3rd edition

# Unmet challenges in high risk hematological malignancies: from benchside to clinical practice

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Starhotels Majestic

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## Biology of high-risk Follicular lymphoma

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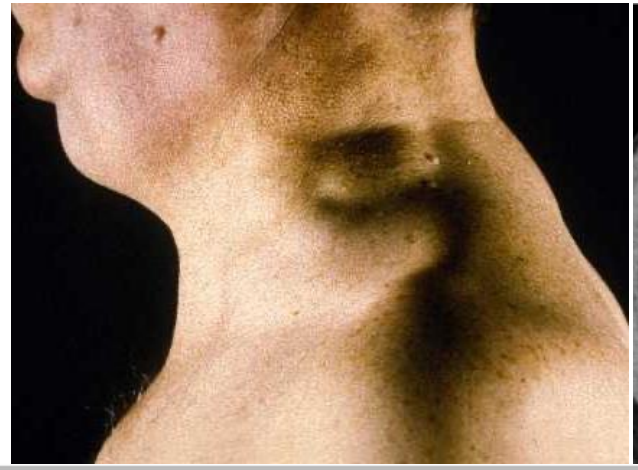
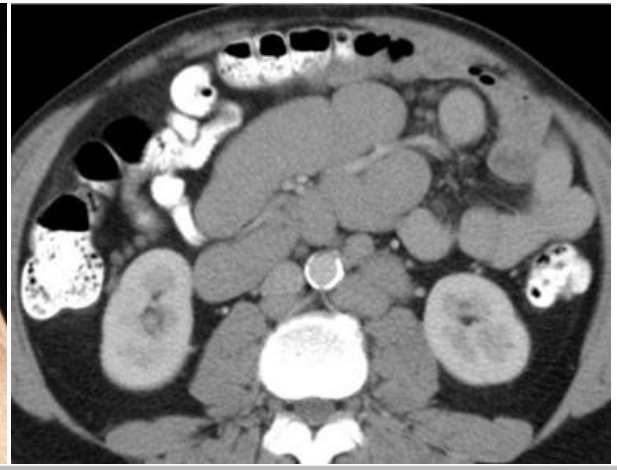
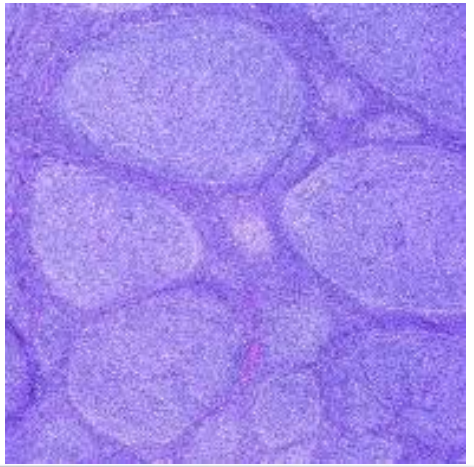
# Conflict of interests

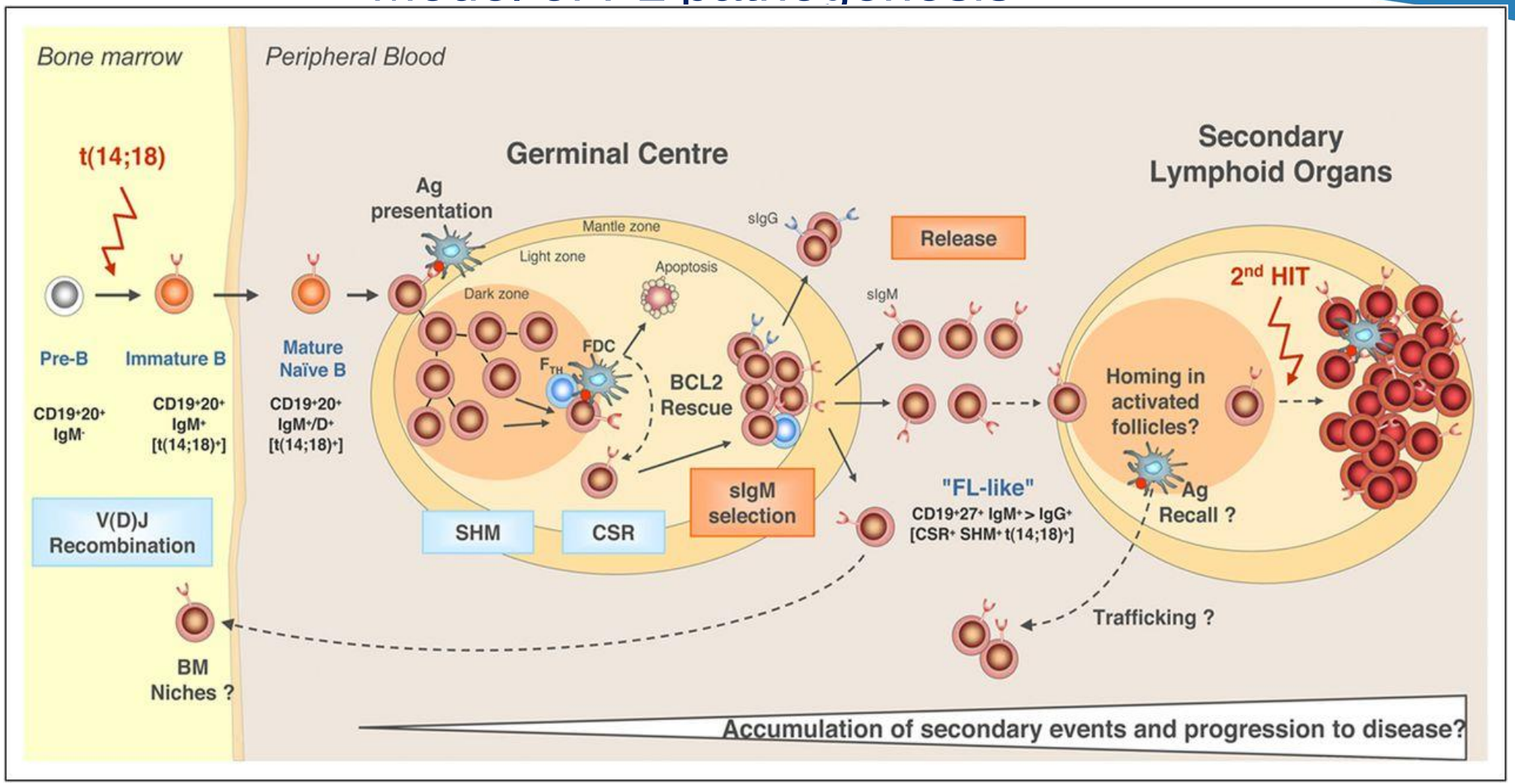
- Scientific research support: Roche, Morphosys
- Advisory Boards: Roche, AbbVie, Lilly, Janssen, BMS, Kite-Gilead, Novartis, BMS

# **Follicular Lymphoma (FL):** A Prototypical GC Neoplasia

- FL is among the most **common** lymphomas worldwide
- FL is a **clinically and molecularly heterogeneous disease**
- Advanced stage disease still considered **incurable**

