Linfoma Follicolare
Basi biologiche

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The big unmet need: from FL biology to FL patients

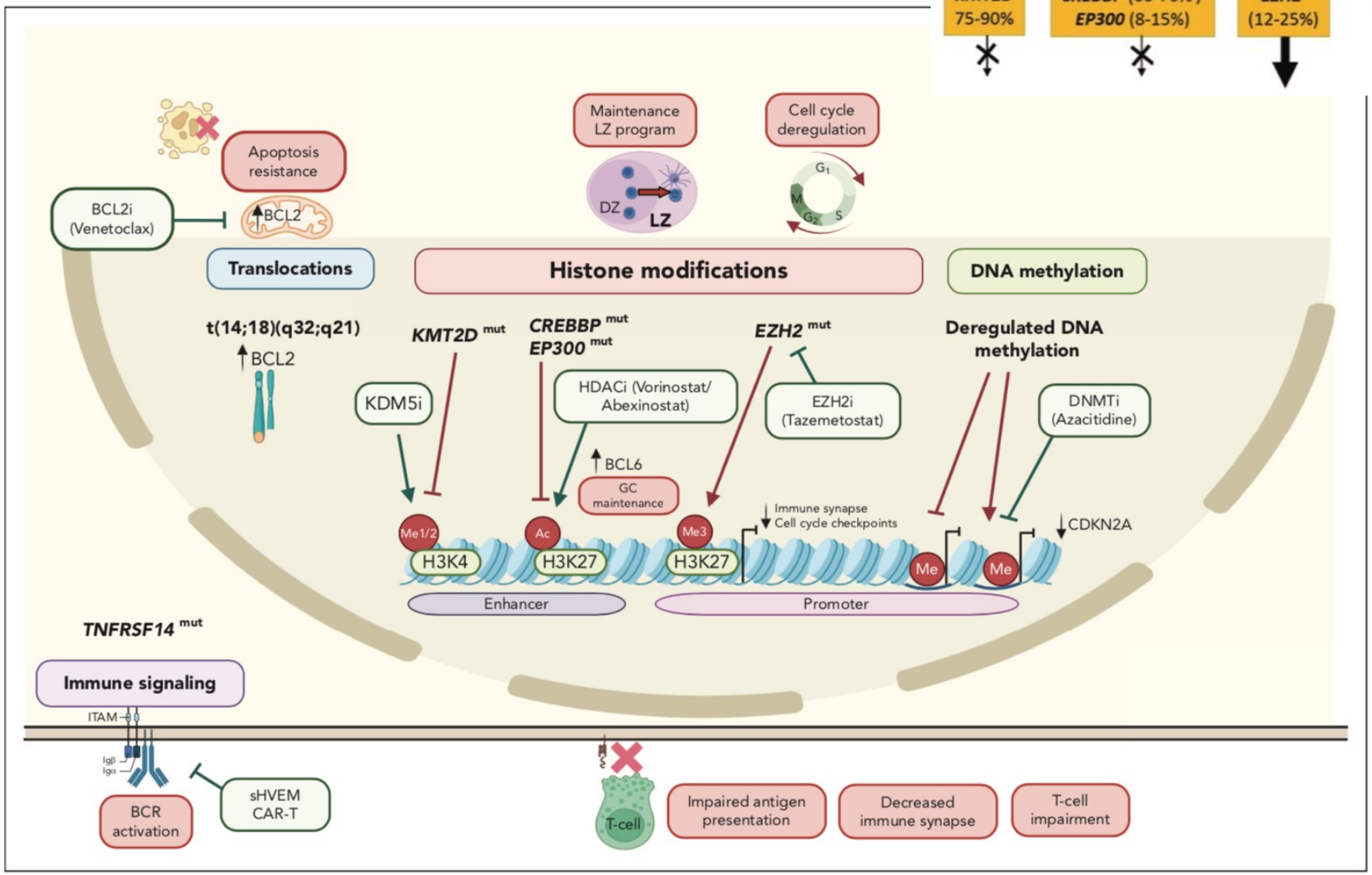
Understanding FL biology
is a critical step to:

1) defining the biological basis of tumor heterogeneity;
2) identifying biomarkers of outcomes;
3) defining targetable pathways.

Clinical unmet needs:

1) predict patients' course or response to treatment
2) Identify high risk patients and improve outcome
3) Predict and prevent transformation

Unlike DLBCL, FL lacks defined genetic or transcriptional subtypes,
adding complexity to understanding the disease and
developing tailored therapeutic strategies.



t(14;18) the primary event that upregulates the antiapoptotic BCL2 protein.

Most frequently mutated genes: chromatin-remodeling genes, which affect **acetylation (CREBBP and EP300**) or **methylation (KMT2D and EZH2)** histone marks in enhancers or promoters.

These modifications affect:

- the **B-cell transcriptome**, promoting increased proliferation and maintenance of the LZ program;
- - the **tumor microenvironment (TME),** leading to T-cell impairment, reduced antigen presentation, and a weakened immune synapse.

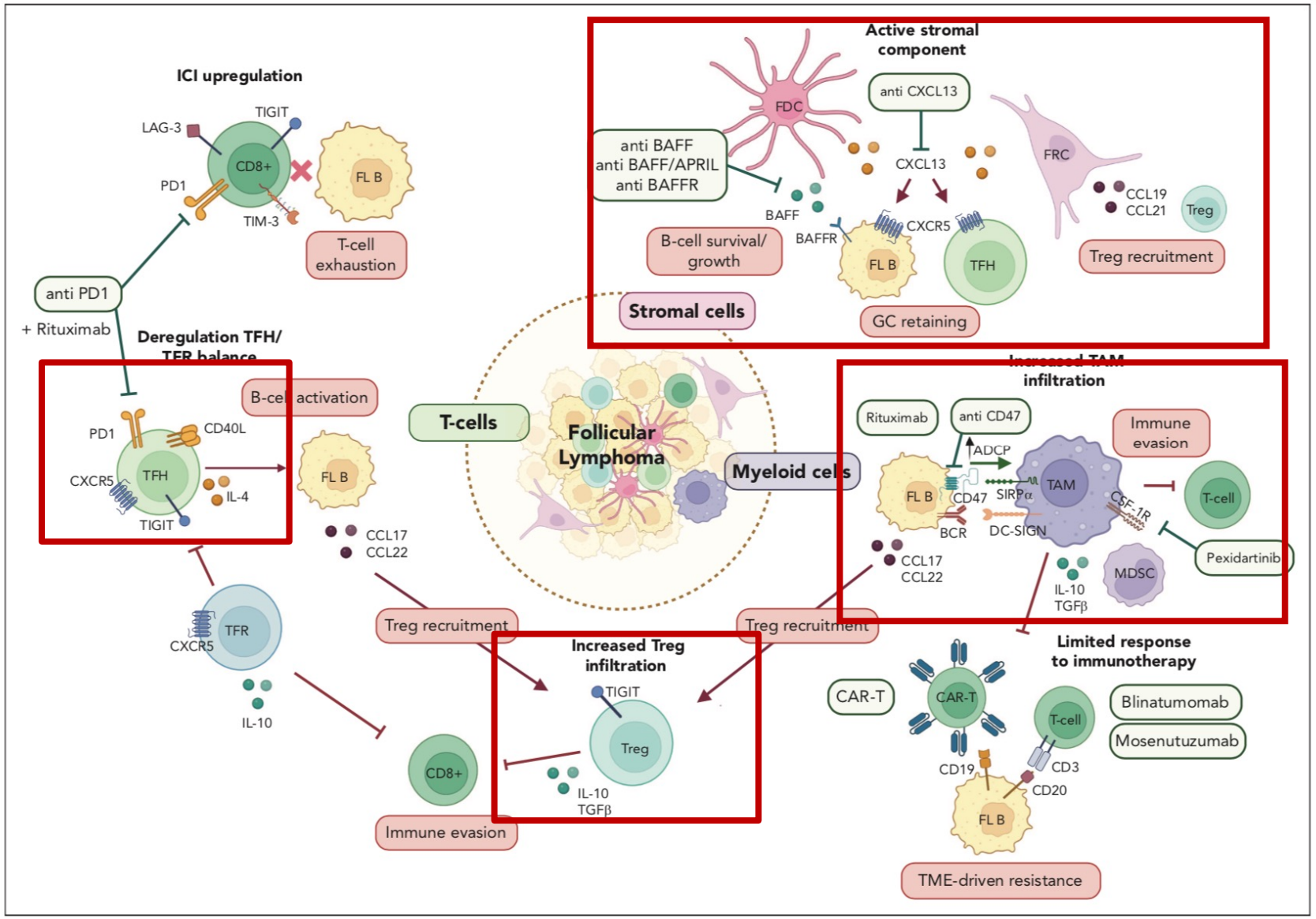
DNA methylation is also deregulated in FL, with both hypomethylating and hypermethylating imbalances reported.

Genetic and epigenetic alterations can be targeted by drugs (green boxes).

TNFRSF14 mutations impair HVEM-BTLA interactions, resulting in **increased BCR activation.**

HVEM, herpesvirus entry mediator; B-and T-lymphocyte attenuator

Araujo-Ayala and Béguelin, Blood 2025

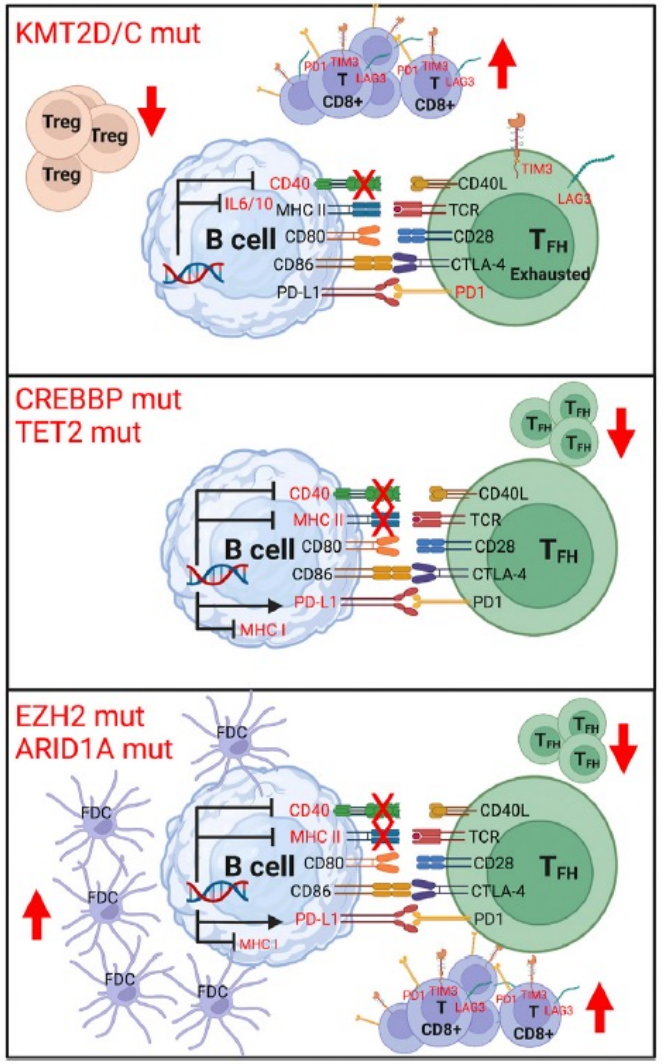


Stromal compartment: FDCs promote FL B-cell survival via BAFF, IL6 and CXCL13 and retain TFH and B cells through the CXCL13-CXCR5 axis.
LSC (lymphoid stromal cells) & **FRC** (fibroblast reticular cells)

FL-supportive TME comprises T cells, myeloid cells, and stromal cells.
T cells, particularly **TFH cells**, support FL B cells (IL4 secretion, CD40L stimulation).
Immunosuppressive **Tregs** are recruited via CCL17/22, produced by TFH-stimulated FL B cells and TAMs.
CCL19/21, produced by FRCs, induce TAM polarization and recruits TFH, which further support FL B cells and recruit Tregs.
The antitumor function of **CD8+ T cells** is compromised by exhaustion (increase TIM3, PD1, LAG3, TIGIT).
TAM infiltration is increased, displaying immunosuppressive properties through IL10 and TGFbeta -> expand Tregs and suppress cytotoxic T cells. Promotes BCR activation.
TAMs polarize into protumorigenic (M2) phenotypes, supporting tumor progression and boosting ADCP, but show a reduced phagocytic capacity related to FL B-cell expression of CD47, a “do not eat me” signal.

Araujo-Ayala and Béguelin, Blood 2025
Laurent et al, Blood 2024

Genomic alterations subvert FL TME



Recurrent genetic alterations allow the escape from immune surveillance and shift some immune and stromal cell subsets towards a supportive phenotype in FL.

