors (POD24**) and **FL transformation in high-grade lymphoma**



- Relapses rarely derive from direct evolution from the dominant clone at diagnosis but from divergent evolution from antecedent clones
- **Suggest the existence of a Precursor population before disease onset which serves as a root to propagate relapse**
- **Targeting the Cancer Precursor cell (CPC) may be key for therapy to be curative**

Okosun et al Nat Genetics 2014
Pasqualucci et al Cell Reports 2014
Green et al PNAS 2015
Kridel et al Plos Med 2016
Odabashian et al Blood 2020

How to use this molecular information in the ‘preclinical’ context ?



Q1 :Could we envision prevention studies / precursor mutation screening approaches in liquid biopsies from at-risk populations ?

- **The Cons**
- Low incidence – Technological challenge (CT DNA profiling at large scale ?)
- Watch & Wait still a SOC in indolent FL …
- **The Pros**
- Improve the predictive power of our biomarker by including more parameters: t(14;18) frequency; CREBBP KAT and at risk SNP ?
- Could help in stratifying individuals at elevated risk of clinical malignancy (ISFN ..)

Implications of CPC characterization in the clinical context

Q2 : If we are to target CPCs to delay/eradicate relapses, how to proceed prospectively?

- FL CPC is still an elusive population that remains to be **isolated and functionally characterized** (phenotypic, spatial and transcriptomic level)
  - Are early CPC present before disease onset = Relapse initiating cells ?

- We need to understand **tumor-Immune relationships and spatial heterogeneity of pre-tumor niches** and how they contribute to the intratumor heterogeneity seen in FL patients

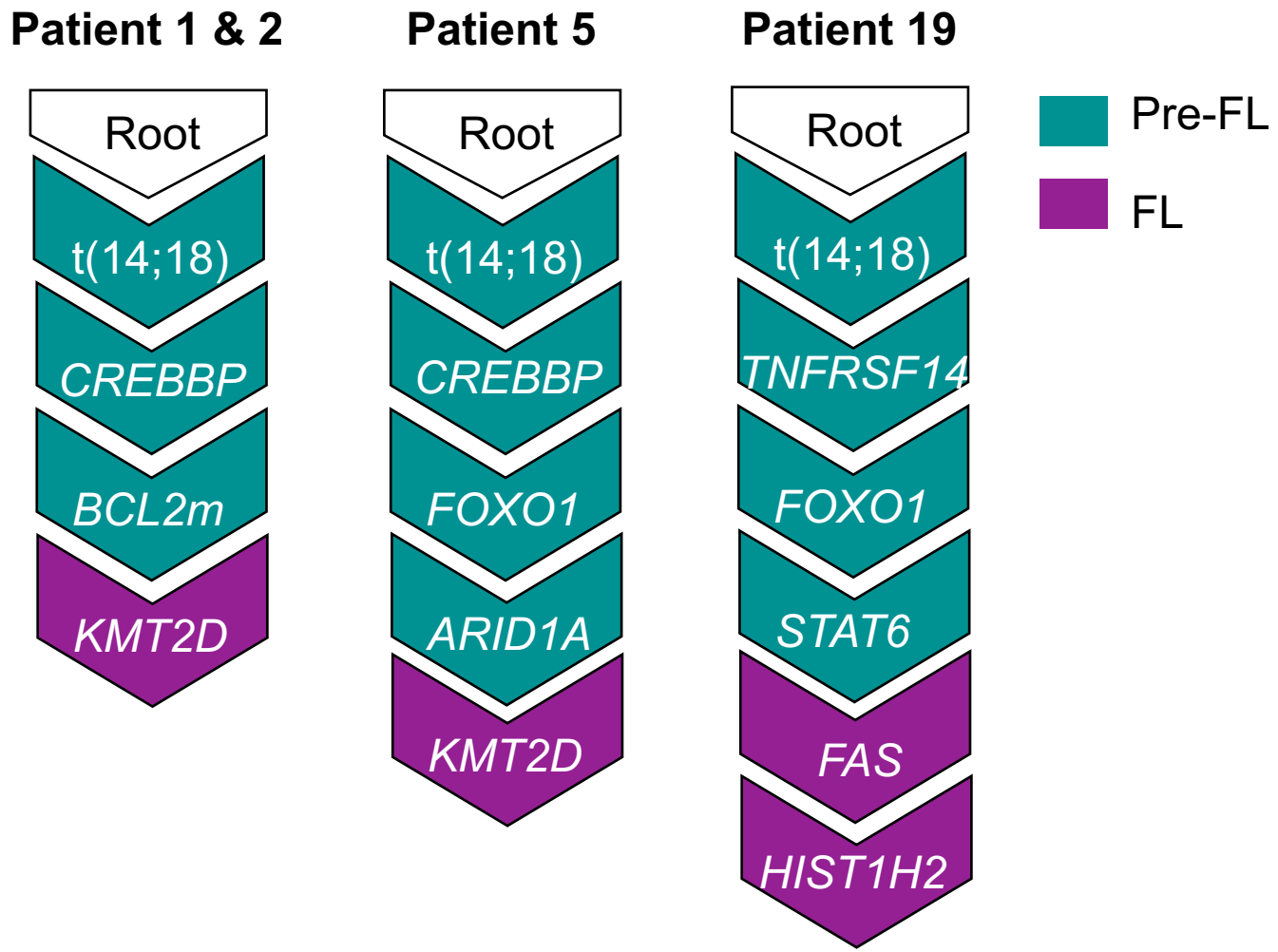
- **Are CPC recurrent mutations linked to specific oncogenic dependencies ?**