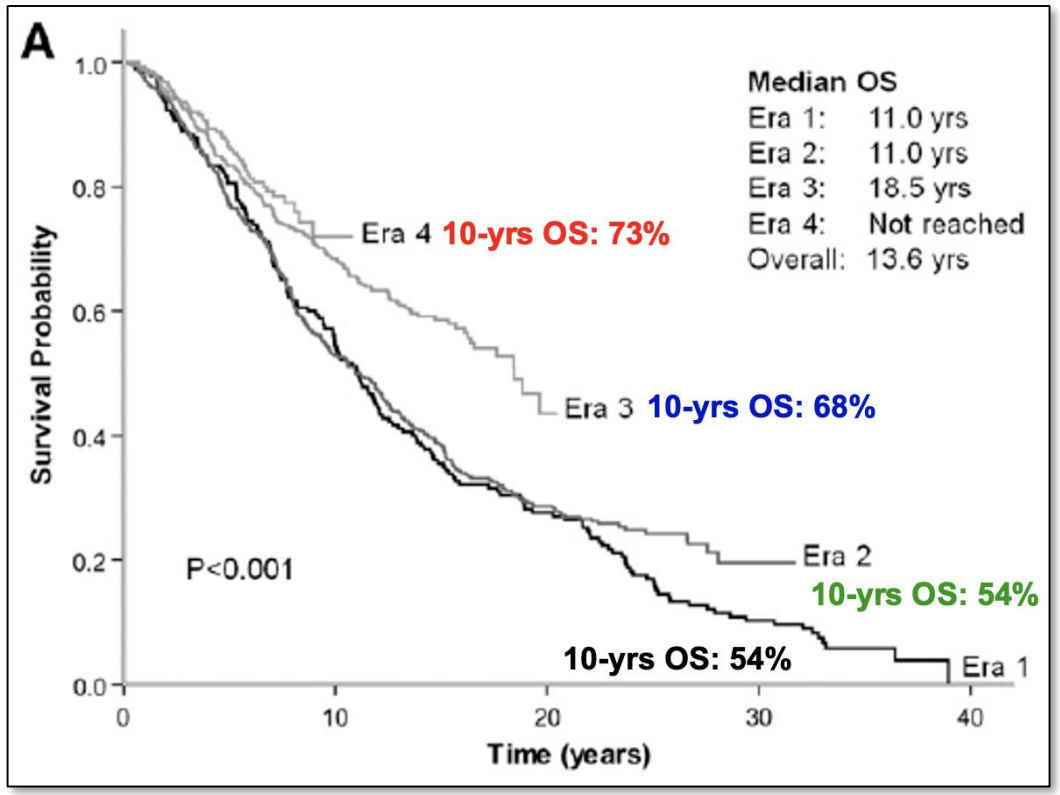
# Model of FL pathogenesis



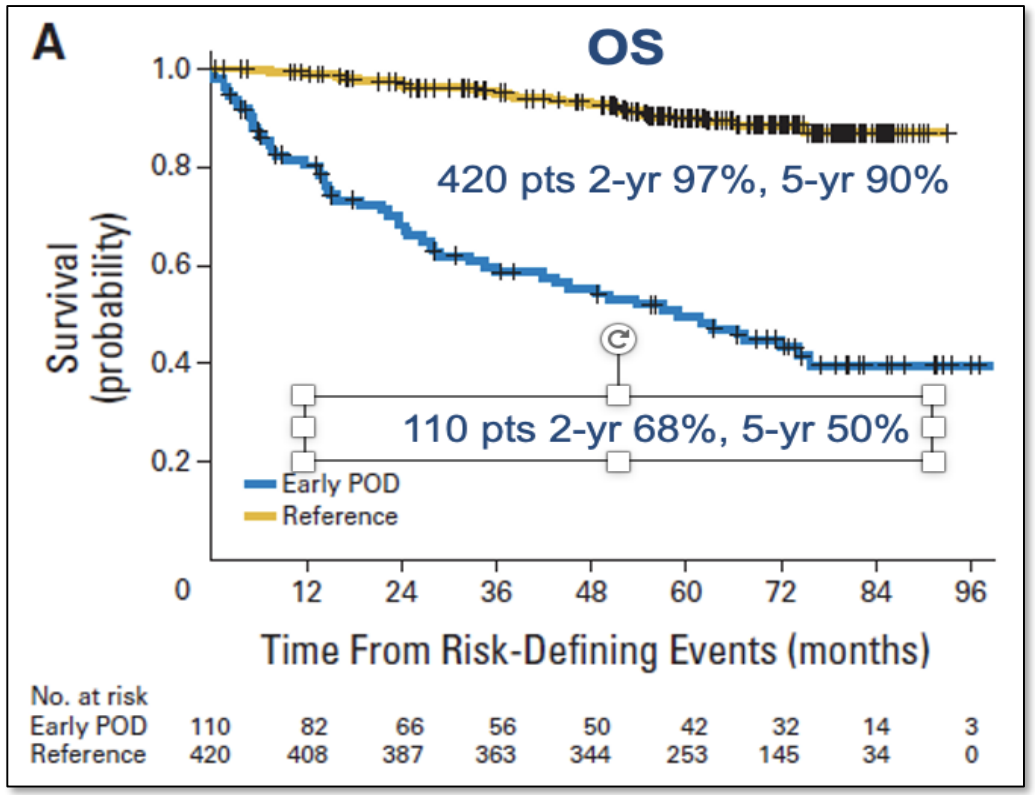



Brad S. Kahl, and David T. Yang Blood 2016;127:2055-2063

# Prognosis and survival in Follicular lymphomas




*Tan D, et al; Blood 2013*




*Casulo C et al, JCO 2015*

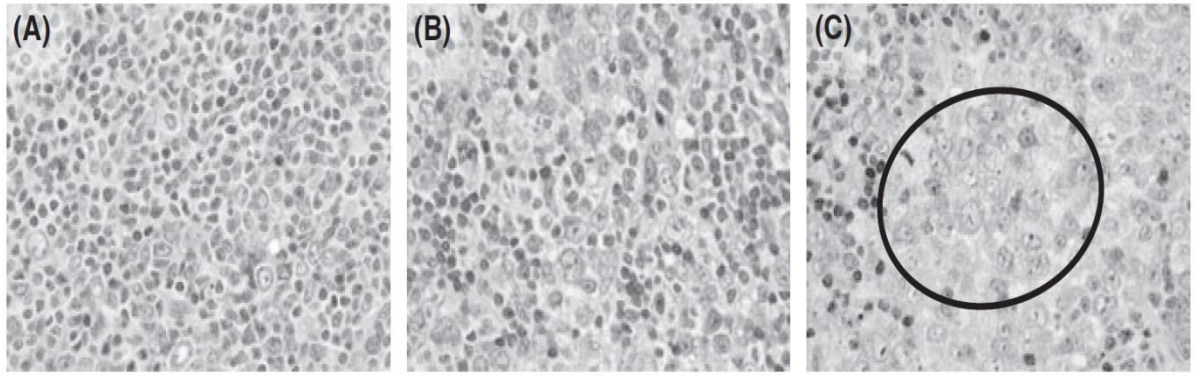
# Biology of high risk Follicular lymphoma

Central questions:

1. What is high risk in FL?
2. How to identify patients at risk?

# Follicular Lymphoma

## Grade IIIa/IIIB



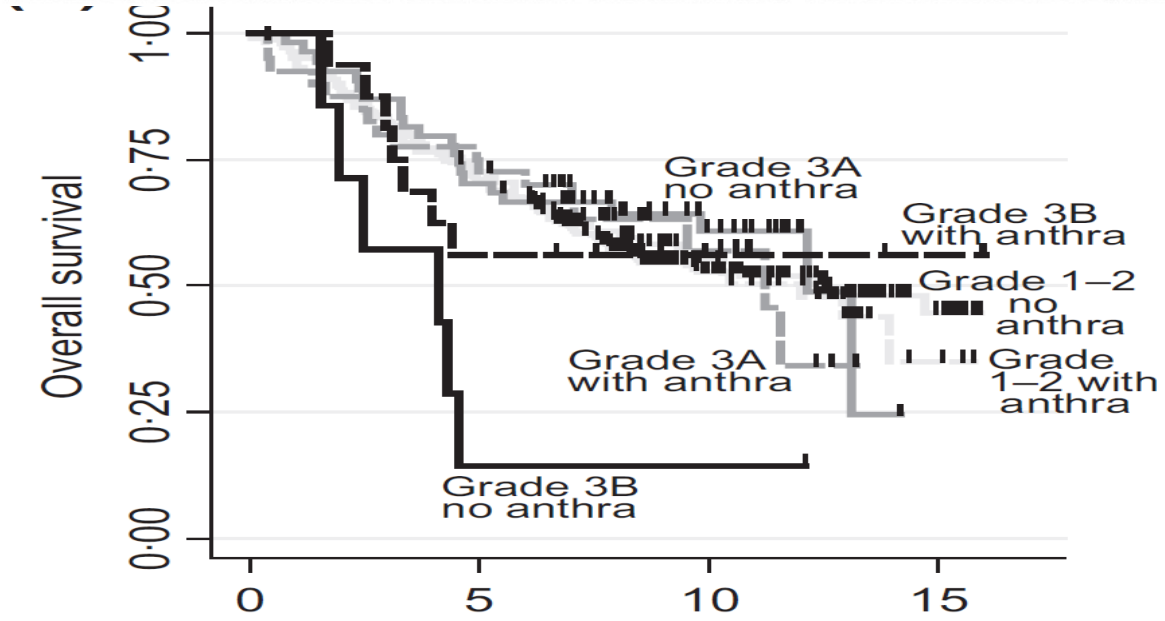




**Table 1. Grading of FL**

| Grade | Description |
|---|---|
| 1 | ≤5 blasts/HPF |
| 2 | 6-15 blasts/HPF |
| 3A | >15 blasts/HPF, centroblasts with intermingled centrocytes |
| 3B | >15 blasts/HPF, pure sheets of blasts |

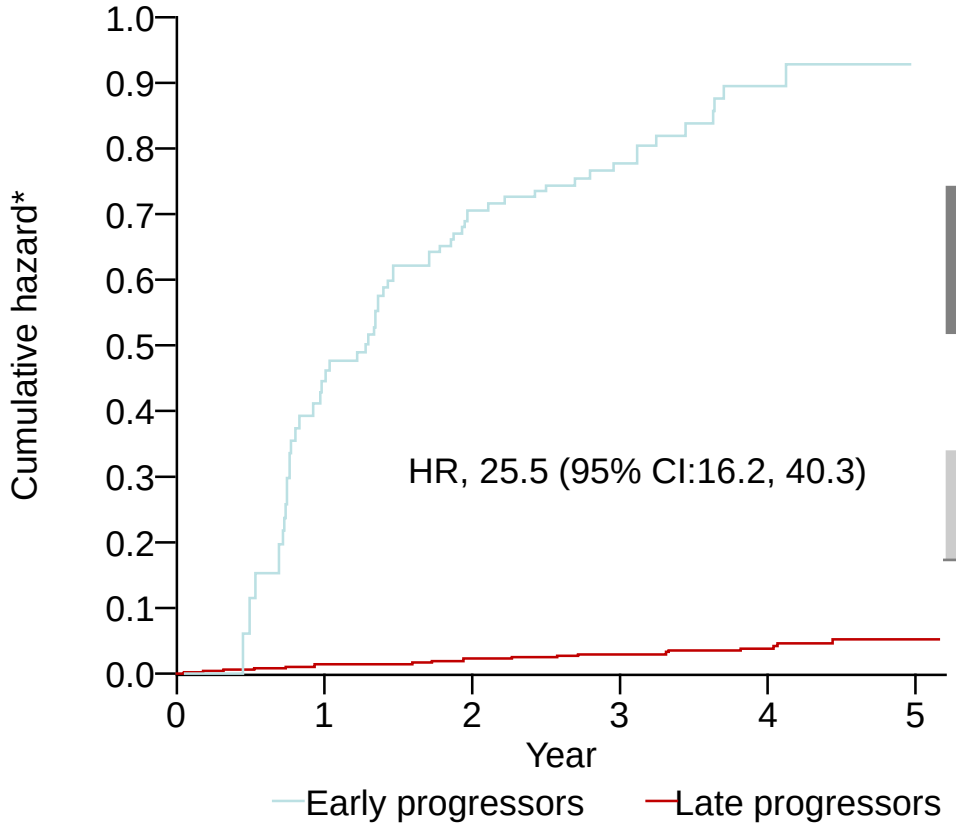
FL, follicular lymphoma; HPF, high-power field.

*Wahlin, BJH 2011*

# Follicular Lymphoma – Grading and Subtyping

| | **before** (Kiel, REAL etc) | **WHO 2000** | **WHO 2008** | **WHO 2017** | **WHO 2022** |
|---|---|---|---|---|---|
| **grading** | no grading (Kiel)<br>provisional (REAL) | Grade 1<br>Grade 2<br>Grade 3A<br>Grade 3B | Grade 1<br>Grade 2<br>Grade 3A<br>Grade 3B | Grade 1/2<br><br>Grade 3A<br>Grade 3B | classic<br><br>large cell type |
| **subtypes** | *none* | *none* | „variants“ (pediatric, intestinal, „in situ“)<br><br>•PCFCL (cutaneous) | „variants“ (testicular, duodenal, „in situ“)<br><br>•PCFCL (cutaneous)<br>•Pediatric type | •In situ follicular B cell neoplasm<br><br>•Paediatric-type follicular lymphoma<br><br>•Duodenal-type follicular lymphoma<br><br>•Gonadal follicular lymphoma |

# Risk of death following a POD24 event




| | N | OS events | Incidence rates/100 patient-years (95% CI) | Median follow-up (years) | Patient-years at risk |
|---|---|---|---|---|---|
| With POD24 event | 155 | 56 | 19.38 (14.9, 25.2) | 1.88 | 289 |
| Without POD24 event† | 1202 | 39 | 1.03 (0.8, 1.4) | 3.32 | 3772.2 |

60% of deaths occur in POD24 patients!