1) Mutations in KMT2D/C suppress CD40, IL10-IL6, NF-kB signaling, with **prevalence of exhausted CD8+ T cells and decrease of Treg cells.**

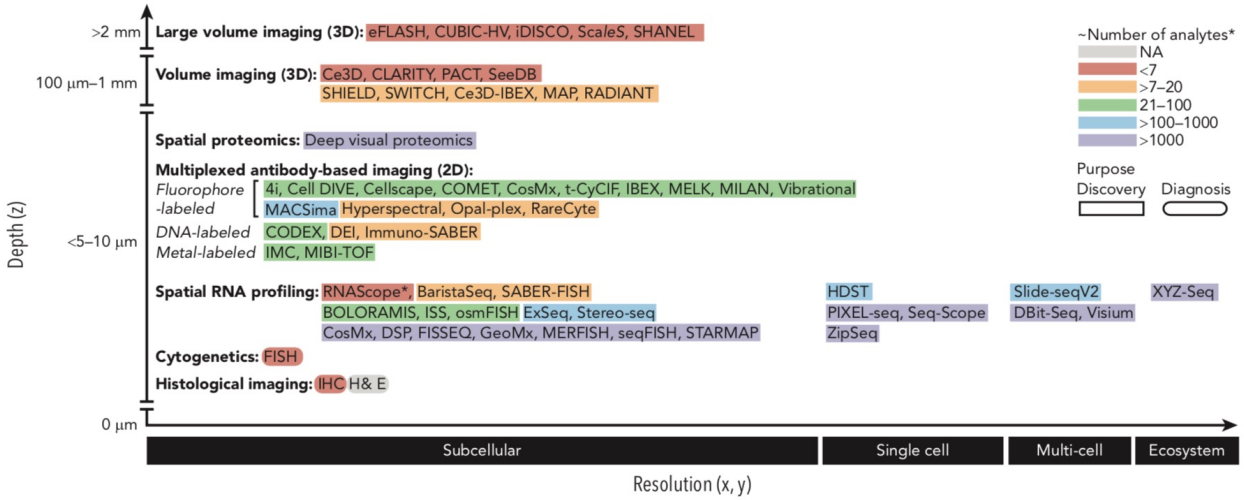
2) Mutations in **CREBBP repress antigen presentation genes (such as MHC class II, its transcription factor CIITA),** increase inhibitory molecules (such as PDL1), with lower infiltration of CD4+ T cells.

3) Mutations in **EZH2** and ARID1A (right lower panel) repress CIITA (master regulator of MHC class II), NLRC5 (transactivator of MHC class I) and CD40, **with decrease in T follicular helper (TFH) cells and increase in follicular dendritic cells (FDC).**

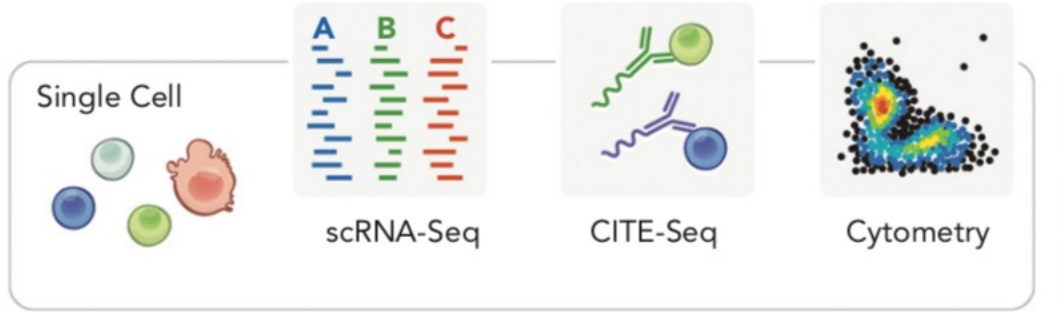
4) The gene encoding **herpesvirus entry mediator A (HVEM)/TNFRSF14** is inactivated by mutations/deletions at 1p36 region in half of FL cases. The disruption of the HVEM–**BTLA (B and T lymphocyte attenuator)** inhibitory axis has two main consequences: i) the BCR is released, thus **activating the BCR pathway and promoting cell-autonomous signals of growth and survival**; ii) increased **recruitment of T follicular helper (TFH)** cells and increased production of TNF family cytokines that activate the lymphoid stroma (follicular dendritic cells and fibroblast reticular cells).

Mondello, Ansell, Nowakowski. Front Genetics 2022

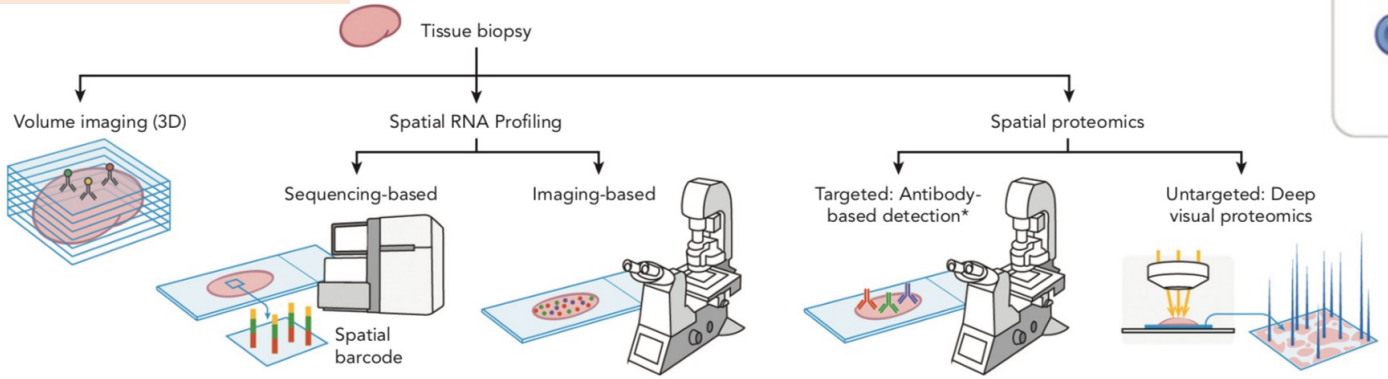
Good News!
FL biology at single-cell and spatial resolution



Bulk analyses



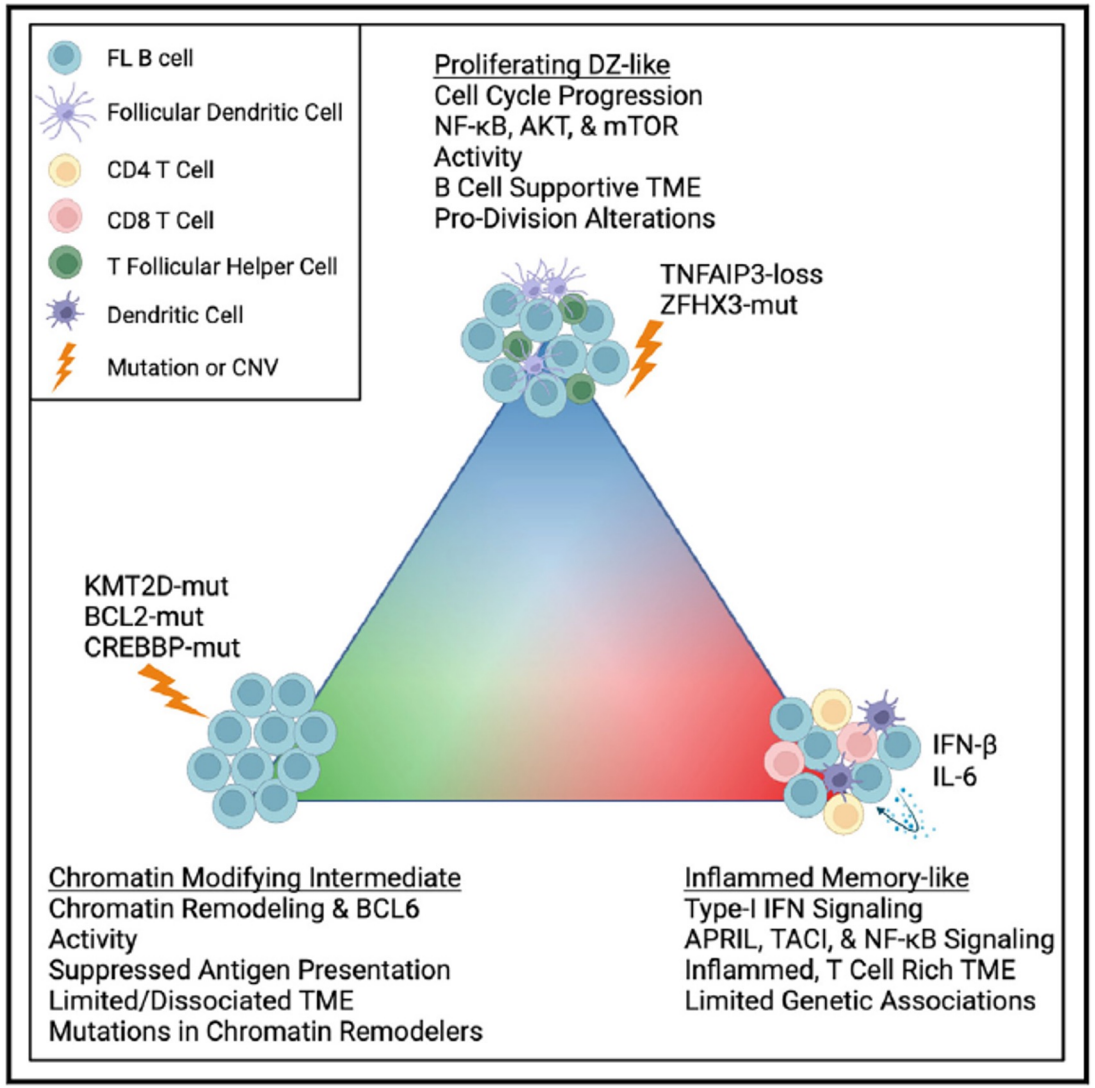
Spatial technologies



Single cell

Radtke & Roschewski. Blood 2024

FL: 3 trascriptional states (bulk RNA seq)



Krull et al, Cell Reports Medicine 2024

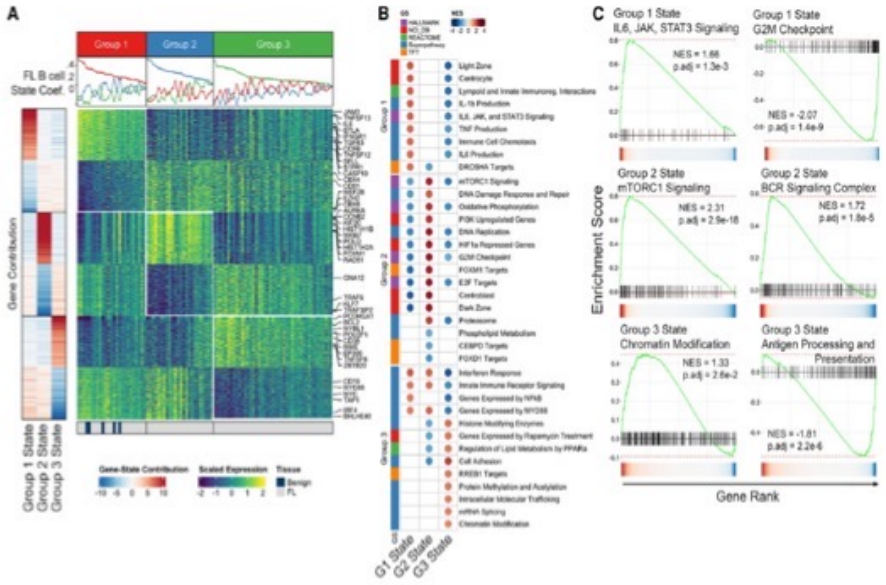
RNA-seq on B cells sorted from 87 FL biopsies
Integrated with immune profiling (CyTOF from cell suspensions, n = 60)
WES on tumor/normal pairs from FL tumors (n = 123), 119 of which had matched RNA-seq from both sorted and bulk data

Validation to 4 independent FL datasets of diagnostic FL biopsies (n = 742) with bulk RNA-seq

- B cells from FL exhibit 3 distinct transcriptional states
- FL B cells differ by enhanced **inflammation (G1), proliferation (G2), or chromatin remodeling (G3)**
- Tumor cell states correlate with unique immune-microenvironment features
- Unique mutation and CNV profiles highlight potential genetic causes of heterogeneity

MAYO Clinic = FL in watch and wait, RT, R-CHT, R monotherapy

Transcriptome and GC programs



G1 state was most enriched in programs related to type 1 interferon (IFN) signaling, IL-6 signaling, MYD88/NF-kB (nuclear factor kB), and repressed cell cycle

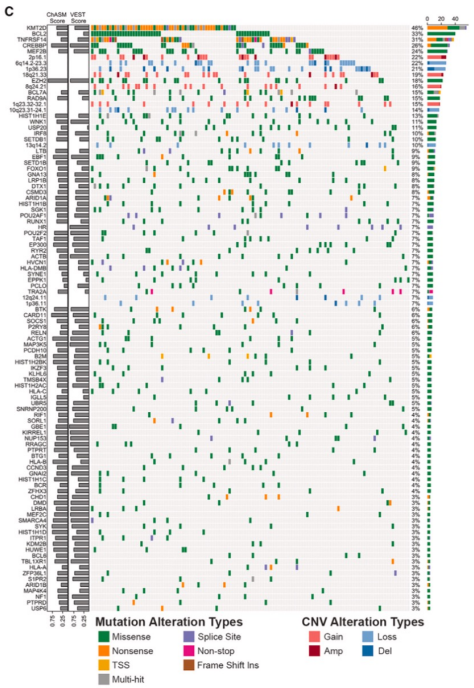
Inflamed-antiproliferative memory-like (INFM)

No significantly enriched variants

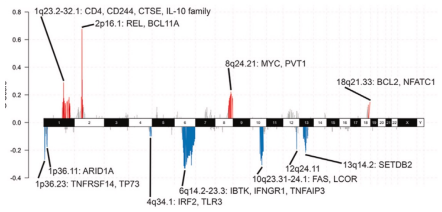
G2 state was dominated by proliferative signals, metabolic reprogramming, m-TORC and B cell receptor (BCR) signaling, and response to stress or damage

Proliferative dark-zone-like (PDZ)

Mutated immunoregulatory genes (*HLA-B* and b*2M*), copy-number losses to 6q14.2–23.3 of the NF-kB inhibitor gene *TNFAIP3*, and genes associated with cell growth (*ZFHX3*) and differentiation (*UBR5*).