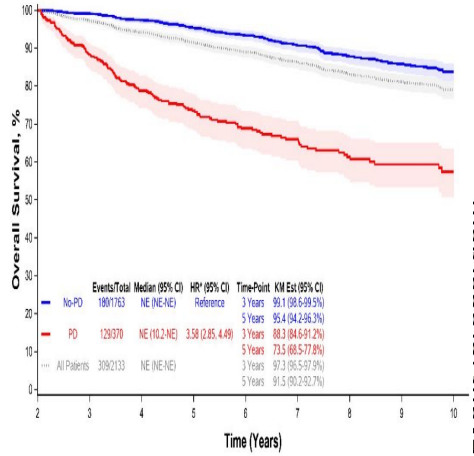
  - **Small inhibitors** (HDAC3i /KDM5i /EZH2i /Venetoclax …) could provide therapeutic benefits by curtailing the repopulating potential of this CPC population, restoring immune surveillance and reduce FL intratumor heterogeneity

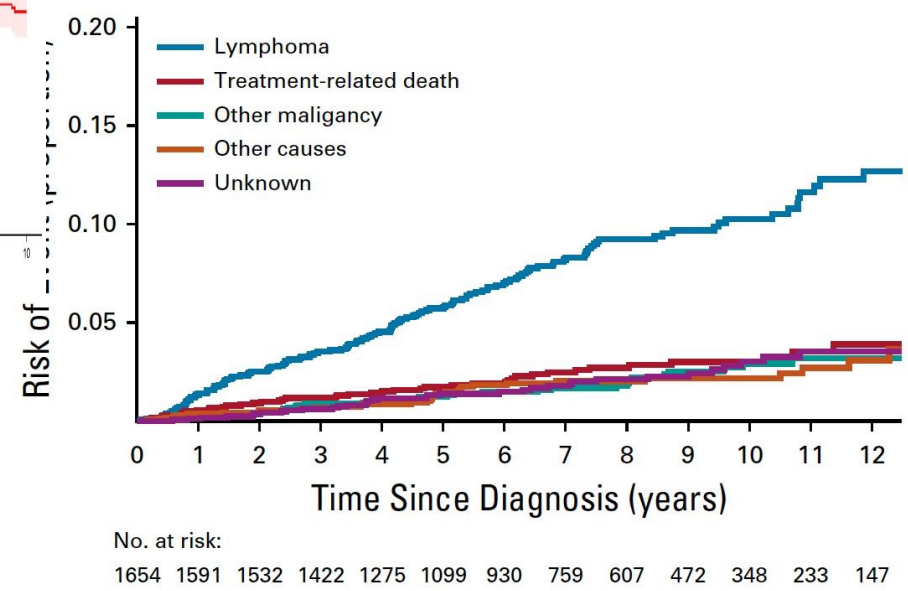
  - Opportunities to combine targeted drugs with **immunotherapies**

What does this tell us about the order of events ?

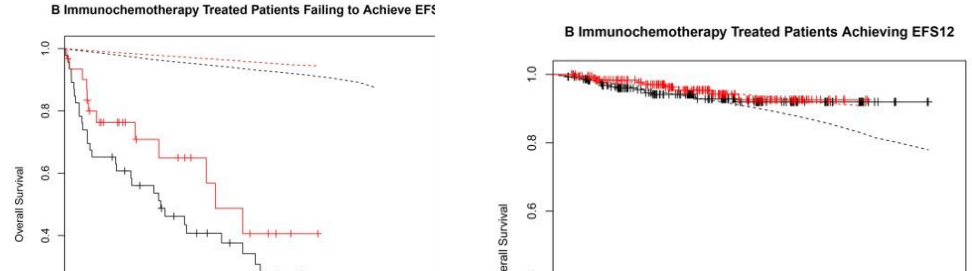
t(14;18) and CREBBP mutation predominates as early genetic events while KMT2D or EZH2 appears to happen later and closer to diagnosis







Matthew J. Maurer, BJH, 2016



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Challenge and opportunities:

If we want to change/intercept the natural history of FL before it occurs or re-emerges, we need to DECRYPT the biology of antecedent subclones

What are the different flavors of FL precursors/CPCs and which one are giving rise to relapses ?

What is the genomic landscape of the CPCs and can we define the kinetics of events acquisition ?

How do CPC mutations cooperate to progressively build FL and generate the large intra-tumor heterogeneity we see at diagnosis ?

Are CPC features linked to specific oncogenic dependencies ? Can we use these insights to propose novel therapeutics to target the CPC?