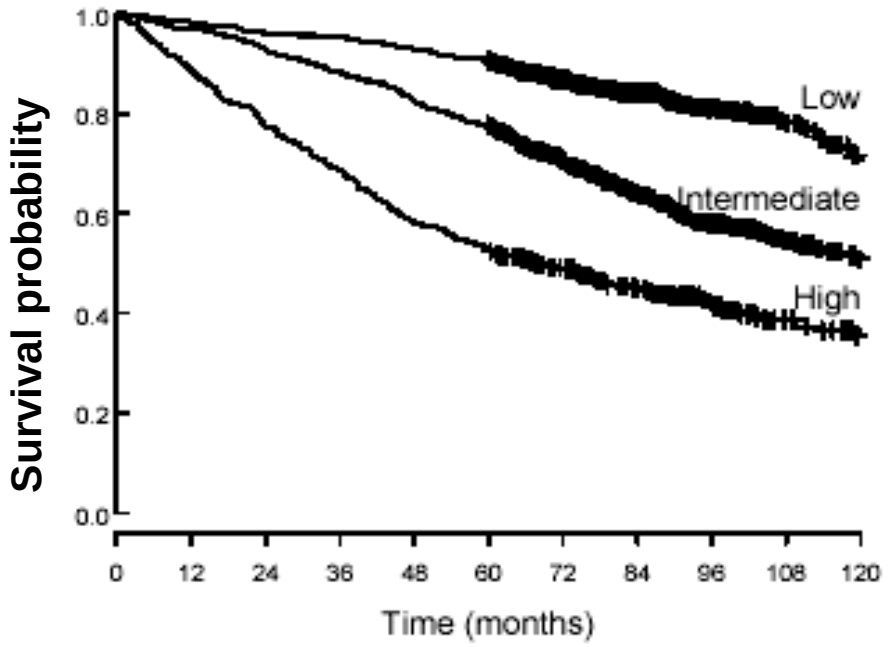
- In GALLIUM, the relative risk of death at any time was 26-fold higher after a POD24 event than in patients without an event

*Cumulative incidence of death following a POD24 event

†Includes all patients in the POD24 group (before they had a POD24 event) and all noPOD24 patients

Seymour Haematologica 2019

# Clinical risk index for follicular lymphomas
# FLIPI (n=1795)




| Parameter | Adverse factor | RR | 95% CI |
|---|---|---|---|
| Age | ≥ 60 y | 2.38 | 2.04 – 2.78 |
| Ann Arbor stage | III – IV | 2.00 | 1.56 – 2.58 |
| Hemoglobin | < 12 g/dL | 1.55 | 1.30 – 1.88 |
| Serum LDH level | > ULN | 1.50 | 1.27 – 1.77 |
| Number of nodal sites | > 4 | 1.39 | 1.18 – 1.64 |

*Solal-Celigny, Blood 2004*

Table 4. FLIPI and PRIMA-PI risk factors

| Parameter | Definition of risk factors | | |
|---|---|---|---|
| | FLIPI 1 | FLIPI 2 | PRIMA-PI |
| Nodal sites | >4 LN regions (definition in[5]) | Long diameter of largest LN >6 cm | — |
| Age | >60 years | >60 years | — |
| Serum marker | Elevated LDH | Elevated B2M | Elevated B2M |
| Stage | Advanced stage III-IV (Ann Arbor classification) | Bone marrow involvement | Bone marrow involvement |
| Haemoglobin | <12 g/dl | <12 g/dl | — |

*Dreyling M Ann Oncol 2021*

# Biology of high risk Follicular lymphoma

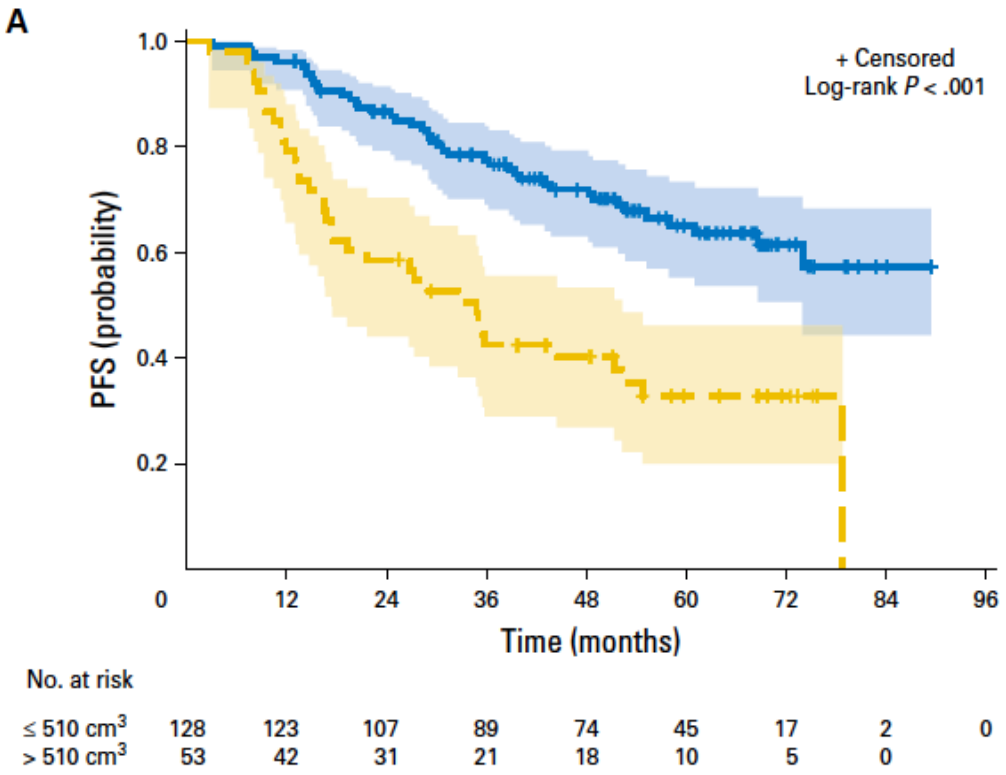
- High risk FL is a function of tumor burden

# Imaging biomarkers for early risk stratification:

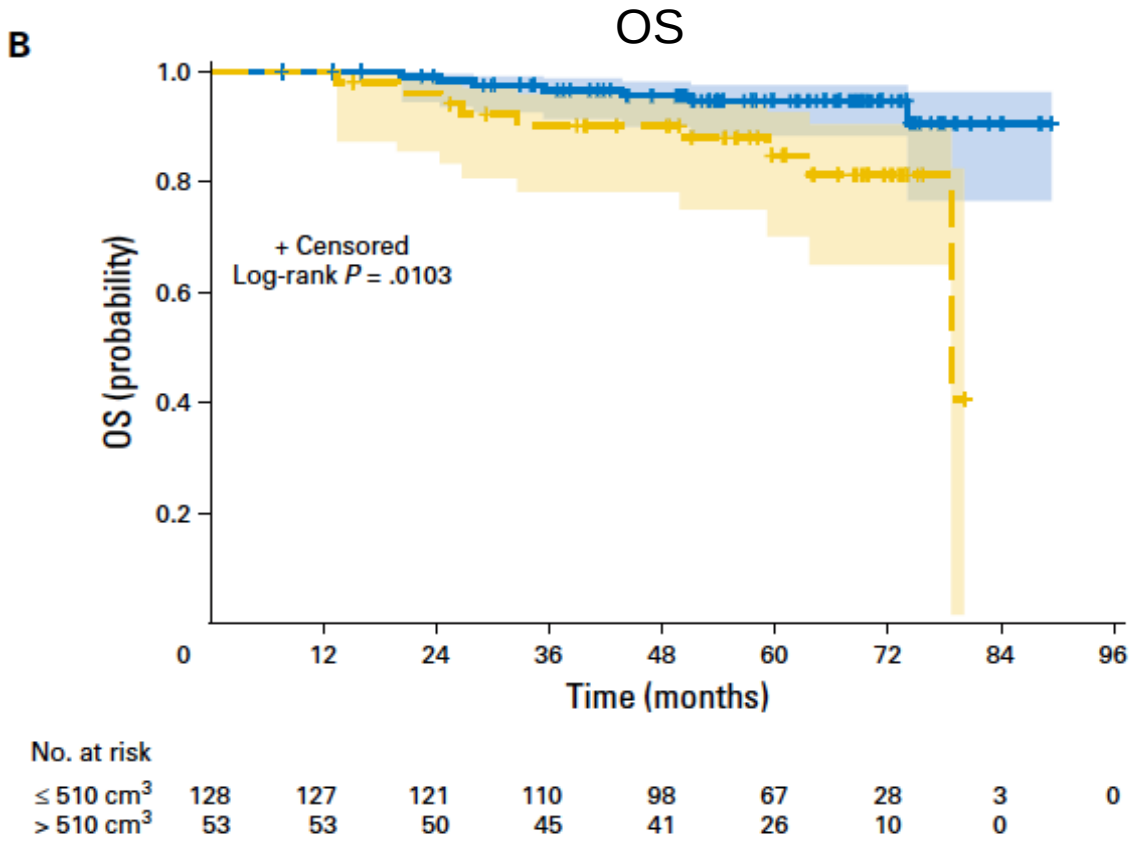
## Baseline metabolic tumor volume in high tumor burden FL