WES

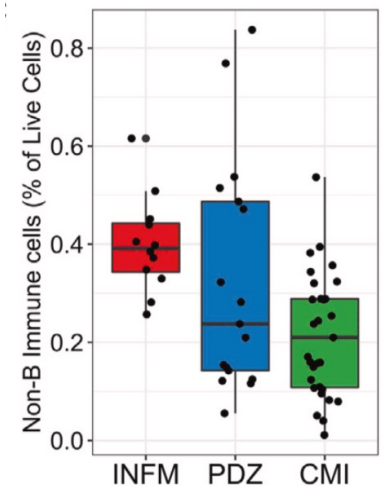


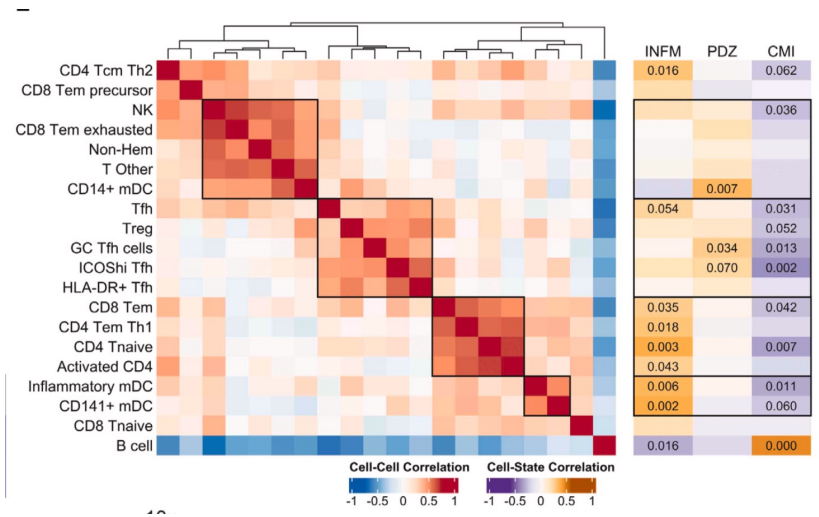
G3 was defined by a positive enrichment in programs related to epigenetic reprogramming, metabolic reprogramming, and cell adhesion, in addition to the negative enrichment of MYD88/NF-kB and inflammation

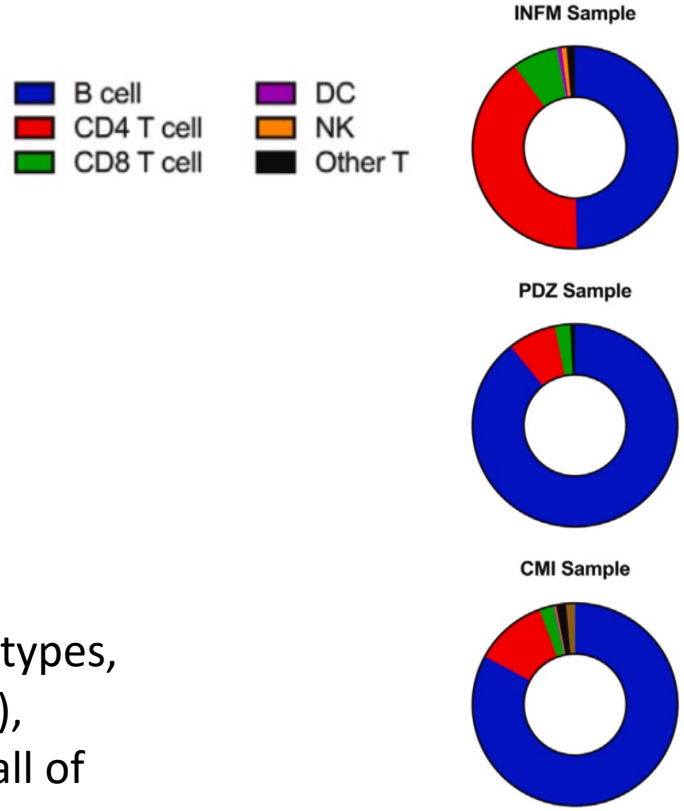
Chromatin modifying intermediate (CMI): 50.4%

Alterations in chromatin-modifying genes (*CREBBP* and *KMT2D)*, mutations in *BCL2*, 10q23.31–24.1 copy-number losses, and copy-number gains at 2p16.1, including *REL*

Krull et al, Cell Reports Medicine 2024







The highest mean percentage of immune cells being detected in INFM (40%) and the lowest being detected in CMI (21%)

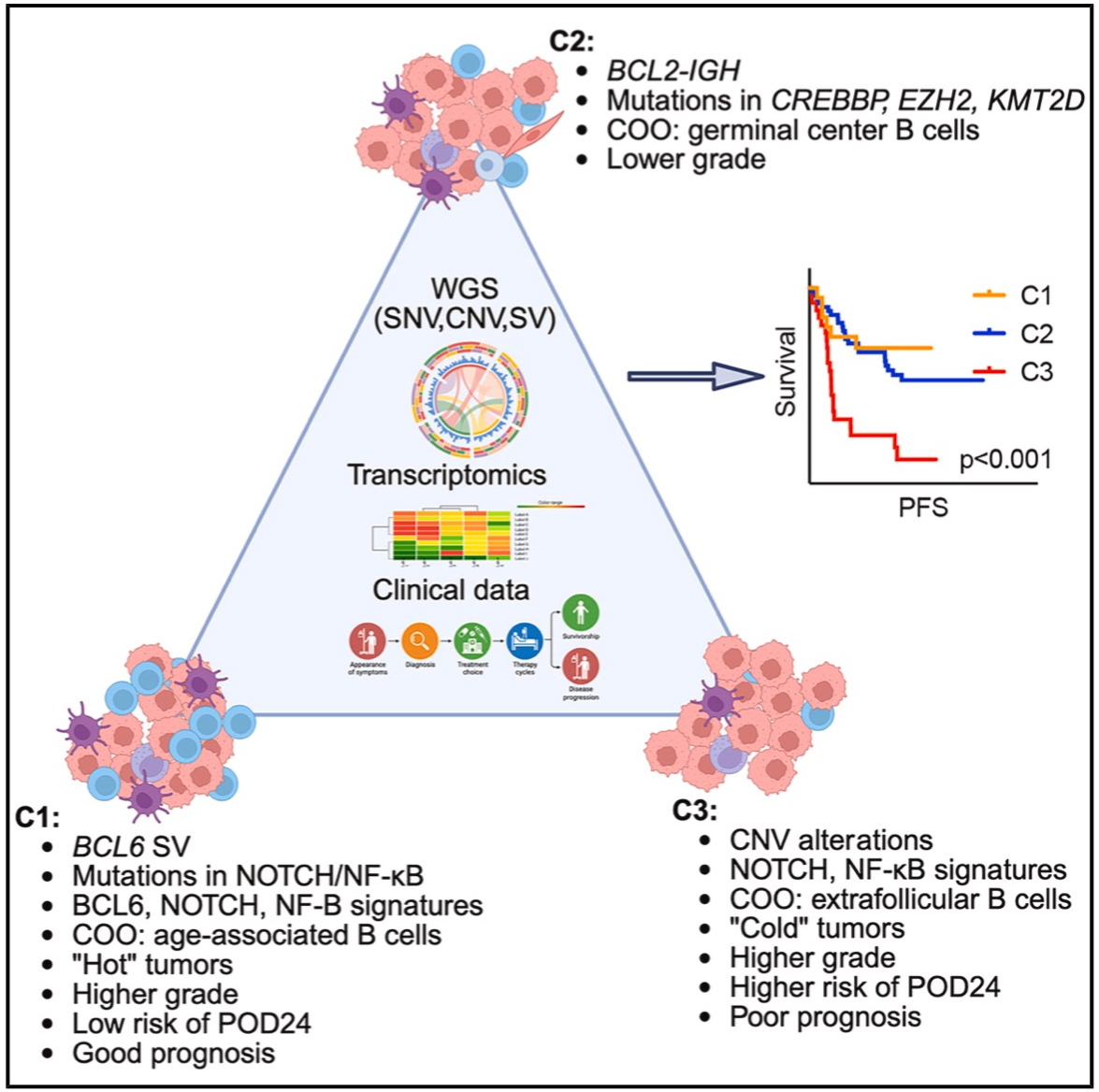
Inflamed-antiproliferative memory-like (INFM):
strong positive relationship with proinflammatory cell types, inflammatory monocyte-derived dendritic cells (mDCs), CD4 Th1 effector memory T cells (Tem) and CD8 Tem, all of which are capable of secreting type 1 IFNs and TNF-a.
Proliferative dark-zone-like (PDZ): supportive Tfh cells and FDC
Chromatin modifying intermediate (CMI): limited TME

No differences in EFS/OS
Nearly 40% of G2 and 30% of G3 experienced POD24
No G1 patients experienced an early event ($p < 0.05$)

INFM = chimeric antigen receptor-T cells or bispecifc antibodies?
PDZ dysregulated mTORC1 -> mTOR inhibition?
CMI = Tazemetostat

Krull et al, Cell Reports Medicine 2024

FL: 3 genetically defined groups (WGS)

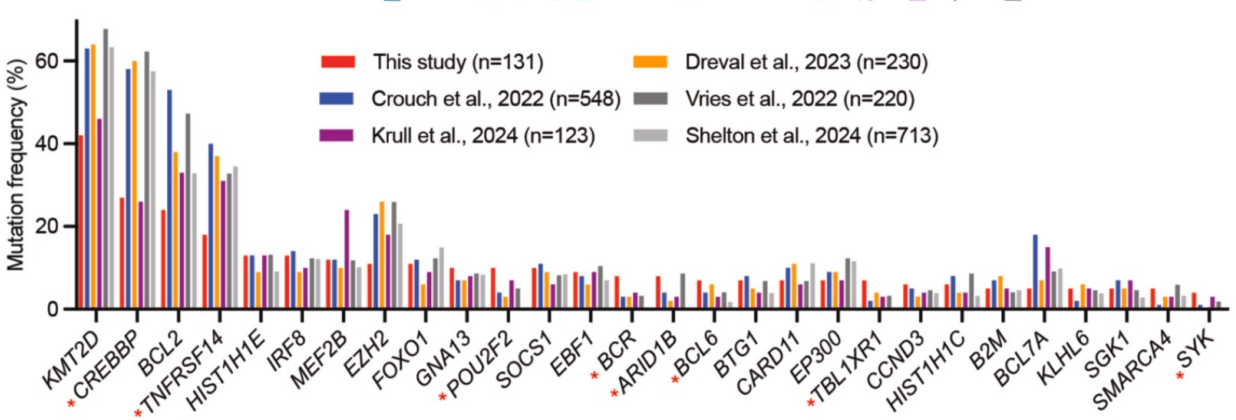


• **Whole-genome sequencing in 131 Chinese FL defines three genetic subtypes of FL**
• Subtypes exhibit distinct mutational and transcriptional patterns and tumor microenvironments
• Subtypes are linked to distinct cell-of-origin characteristics and patient outcomes
• Validation in independent Western cohort (n= 227), Dreval et al Blood 2023

No correlation with FLIPI, m7-FLIPI, PRIMA-PI

BCL2::IGH translocations were present in approximately 47% of the patients in our cohort
Included FL G3b

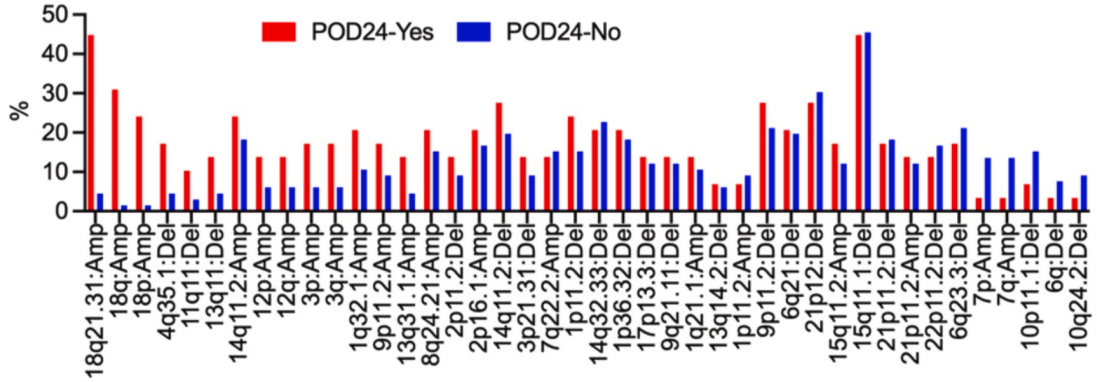
Ren W et al. Cell Reports Medicine 2025

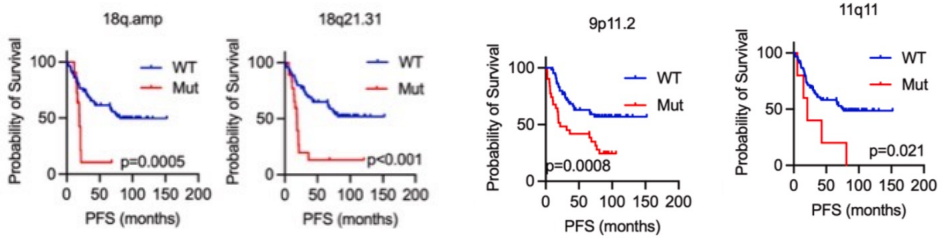


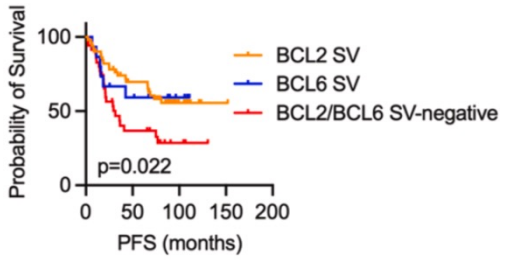
75 significantly mutated genes (SMGs) in the coding regions, each affected by nonsilent mutations in at least 4 samples (>3%)
Each FL harbored 7 SMG