N=185 patients from 3 prospective trials (LYSA, FIL)

PFS




OS

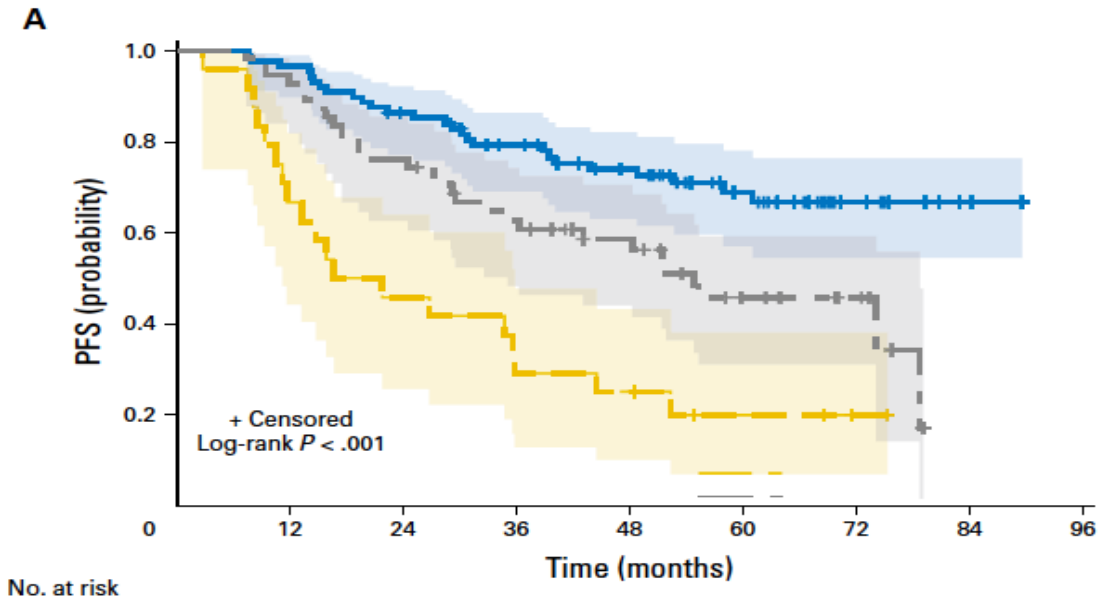



*Meignan JCO 2016*

# Imaging biomarkers for early risk stratification:
## Baseline metabolic tumor volume in high tumor burden FL

N=185 patients from 3 prospective trials (LYSA, FIL)

PFS according to TMTV and FLIPI2



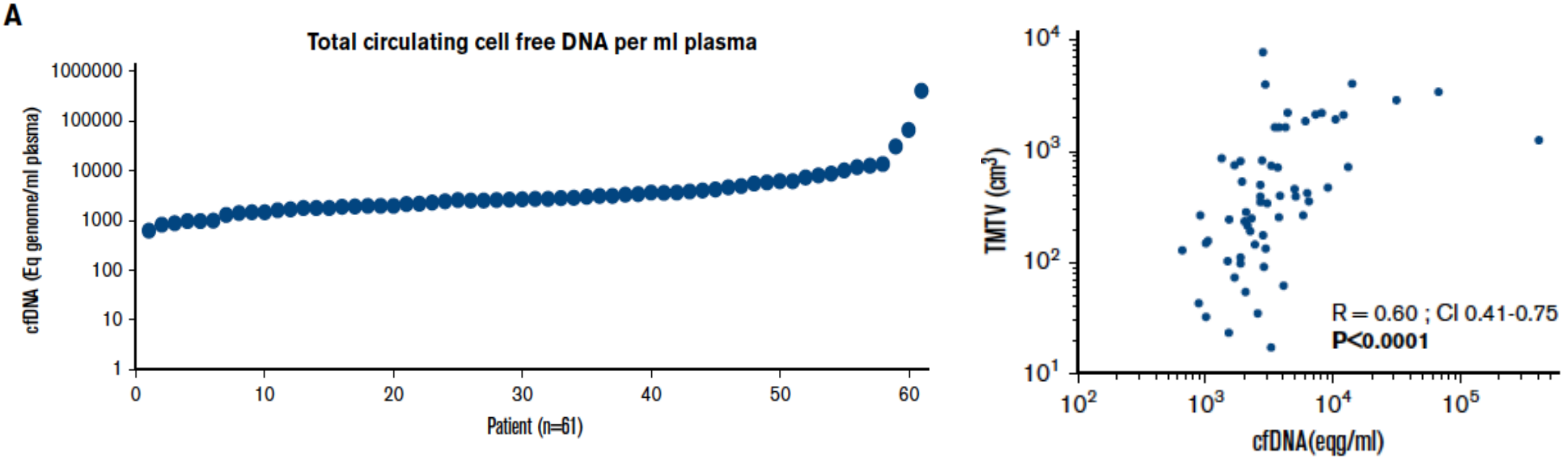

No. at risk

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 88 | 85 | 74 | 63 | 53 | 33 | 13 | 2 | 0 |
| 2 | 24 | 16 | 11 | 7 | 6 | 3 | 1 | 0 | |
| 3 | 55 | 51 | 41 | 32 | 25 | 14 | 6 | 0 | |

| | No. of Patients | Event | Censored | Median Survival (95% CI) |
|---|---|---|---|---|
| TMTV ≤ 510 and FLIPI2 0-2 | 88 | 29.5% (26) | 70.5% (62) | NR |
| TMTV > 510 and FLIPI2 3-5 | 24 | 79.2% (19) | 20.8% (5) | 19.2 (11.2 to 35.7) |
| TMTV > 510 or FLIPI2 3-5 | 55 | 52.7% (29) | 47.3% (26) | 54.7 (35.1 to 78.7) |

*Meignan JCO 2016*

# TMTV, CTC and cfDNA: distinct prognostic value in follicular lymphoma

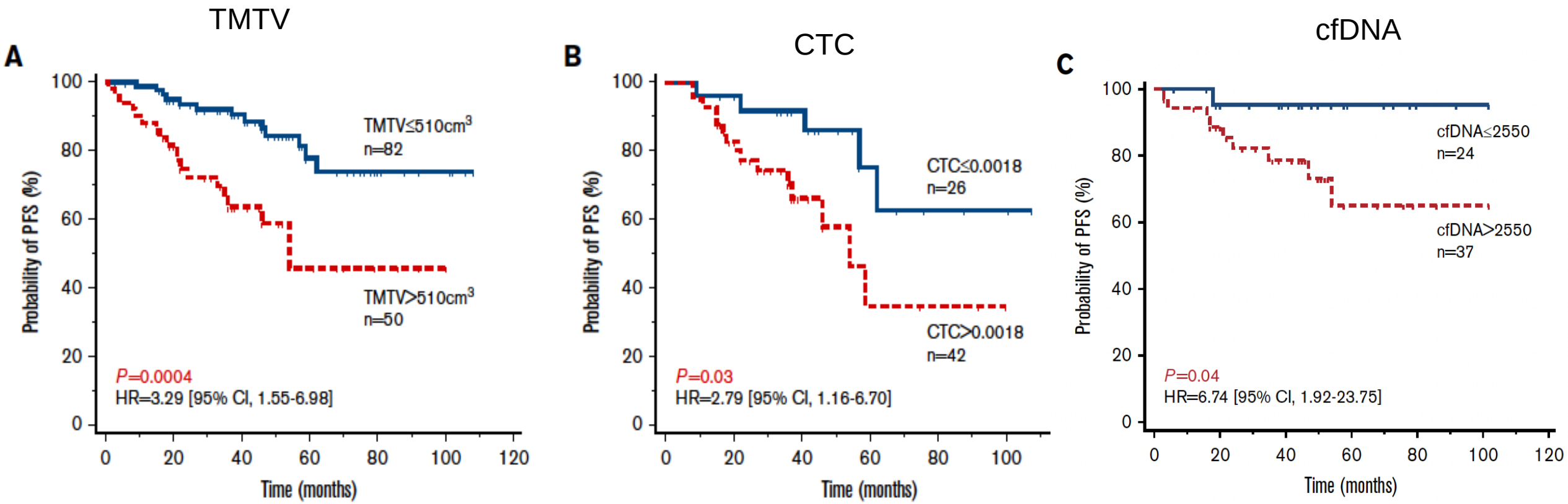



Correlation of TMTV and cfDNA at diagnosis

*Delfau-Larue Blood adv 2018*

# TMTV, CTC and cfDNA: distinct prognostic value in follicular lymphoma

**PFS**



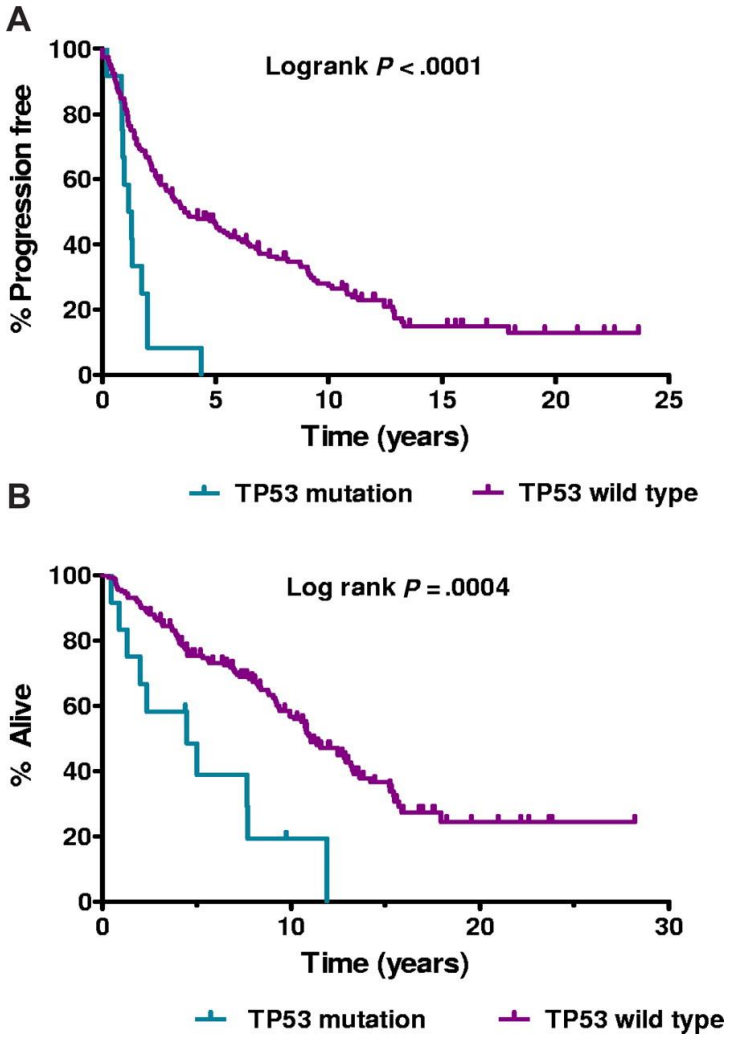

*Delfau-Larue Blood Adv 2018*

# Biology of high risk Follicular lymphoma

- High risk FL is a function of tumor burden
- Genetic alterations

# Progression free Survival dependent on TP53 mutational status

Heterozygous *TP53* mutation was detected in 12 of 185 (6%) analyzed cases and is associated with poor prognosis




Derville O'Shea et al. Blood 2008;112:3126-3129

blood

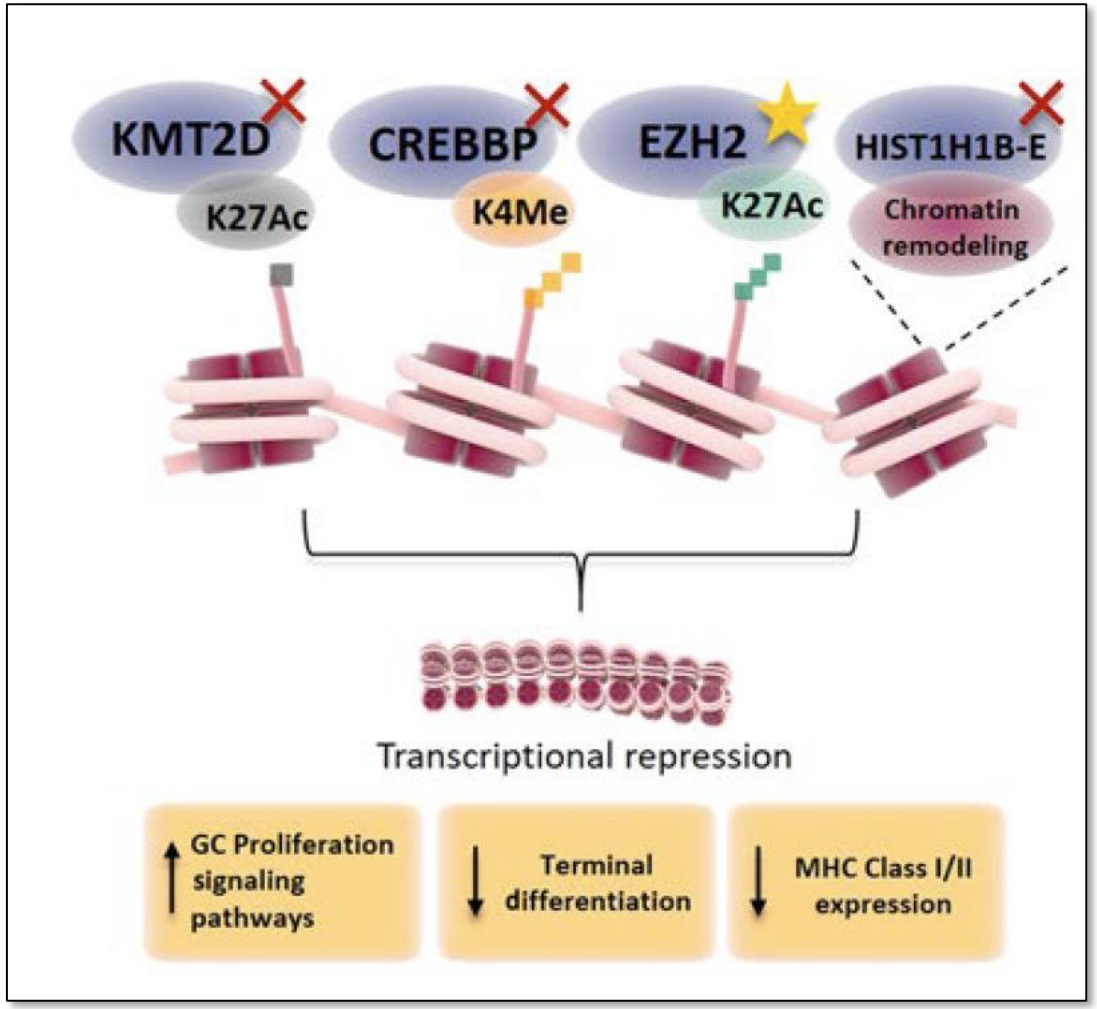
# Mutational landscape in follicular lymphoma