POU2F2, *ARID1B*, *TBL1XR1*, *SYK*, *FAS*, *KLF2*, *CXCR4*, *BCL10*, and *CD70*, were more frequently mutated

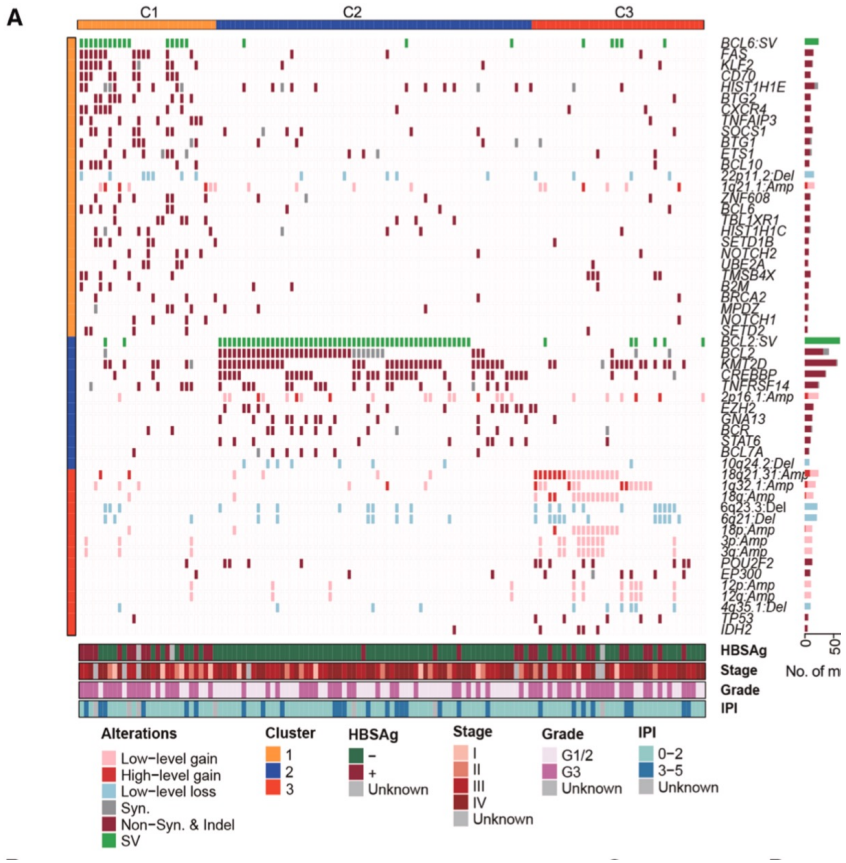
Genes that are most frequently altered in FL, such as *KMT2D*, *CREBBP*, *BCL2*, *TNFRSF14*, and *EZH2*, exhibited significantly lower mutation frequencies in our cohort than in the five previously reported Western cohorts





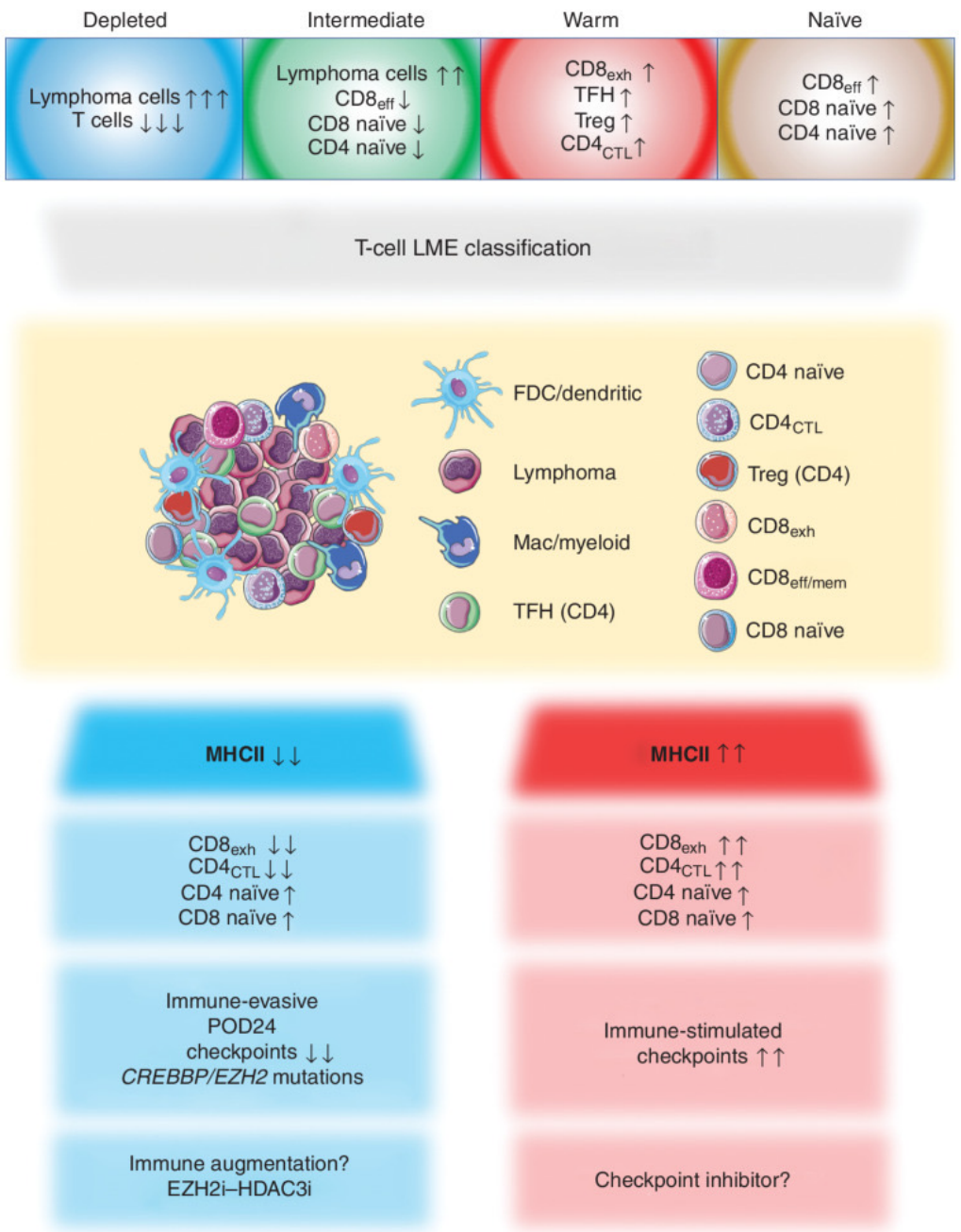


Gain of 18q21.31/MALT1 and loss of 11q11/9p11.2 were closely associated with inferior PFS in patients treated with R-CHOP



MVA including age, IPI, stage, grade, M7- FLIPI, confirmed that the genetic subtypes independently predicted treatment outcomes in R-CHOP-treated patients (p = 0.007). Reproduced similar clusters in validation cohorts, but PFS differences across these clusters were not significant

	C1 (n=29)	C2 (n=66)	C3 (n=36)
Clinical features	✓ High grade ✓ Low risk of POD24 ✓ Good PFS	✓ Low grade ✓ Low risk of POD24 ✓ Moderate PFS	✓ High grade ✓ High risk of POD24 ✓ Poor PFS
Mutation profiles and mutagenesis	✓ BCL6 SV ✓ Mutations in NOTCH/NF-κB signaling ✓ IgM isotype ✓ POLH/AID	✓ BCL2 SV ✓ Mutations in histone genes ✓ IgG/IgA isotype ✓ POLH/AID	✓ No BCL6/BCL2 SV ✓ CNV affecting PI3K, NF-κB signaling ✓ IgM isotype ✓ AID
Similar to DLBCL subtypes	✓ BN2/C1	✓ EZB/C3	✓ A53/C2
Expression signatures	✓ BCL6, MYC ✓ NOTCH, PI3K ✓ BCR/ NF-κB, IRF4 ✓ ABC-DLBCL	✓ GCB-DLBCL ✓ B-cell state S1	✓ MYC, NOTCH, PI3K ✓ BCR/ NF-κB, IRF4 ✓ B-cell state S5 ✓ ABC-DLBCL
TME	✓ T-cell exhaustion ✓ Inflamed TME ✓ Hot tumor	✓ Stromal signature	✓ Less inflamed TME ✓ Cold tumor
Potential cell-of-origin	✓ Age/autoimmune-associated B cells	✓ GC B cells	✓ Extrafollicular B cells
Potential drug targets	✓ PI3K inhibitor ✓ BTK inhibitor ✓ IRF4 inhibitor ✓ Immune therapy	✓ BCL2 inhibitor ✓ EZH2 inhibitor	✓ PI3K inhibitor ✓ BTK inhibitor ✓ IRF4 inhibitor



FL: 4 TME states (scRNA seq)

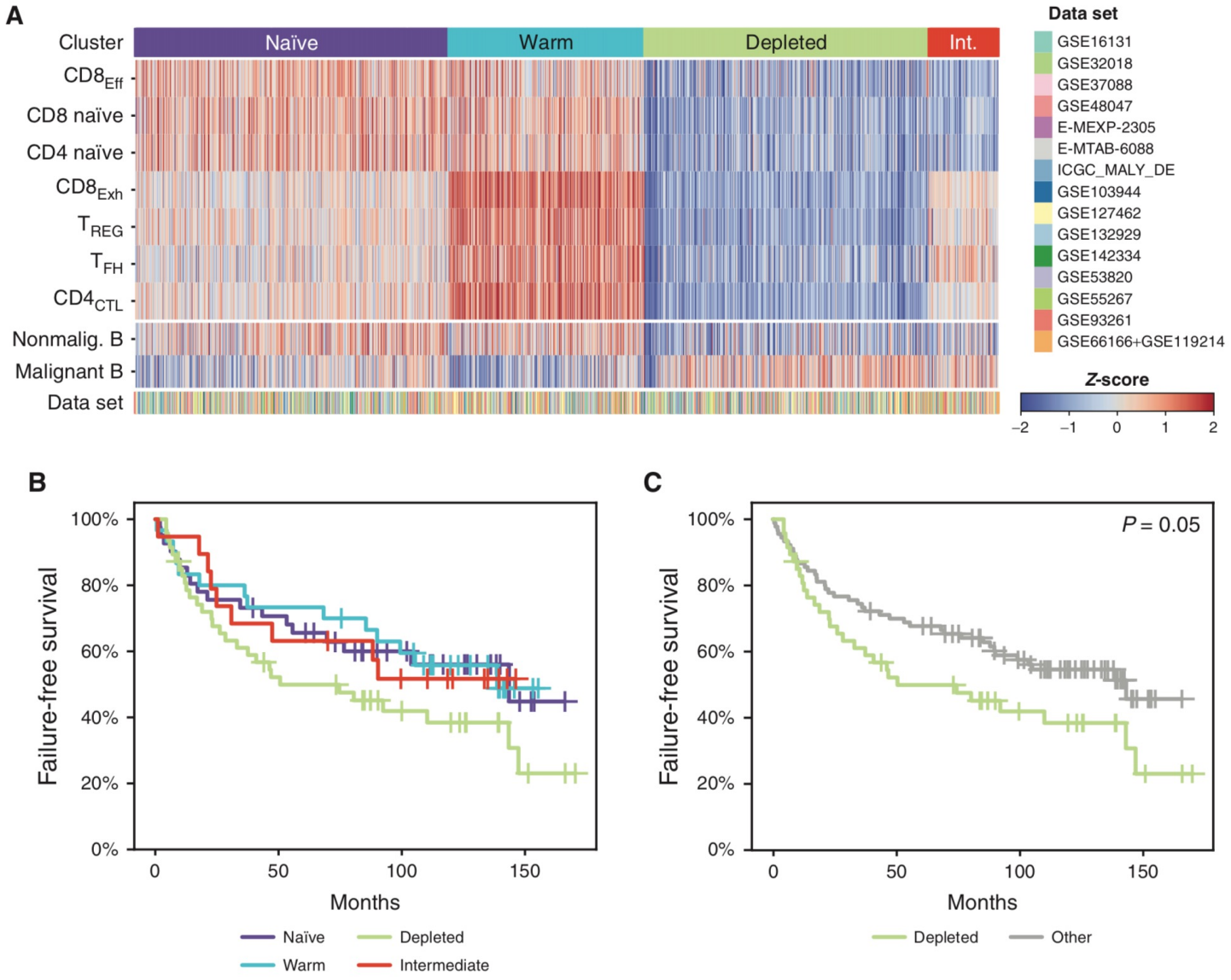
Single-cell RNA-seq in 20 FLs; BCR and TCR; WES (n=19), flow
11 previously untreated and 9 relapsed FLs
(median 1 line of prior therapy; range, 1–6), grades 1 to 2 (n = 14) or 3A (n = 6)

Variable proportions of CD4: naive, Tregs, TFH (usually CXCR5+PD1+) and **CD4 CTL cytotoxic**
CD8 naive, effector, exhausted

4 major subtypes of FL based on the expression of major histocompatibility complex class II (MHC II) genes and the presence of distinct T-cell subsets in the TME.

MHC II high tumors exhibit **increased proportions of exhausted** CD8+T cells (expressing high TIGIT and LAG3) and **cytotoxic CD4 cells (expressing OX40, GZMK and CTLA4)**
Patients with mutation-driven **loss of MHC II expression (58%)** display reduced numbers of activated CD4+ and CD8+ T cells in the TME (potential **immune evasion**).

Han G, et al. Blood Cancer Discov. 2022



Deconvolution from bulk GEP or RNA-seq in 1269 FL from 15 public databases

FFS for 137 R-CHOP-treated FL patients are shown according to subtypes
Poor prognosis: FL T-depleted

Immune augmentation to restore antigen presentation in **MHC II low** or checkpoint inhibitors to reinvigorate T cells in **MHC II high**?

Han G, et al. Blood Cancer Discov. 2022

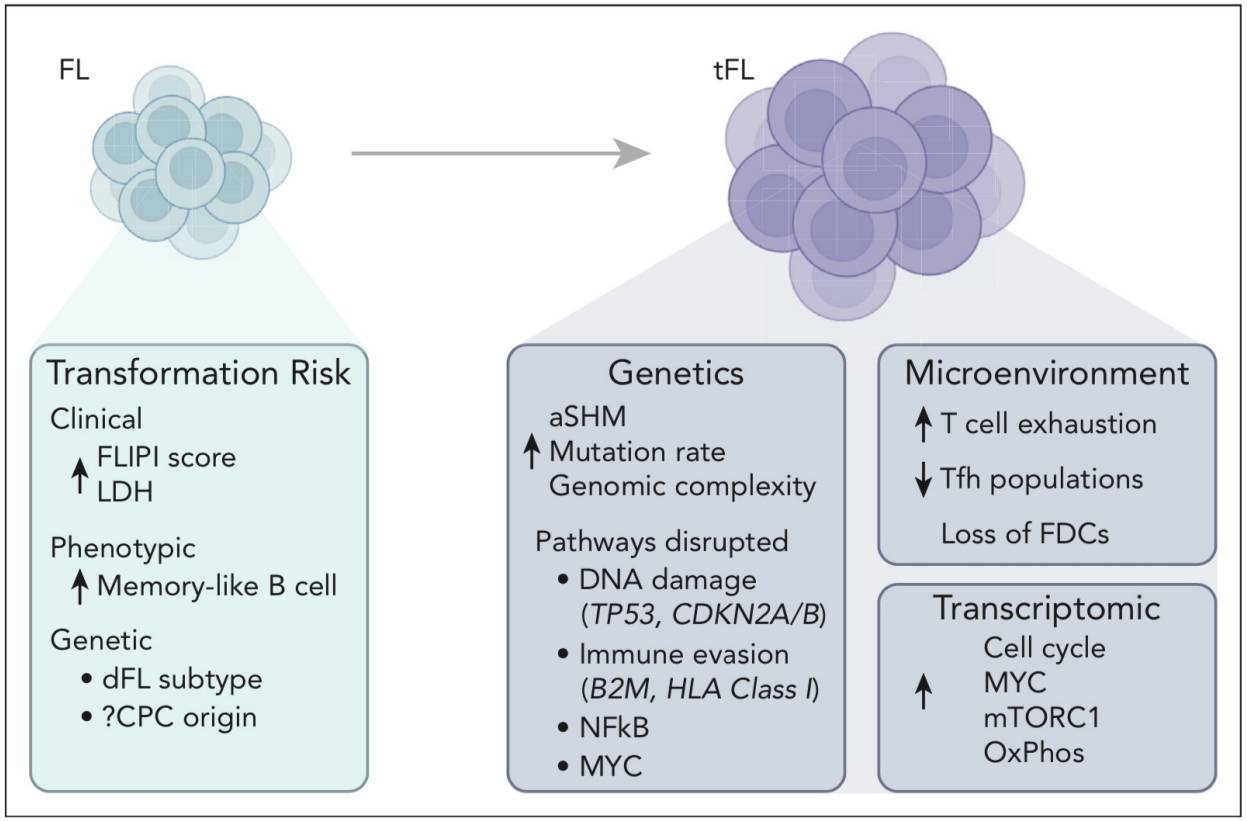
scRNAseq in FL: Multicellular echosystems in FL?

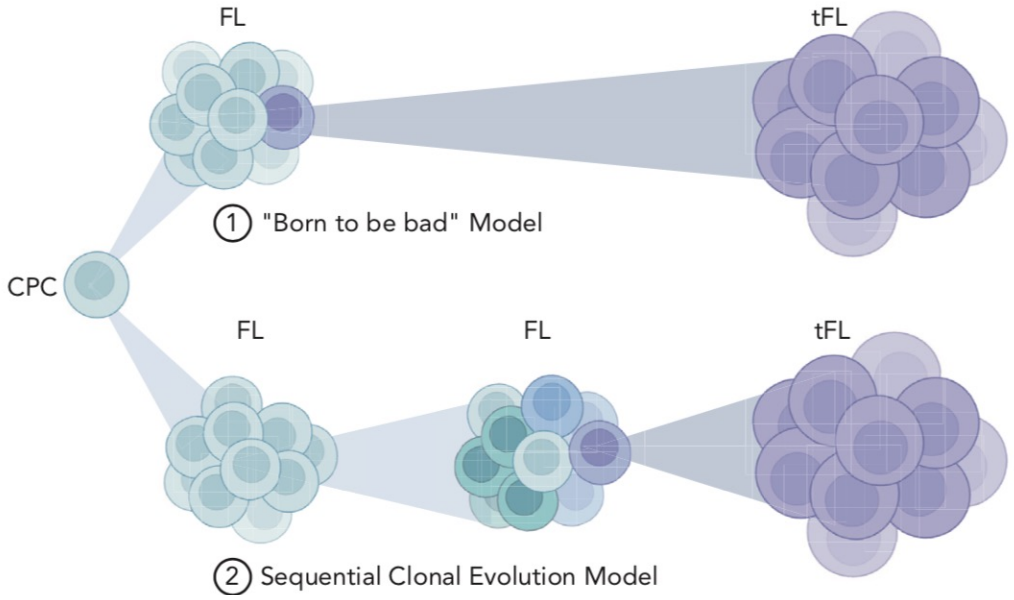
- Single-cell ATLAS of 107 FL samples from a real-world series: 60 newly-diagnosed, untreated patients, with 17 POD24; 39 relapses and 8 tFL.
- **10 malignant archetypes (similar cell content)** representing the main sources of intra-tumor heterogeneity, **co-occurr in each pt**, each associated with a specific differentiation stage: memory- like (Arch2,3,6,9), GC-like (Arch7,8), MYC/NFKB-driven (Arch5), pre-B-like (Arch10), cell cycle (Arch1), and plasma cell-like (Arch4).
- **Each pt has a unique composition, where multiple archetypes co-exist.**
- **3 distinct multicellular echosystems (MCEs) with unique archetype/TME compositions and clinical outcomes**.
- **MCE1 (**Arch 1,3,6,7,10) = high IgM expression and a supportive T cell effector niche, showed enrichment in POD24 patients (27.5%) and shorter PFS
- **MCE3 (**enriched in Arch8) = IgG expression and depleted in POD24 patients (6%).

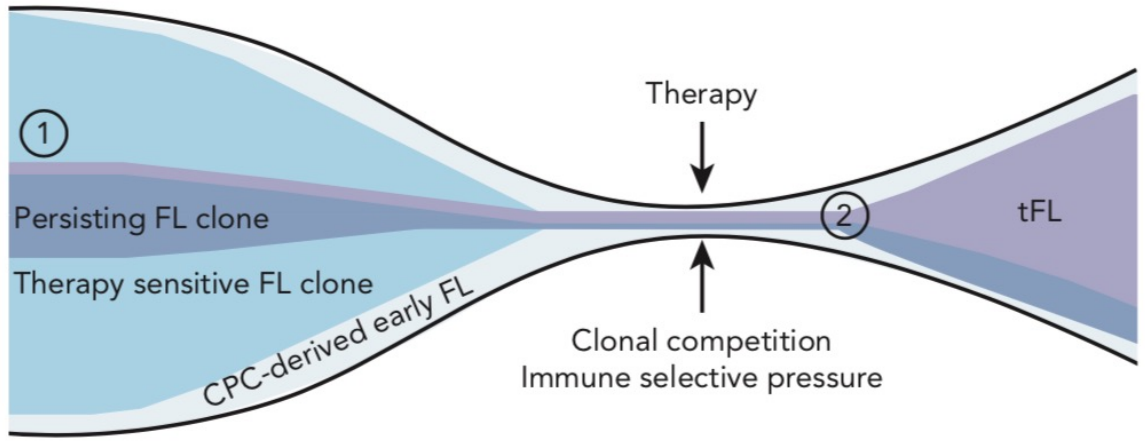
- **Validation of MCE gene signatures in bulk RNA-seq dataset** in 2 frontline trials: RELEVANCE (n = 273) and PRIMA (n = 135).
- In both trials' R-chemo arms, **MCE1** patients had significantly shorter PFS than MCE2 or 3 ($p \leq 0.0001$).
- In the R2 arm, PFS was similar across MCEs ($p = 0.72$), **suggesting R2 may benefit MCE1 patients more than R-chemo.**
- MCE scoring system remained the strongest prognostic factor in MVA (PFS: HR = 3.3, $p = 0.0002$; EFS: HR = 2.8, $p = 0.001$; POD24: OR = 5.1, $p \leq 0.03$).

Sarrabay ASH 2024; ICML 2025

An updated understanding of FL transformation



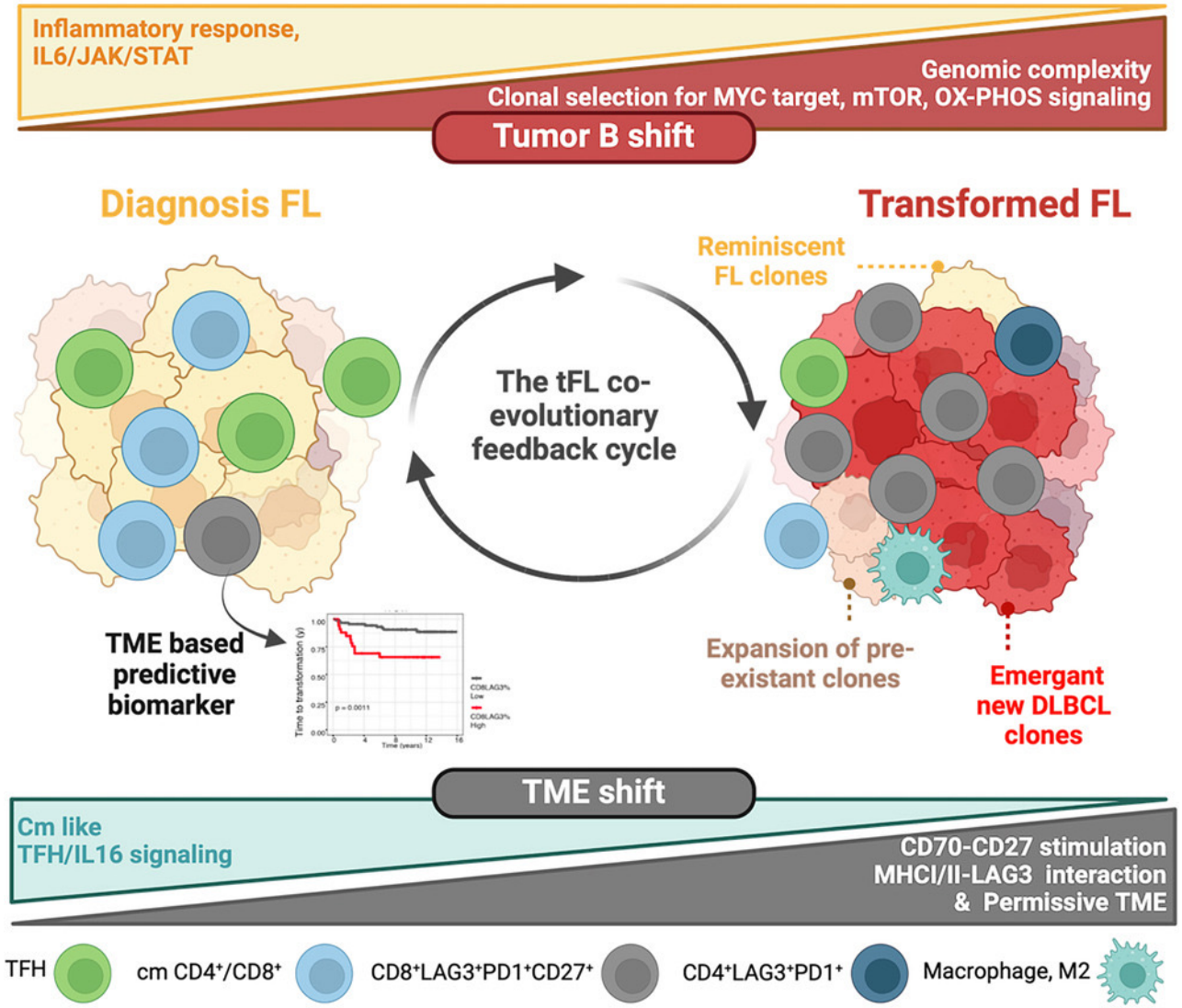




TP53 biallelic inactivation 15-30%
CDKN2A/B 20-30%
Immune evasion genes 20-25%
MYC 25%

Parry & Okosun. Blood 2025

tFL: scWGS and scWTS



Sarkozy C, et al. Cancer Cell. 2024

Single-cell whole genome sequencing (scWGS) and transcriptome sequencing (scWTS) of 11 paired pre/post-transformation patient samples and scWTS of additional samples from patients without transformation

The genomic and transcriptomic divergence of clones: **patients who transform earlier** showed greater clonal similarity, whereas **later transformations are more biologically diverged**.

tFL cells: upregulated transcriptomic pathways in cell cycle, MYC signaling, mTORC1 signaling, and oxidative phosphorylation, mirroring findings of CLL transformation

TME in tFL: decrease in central memory and T follicular helper (Tfh) cells + increased exhausted T-cell populations. Multicolor immunofluorescence validated **higher CD8+LAG3+PD1+ T cells near malignant B cells in tFL biopsies.**

The frequency of CD8+LAG3+cells in the nontumor population correlated with time to transformation in 2 independent CIT-treated FL cohorts.