## Main classes of mutations in follicular lymphoma

| Mutational class | Frequency | Mutated/altered genes |
|---|---|---|
| Epigenetic modifiers | >90% | *KMT2D*, *CREBBP*, *EZH2*, *EP300*, *HIST1H1B-E* |
| Immune regulation | ~28%-40%; 6% | *TNFRSF14*, *CTSS* |
| mTOR signaling | >30% | *RRAGC*, *ATP6V1B2*, *ATP6AP1*, *SESTRIN1* |
| Cell cycle regulators | ~10%-15% | *CDKN2A/B*, *CCND3*, *MYC*, *TP53* |
| Jak/STAT signaling | ~20% | *STAT6*, *SOCS1* |
| NFκB signaling | >15% | *CARD11*, *TNFAIP3*, *CD79A/B*, *MYD88* |

Mutations in epigenetic regulators affect respective histone modifications with a convergence on specific downstream functional effects

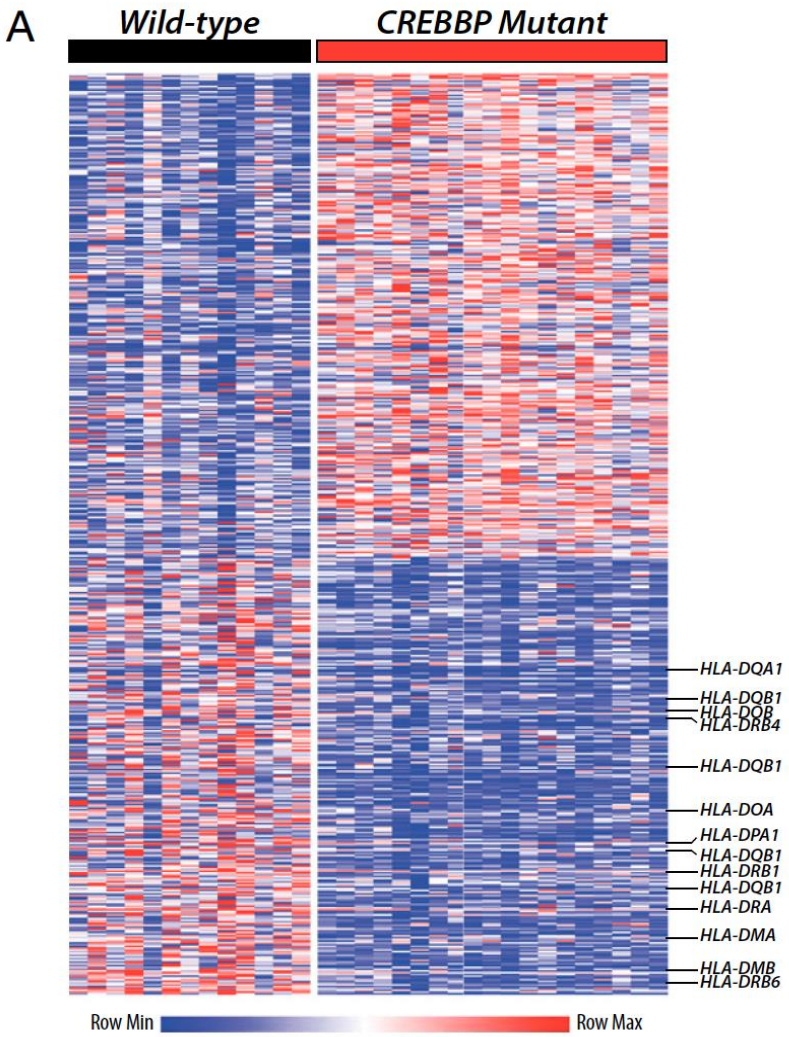
## Epigenetic modifications in FL



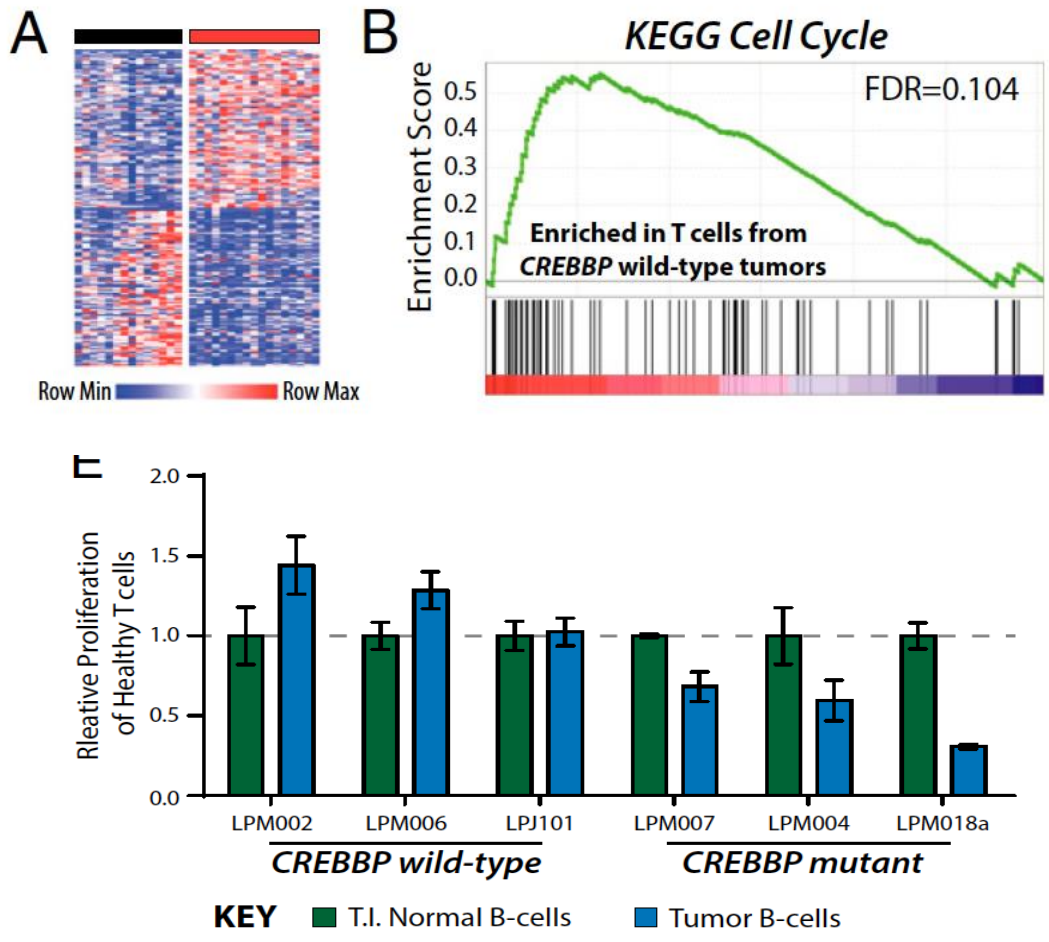

Perrett et al. ASH Hematol Educ Program 2022 (1):688-694

# CREBBP mutation as an early event in FL evolution that contributes to immune evasion via decreased antigen presentation.