Back to FL patients: are we closer to prognostic/predictive biomarkers in FL?

Composite clinico-biologic risk scores	ref	method	model
TME risk models	Dave, NEJM 2004	1974-2001, pre-Rituximab GEP	IR1 T-cells and monocytes = FAVORABLE (CD7, CD8B1, ITK, LEF1, STAT4; ACTN1, TNFSF13B) IR2 monocytes and dendritic cells = UNFAVORABLE (TLR, FCGR1A, SEPT10, LGMN, C3AR1)
	Mondello, Blood Cancer J 2021	2002-2012	Combines FLIPI + CD4+ T intrafollicular expression (activated central memory T) EFS and EFS24
	Tobin, JCO 2019	Nanostring 12 genes	Immmune infiltration low (ie, low PD-L2) FL vs immune infiltration high (high PD-L2) FL POD24 45.7% vs 16.3% HR 4.32
Gene-expression based risk models	Huet, Lancet Oncol 2018	23-gene expression (PRIMA-23)	POD24 prediction: sensitivity of 43% and a specificity of 79%, PPV of 38% and NPV of 82% Treatment dependence
Gene mutations based risk scores	Pastore, Lancet Oncol 2015	m7-FLIPI Targeted seq	Model = HR FLIPI + ECOG PS >1 + EZH2, ARID1A, MEF2B, EP300, CREBBP, FOX01, CARD11 Treatment dependence
	Jurinovic, Blood 2016	POD24-PI	FLIPI, EP300, FOXO1, EZH2 m7-FLIPI HR: high risk of POD24 (OR 5.82-4.76) 22%-30% of POD24 not predicted by any

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Check for updates

Identification of genetic subtypes in follicular lymphoma

Victoria Shelton[1], Rajesh Detroja[1], Ting Liu[1], Keren Isaev[1], Anjali Silva[1,2], Verena Passerini[3], Mehran Bakhtiari[1], Lourdes Calvente[1], Michael Hong[1], Michael Y. He[1], Saloni Modi[1], Samantha A. Hershenfeld[1], Maja Ludvigsen[4,5], Charlotte Madsen[5], Stephen Hamilton-Dutoit[6], Francesco Annibale d'Amore[4,5], Marianne Brodtkorb[7], Nathalie A. Johnson[8], Tara Baetz[9], David LeBrun[10], Josh W. D. Tobin[11,12], Maher K. Gandhi[11,12], Andrew J. Mungall[13], Wei Xu[14], Susana Ben-Neriah[15], Christian Steidl[15], Jan Delabie[16], Rosemarie Tremblay-LeMay[16], Opeyemi Jegede[17], Oliver Weigert[3,18,19], Brad Kahl[20], Andrew M. Evens[21] and Robert Kridel[1]✉

Cluster Development Cohort (n=713)

FFPE biopsies

1. A retrospective, multicenter cohort of patients with limited-stage disease, who were treated with radiation only, or with advanced-stage disease, treated with immunochemotherapy, accrued from Toronto, Montreal, Kingston, Aarhus, Oslo, and Brisbane (referred to as the "multicenter cohort", n = 203);
2. A retrospective cohort of patients from existing data from a published study in which treatments were heterogeneous (referred to as the "PLOSMED cohort", n = 242) [24];
3. The E2408 trial (NCT01216683), a randomized phase II trial in which high-risk untreated patients received induction therapy with bendamustine and rituximab, with or without bortezomib, followed by maintenance with rituximab, with or without lenalidomide (n = 83) [25];
4. The E4402 trial (NCT00075946), a randomized phase III trial in which patients with low-tumor burden were treated with single-agent rituximab, followed by rituximab maintenance or re-treatment, as needed (n = 185) [26].

Blood Cancer Journal (2024)14:128 ; https://doi.org/10.1038/s41408-024-01111-w

Validation Cohorts

- Gallium trial (n=418)
- PLOSMED cohort (n=116)

FL with transformation

Two different gene panels (entire coding regions of 86 and 71 genes) -> **57 genes in common**

+

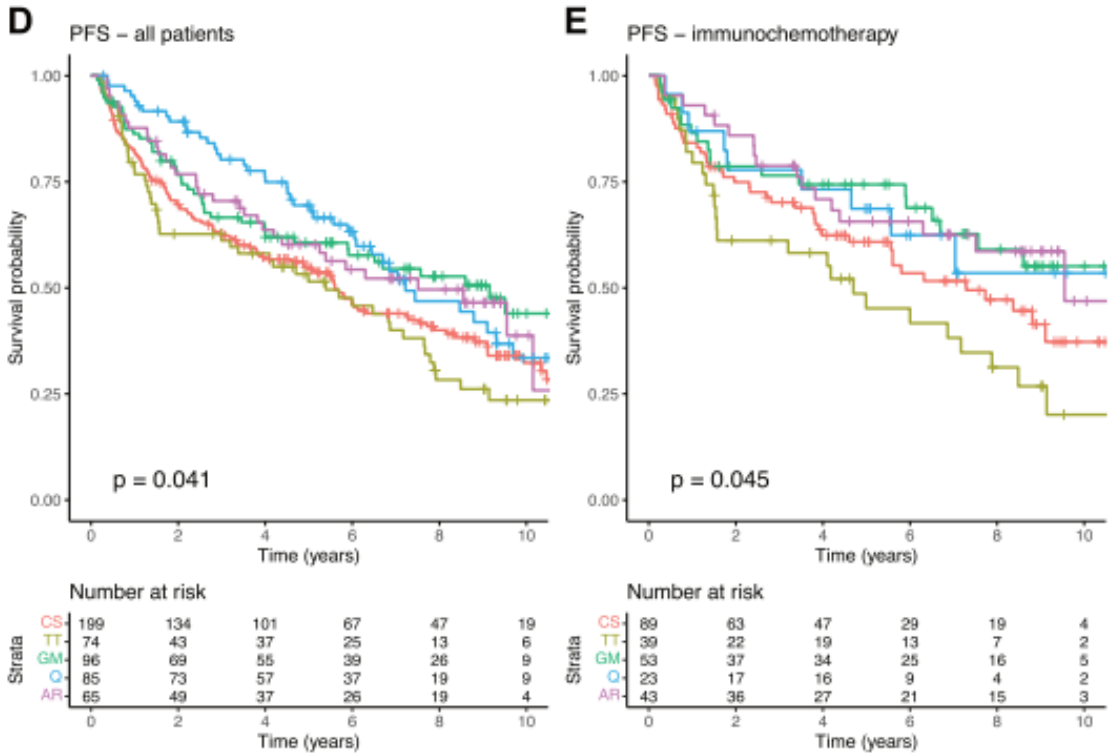
Clustering methods as for Akaike Information Criterion (AIC)

FlexMix model for validation with Gallium

Methylation (n=328 FL vs 10 DLBCL vs 5 RLN)

RNA-seq data (n = 282 FL) to identify B-cell receptor sequences within the bulk transcriptome

Subtypes	N° = 713	Mutations enriched	Other		5y-PFS (n=519)
CS	257	CREBBP, STAT6, and TNFRSF14	**EZH2 (28%)**	Grade 3A (7%)	
TT	101	TNFAIP3, encoding a negative NF-κB regulator, with 28/34 (82%) predicted to disrupt protein sequence. TP53, MYD88	EZH2 (15%)	**Grade 3A (24%)** t(14;18) 71%	45.2% vs. 66.0% remaining p = 0.008 POD24 after CIT 41% vs 22% p = 0.033
GM	137	GNA13 and MEF2B	**EZH2 (34%)** **Highest mutation burden**, due to SHM (BCL7A, BCL2, HIST1H1C, DTX1) and IGH variable region	**t(14;18) 90%**	
Q	124	Weak HLA-DMB and NOTCH1	EZH2 (5%) Lowest mutation burden	Grade 3A (9%) t(14;18) 65% **Stage I 35%** Lowest proliferative history (methylation)	
AR	94	mTOR signaling pathway (ATP6AP1, RRAGC, ATP6V1B2) and CTSS	EZH2 (7%)	**t(14;18) 84%** **Stage III/IV 90%** **>4 nodal sites 74%** IGHM- expressing cases (30/34, 88.2%)	



A modest, but significant outcome association between genetic clusters and PFS was observed in 247 patients treated with immunochemotherapy (log-rank p = 0.045).

In a Cox MVA that adjusted for the FLIPI, the hazard ratio for progression or death was 1.8 (95% confidence interval 1.1–2.7, p = 0.014) when contrasting cases from TT against all other patients.
TT was associated with inferior PFS in the FLIPI high-risk, but not low-intermediate-risk patients

Gallium: TT was associated with unfavorable PFS (log-rank p = 0.027). In a multivariable Cox regression model that considered clinical risk factors, the chemotherapy backbone, and the type of anti-CD20 antibody, HR for PFS was 1.6 for patients assigned to TT (95% confidence interval 1.05–2.4, p = 0.028).

«The molecular and cellular mechanisms responsible for the clinical heterogeneity of follicular lymphoma are unknown»
(Dave et al, NEJM 2004)

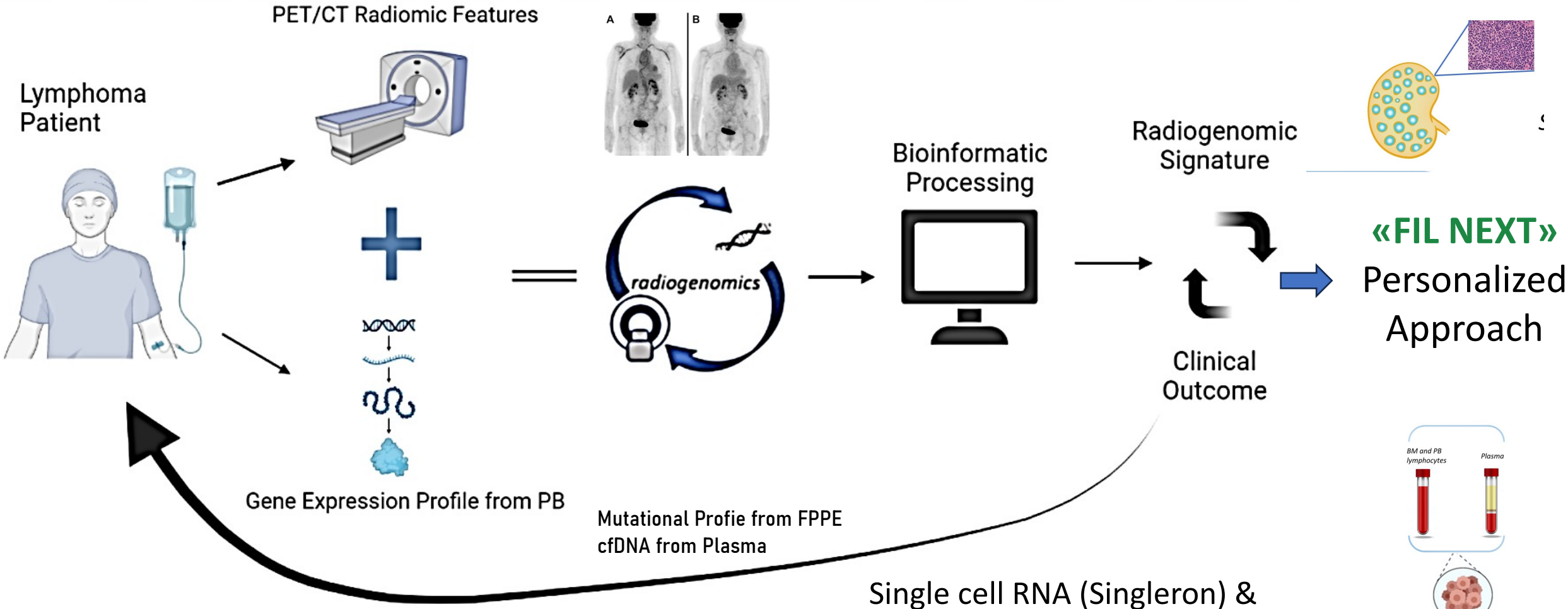
Conclusions

- Recent advances in high-throughput bulk sequencing, scRNA/scWGS and spatial transcriptomics uncovered genetic, epigenetic, and immunogenetic features of FL
- Treatment decisions in FL remain largely agnostic concerning the underlying tumor biology
- Need to move from bench to bedside
- If biology becomes a targetable vulnerability in FL, **the eradication of the common progenitor cells (CPC)** can be the aim, changing the paradigm of watch and wait strategy and treat only at progression at diagnosis and at relapse -> cure?

CSBB: translational studies in FL from 2026

upcoming publications: CHIP, EZH2, PET & BOM (FOLL12)

FONDAZIONE ITALIANA LINFOMI



Single cell RNA (Singleron) &
single-cell DNA (Tapestri)

Gruppo Bioinformatico
Gruppo Immunoflow

FOLL-PREDICT: High-risk FL prediction through ctDNA

FOLL19 ancillary study

Giovanni Manfredi Assanto, Riccardo Moia

Commissione studi biologici e bioinformatici
Commissione Linfomi indolenti

Bando Roche per la Ricerca 22 luglio 2025

Work Flow

FOLL19 Trial

Patient selection with current FU

Cohort

Work-Flow

30 Patients with POD24 or HT

30 Non POD24 matched by propensity score for clinical risk and treatment

NGS CAPP-Seq Personalized panel of 201 genes[1-2]

ctDNA

Diagnostic lymph node

Predictive panel

Expansion

Objective
Risk prediction of POD24/HT through non-invasive mutational analysis on baseline plasma

Rationale
Plasma could better recapitulate FL heterogeneity than single lymph-node

1) Shelton V., et al. Blood Cancer J. 2024 Aug 7;14(1)
2) Moia R. et al. Blood Adv. 2025 Apr 8;9(7):1692-1701.

BANDO Giovani Ricercatori FIL 2025

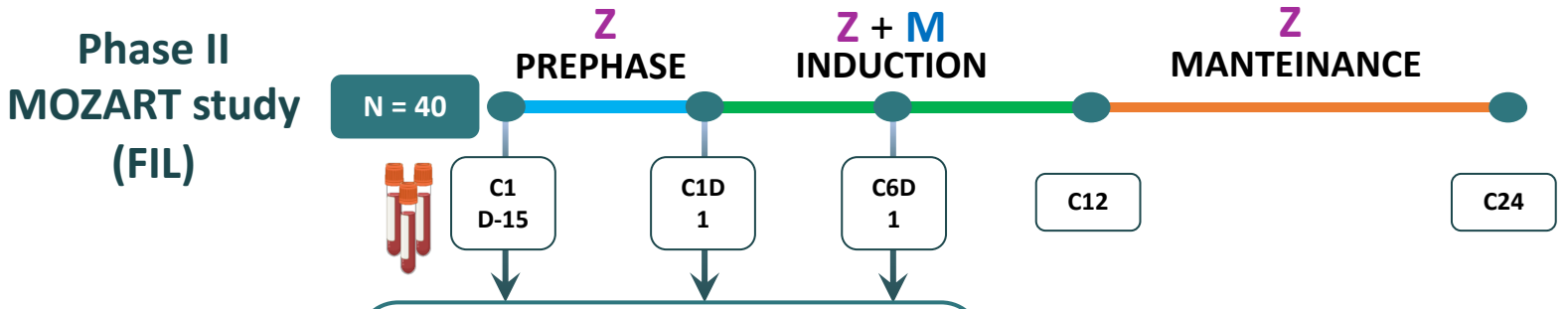
IMMUNO - MOZART

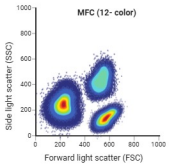
Multilayer immune-modulation analysis in R/R patients with FL treated with zanubrutinib (Z) in combination with mosunetuzumab (M)

Rationale:

- R/R FL is a **clinical unmet need**
- Outcome of **T-cell engager therapies (BsAbs)** is related to T-cell fitness
- **BTKi** could modulate immunity by delaying T cell exhaustion
- Assessing **immune cell fitness** becomes crucial in this subset
- **Lack of predictive biomarkers** is an unmet need in this setting

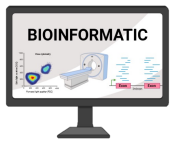
Phase II MOZART study (FIL)





MFC (12-color):
Monitor the kinetics of immune-cell subpopulations
(CD4+ Treg, CD4+ T, CD8+ T and NK cells)

GEP (bulk RNA-seq):
Assessment of immune exhaustion and senescence



Bioinformatic integration:
MFC, GEP, MRD and PET-CT

Objectives:

- Association with response (Metabolic and MRD) and 2y-PFS
- Adjuvant effect of BTKi during BsAbs therapies
- Detect exhaustion of CD4+ T cell populations
- Identify early loss of response to BsAbs

Expected outcomes:

- Predictive biomarkers of treatment failure/response to bsAbs
- Provide tool to drive treatment decision (CAR-T vs bsAbs)
- Identify immune pathways to restore
- Generate hypothesis to validate in larger clinical trials

BsAbs, bispecific monoclonal antibodies; MFC, multiparametric flow-cytometry; GEP, Gene expression profile; MRD, measurable residual disease

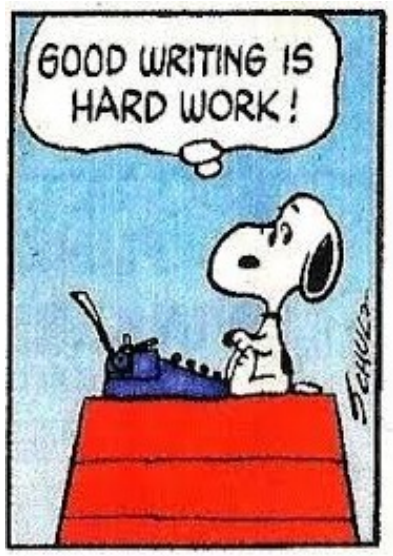
FOLL12 | FOLL19 | FOLL-NEXT

Time for thinking

Time for writing

Grants

Massello, Giugno 2023



Tremiti, Maggio 2025

Brescia, Ottobre 2024


thank you
gracias
danke
merci
obrigado
grazie
ngiyabonga
teşekkür ederim
спасибо
Баярлалаа
рахмат
謝謝
dank je
bedankt
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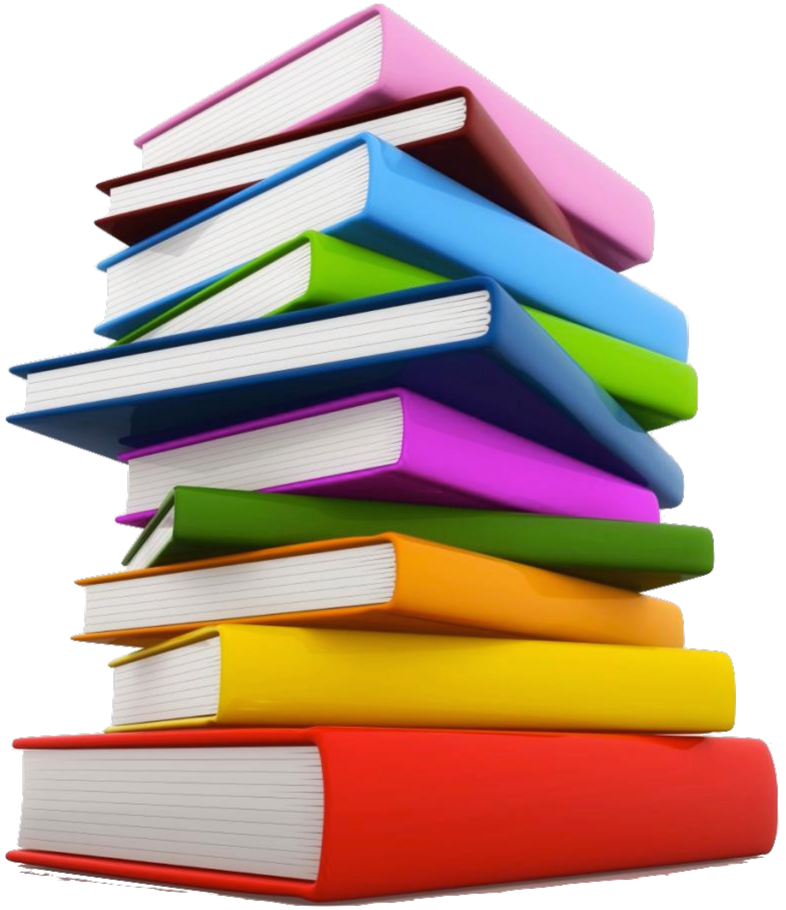


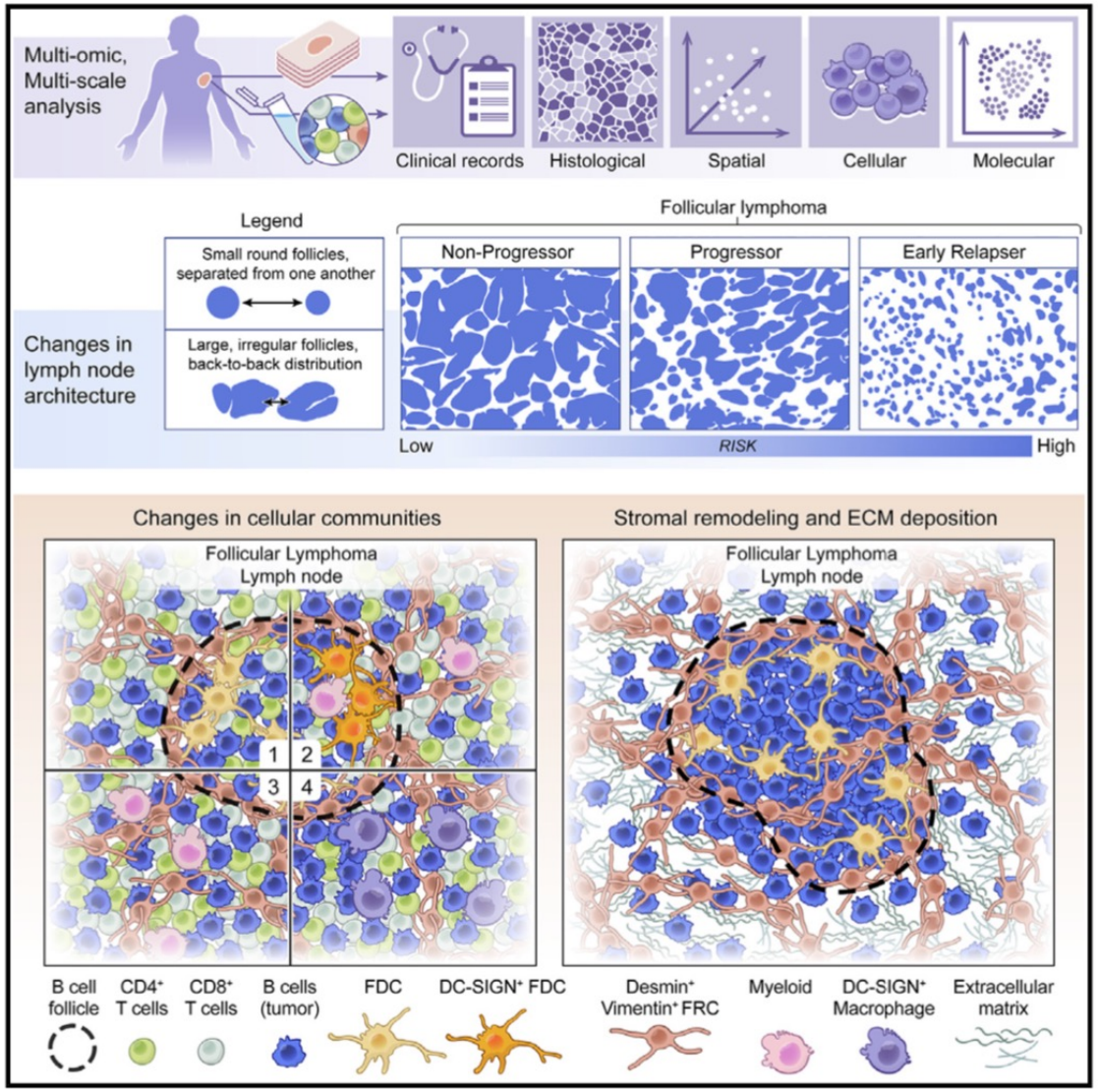
Grazie!

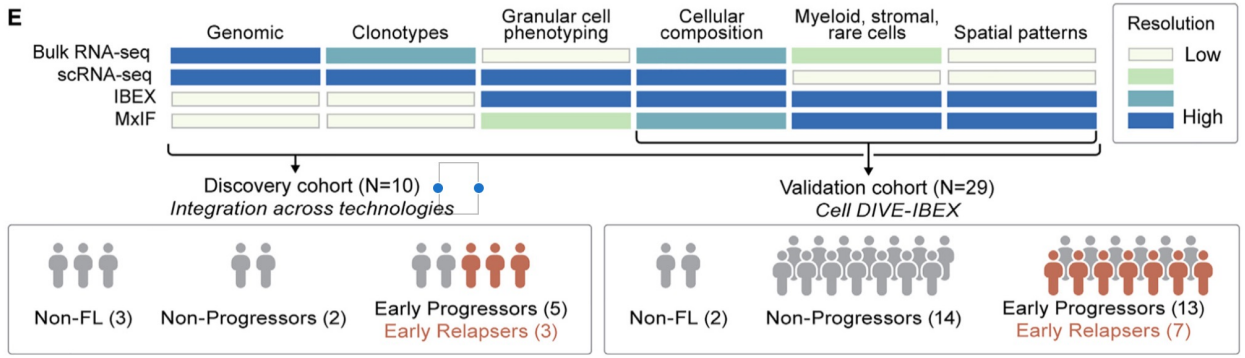
Metanalisi dei papers con «Gene mutations based risk scores» nel linfoma follicolare (gruppo Bioinfo, IDG, GMA)

	Referenza	doi
1.	Pastore A, Jurinovic V, Kridel R, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015 Sep;16(9):1111-1122.	doi: 10.1016/S1470-2045(15)00169-2.
2.	Jurinovic V, Kridel R, Staiger AM, et al. Clinicogenetic risk models predict early progression of follicular lymphoma after first-line immunochemotherapy. Blood. 2016 Aug 25;128(8):1112-20.	doi: 10.1182/blood-2016-05-717355.
3.	Crouch S, Painter D, Barrans SL, et al. Molecular subclusters of follicular lymphoma: a report from the United Kingdom's Haematological Malignancy Research Network. Blood Adv. 2022 Nov 8;6(21):5716-5731.	doi: 10.1182/bloodadvances.2021005284.
4.	Dreval K, Hilton LK, Cruz M, et al. Genetic subdivisions of follicular lymphoma defined by distinct coding and noncoding mutation patterns. Blood. 2023 Aug 10;142(6):561-573.	doi: 10.1182/blood.2022018719.
5.	Russler-Germain DA, Krysiak K, Ramirez C, et al. Mutations associated with progression in follicular lymphoma predict inferior outcomes at diagnosis: Alliance A151303. Blood Adv. 2023 Sep 26;7(18):5524-5539.	doi: 10.1182/bloodadvances.2023010779.
6.	Shelton V, Detroja R, Liu T, et al. Identification of genetic subtypes in follicular lymphoma. Blood Cancer J. 2024 Aug 7;14(1):128. DATA AVAILABILITY Genomic data for cases from the multicenter cohort have been deposited at the European Genome-phenome Archive (https://ega-archive.org/, accession number EGAS50000000435). The code for this study has been made available on GitHub: https://github.com/kridel-lab/FLOMICS.	doi: 10.1038/s41408-024-01111-w.

Early progressed FL

Excisional biopsies from untreated FL patients, analyzed by bulk WES, RNA sequencing (RNA-seq), single-cell RNA-seq (scRNA-seq), IBEX imaging*
In addition, FFPE samples for routine diagnostic pathology and multiplexed immunofluorescence (MxIF) imaging





- **Tumor cells** in high-risk FL patients exhibit enhanced B cell receptor signaling, glucose and glutamine metabolism. FOXP1 expression
- Loss of FDC, enhanced stromal remodeling and extracellular matrix (ECM) deposition in aggressive clinical cases
- Stromal «communities»: desmin+ vimentin+ fibroblasts
- Distinct follicular growth patterns (smaller, irregularly shaped, separated) observed in patients 20 months before relapse

*Bulk RNA-seq does not allow for molecular dissection of rare cells

Radtke et al. Cancer Cell 2025

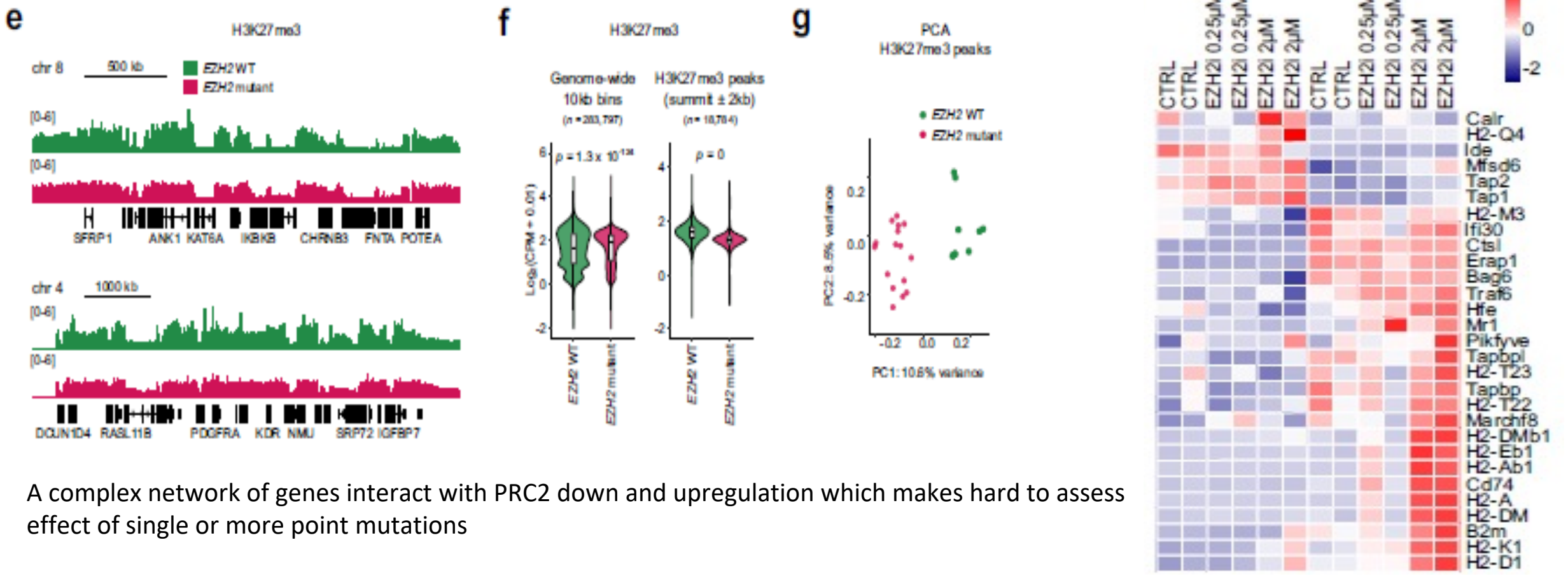
reference	N° FL	Seq approach	Clusters	Note	5y-PFS (n=519)
Crouch S, From UK's population-based Haematological Malignancy Research Network (HMRN) Blood Advances 2022	N= 548 (538 with follow-up and 96 DLBCL transformation) 2004-2012 41% CHT 17.5% RT only 39.4% W+W Rit 1%	Targeted seq FFPE 293-gene panel pan-hematologic malignancy Thirty-one genetic features were used in the cluster analysis;	1. FL_aSHM, high burden of aberrant somatic hypermutation 2. FL_STAT6, STAT6 & CREBBP and low aSHM 3. FL_Com, absence of the 1) and 2) and enriched of KMT2D mut	Independent of translocation status. Of transformed cases with paired samples, 17 of 26 had evidence of branching evolution.	Poorer OS in the aSHM group (P = .04) was associated with older age; however, overall tumor genetics provided limited information to predict individual patient risk.
Dreval K, Vancouver, Blood 2023	423 patients to compare the **protein coding and noncoding mutation landscapes of** untransformed FL, transformed FL, and de novo DLBCL.	WGS	1. DLBCL-like (dFL) 2.Constrained FL (cFL) Lower mutational burden, less aSHM, enrichment of CREBBP KAT missense mut, mut in mTORC1 signaling-associated genes (RRAGC, ATP6V1B2, and ATP6AP1)		cFL status, whether assigned with this full classifier or a single-gene approximation, is associated with a reduced rate of HT.
Russler-Germain DA, Alliance A151303, Blood Advances 2023				**Mutations associated with progression (MAP)** (CREBBP, STAT6, TP53, IGLL5, B2M, SOCS1, and MYD88) confer adverse risk when found at FL diagnosis	Low-risk m7-FLIPI is predictive of prolonged remissions with standard rituximab plus chemotherapy in newly diagnosed FL

- all with matched normal genomes.

- We identified 88 SMGs displaying variable frequency across analyzed groups. Using the diagnostic FL samples, a random forest (RF) classifier was trained to distinguish de novo DLBCL from FL, and this identified 2 genetic subgroups within FL. The constrained FL group (cFL) is highly enriched for missense mutations in the lysine acetyltransferase (KAT) domain of CREBBP, as well as mutations in RRAGC, ATP6AP1, and ATP6V1B2, and was less likely to undergo HT. In contrast, the remaining DLBCL-like FL (dFL) are further characterized by increased rates of aSHM and higher risk of transformation.

Future perspective: It is hard to predict mutations effect on H3K27me3 activity

Constitutive activation of EZH2 brings to increased levels of Histone Methylase H3K27me3 which in augmented quantity can spread over broader portiong of genome leading to different epigenetic modulation



A complex network of genes interact with PRC2 down and upregulation which makes hard to assess effect of single or more point mutations

Romero P., et al. Nat. Commun. 2024 Apr 24;15(1):3452

- Isshiki Y, Chen X, Teater M, et al. EZH2 inhibition enhances T cell immunotherapies by inducing lymphoma immunogenicity and improving T cell function. Cancer Cell. 2025; 43(1):49-68.e9.

FIS3 (Ferrero)

- ***WPI – Tumor Cell Dynamics (Torino, Roma)***
- **Screening of Somatic Mutations**

- Analysis of somatic mutations, including subclonal variants, in gDNA from FFPE lymph node sections and in plasma-derived cell-free DNA (cfDNA) by next-generation sequencing (NGS)
- Comparative study to evaluate the predictive power of mutation detection across different tissues

- **MRD Analysis by IG Rearrangements and Prognostic Role of IG Repertoire**

- Optimization and validation of NGS approaches to detect heavy and kappa light chain gene rearrangements in BM and PB samples
- Comparison of the performance of NGS-based MRD detection vs the current gold standard (PCR-based detection of *BCL2::IGH* rearrangements) in follicular lymphoma (FL)
- Analysis of the diversity and patterns of IG rearrangements in FL samples by NGS to evaluate their impact on prognosis

- **Development of non-invasive MRD Tools to be tested in plasma samples (cfDNA)**

- - Design and testing of IG- and mutation-based targeted NGS sequencing approaches to detect MRD in plasmatic cfDNA
  - Refinement of the novel developed MRD detection approaches in cfDNA vs the benchmark represented by gDNA (PB/BM)

WPII – Tumor Microenvironment Dynamics (Torino, Roma, Pisa)

Characterization of Tumor Microenvironment (TME)

- Development of a spatial mapping of TME and single-cell gene expression profiling of FL cases
- Comparison of cellular compositions of relapsed and not relapsed FL cases

Role of Myeloid Clonal Hematopoiesis (M-CH)

- NGS-based analysis of M-CH mutations contributions to the tumor myeloid microenvironment and its potential as a predictor of FL outcome
- Evaluation of M-CH's influence on mutation-based MRD analysis as a source of noise

Microbial Profiles Analysis

- Identification and characterization of microbial species present in plasma and assessment of their relationship with FL progression or therapy resistance by shotgun sequencing

Pharmacogenomic Analysis

- Performing pharmacogenomic studies by specific genotyping assays to discover single nucleotide polymorphisms (SNPs) associated with refractoriness to treatment or relapse

WPIII – Computational Analysis (Torino)

Development of Computational MRD Analysis Tools

- Implementation and validation of interpretable computational pipelines for the integration and multilayer analysis of MRD data from multiple sources (gDNA, cfDNA, PET/CT, etc.)

-Definition of a custom scoring prediction based on microbiota signature

- Development of synthetic patient data

FROM FOLL-12 to FOLL-BIO

Each funded with single grant applications (FIL-CLUB; FIL BGR; companies….)

1) **Mutational EZH2 («FOLL-EZ»)** -> RI: S. Ferrero & FIL MRD Network
2) **Gene expression studies & RNA-seq («FOLL-EZ»)** -> Sabino Ciavarella (Bari)

3) **Retrospective FFPE collection, histological revision and correlative studies** -> RI: Maurilio Ponzoni & Luisa Lorenzi (CP)
4) **FL grade 3A substudy** -> RI: Alessandro Pulsoni/Maurilio Ponzoni (Roma, CP)

5) **Novel molecular markers for MRD (other than IGH & BCL-2)** -> RI: Elisa Genuardi (Torino)

6) **CHIP** -> RI: Riccardo Moia (Novara) & Ilaria Del Giudice (Roma)
7) **Pharmacogenomics and constitutional genomics** -> RI: Sara Galimberti, Antonello Di Paolo (Pisa)
8) **Microbiome in PB** -> RI: Francesca Cordero & Simone Ragaini (Torino)

PENDING

FOCUS SU BISOGNI, IDEE E METODOLOGIE - «TOP Challenges» nel Linfoma follicolare - Liquid compartment

Personalize

- Lack of biomarkers to refine prognosis (beyond FLIPI-FLIPI 2)
- Lack of biomarkers predicting POD24 and transformation or markers of resistance
- Lack of biomarkers to indicate chemotherapy backbone in first-line
- Era of immunotherapies -> immunoFLOW & single-cell technologies

PRESENT

- MRD in FL on gDNA (+ PET/CT)
- PET and MRD are the most solid tools so far available to evaluate treatment efficacy (integrate)
- CHIP, pharmacogenomics (NGS)

NEXT:

- A molecular marker for all patients **(NGS)**
- Liquid biopsy in FL **(NGS)**
- Integration with tissue compartment (CP)
- Microbioma
- Bioinformatics: kinetic models of MRD analysis; metanalysis of biologic data; multi-omics

NEXT FUTURE

- ImmunoFLOW
- Single cell RNA (Singleron) & single-cell DNA (Tapestri)
- Bioinformatics
- Integration with tissue compartment (CP)