Decreased MHC class II expression associated with CREBBP mutations



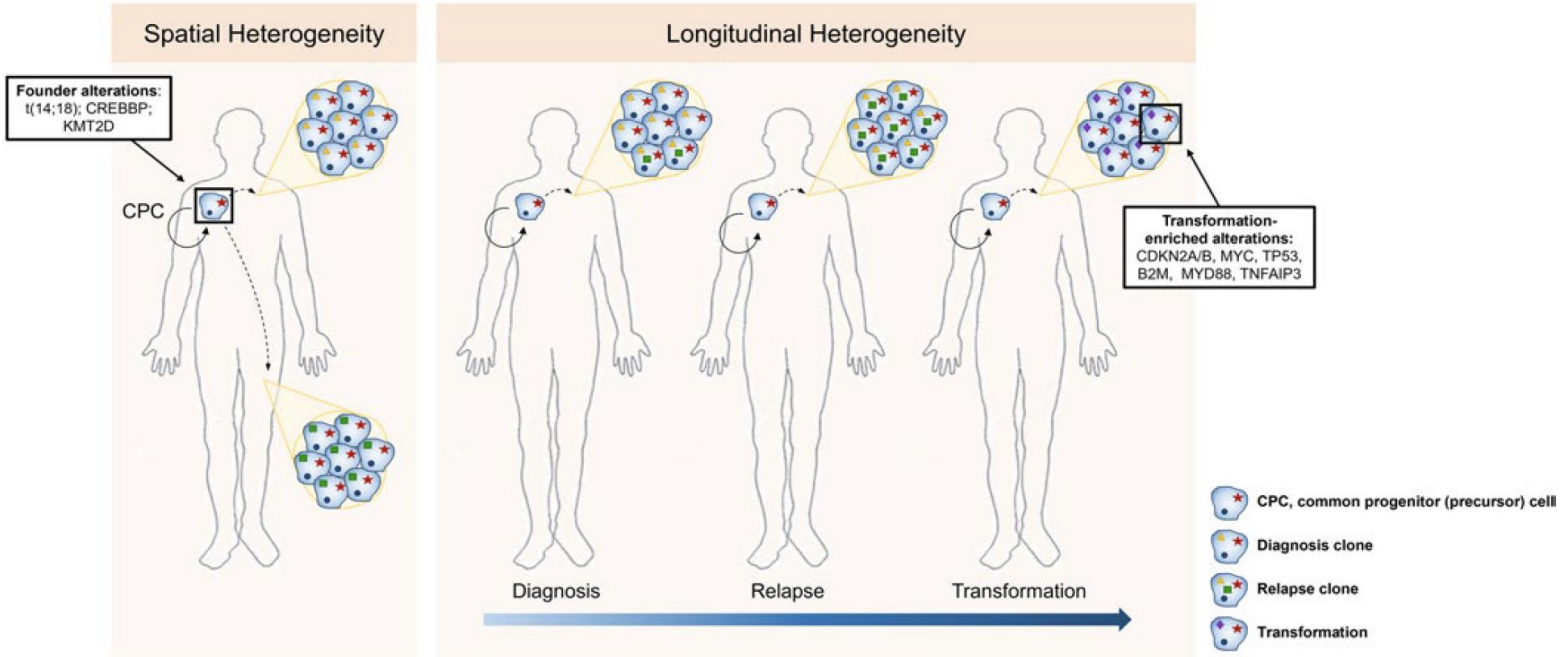

Decreased proliferation signature of T cells associated with CREBBP mutant B-cells



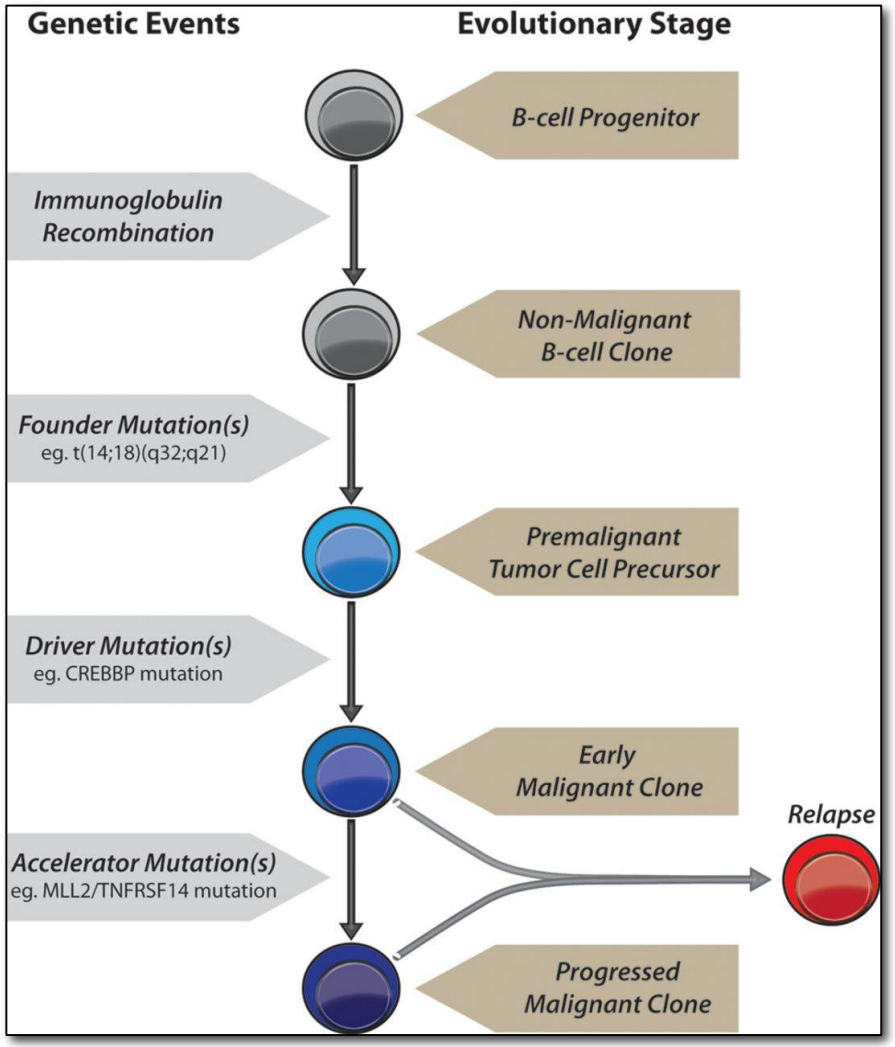

Green PNAS 2015

# Spatial and longitudinal genetic heterogeneity in FL

The cellular composition of the FL TME varies spatially and during different stages of the disease,




Perrett et al. ASH Hematol Educ Program 2022 (1):688-694




Green Blood 2013

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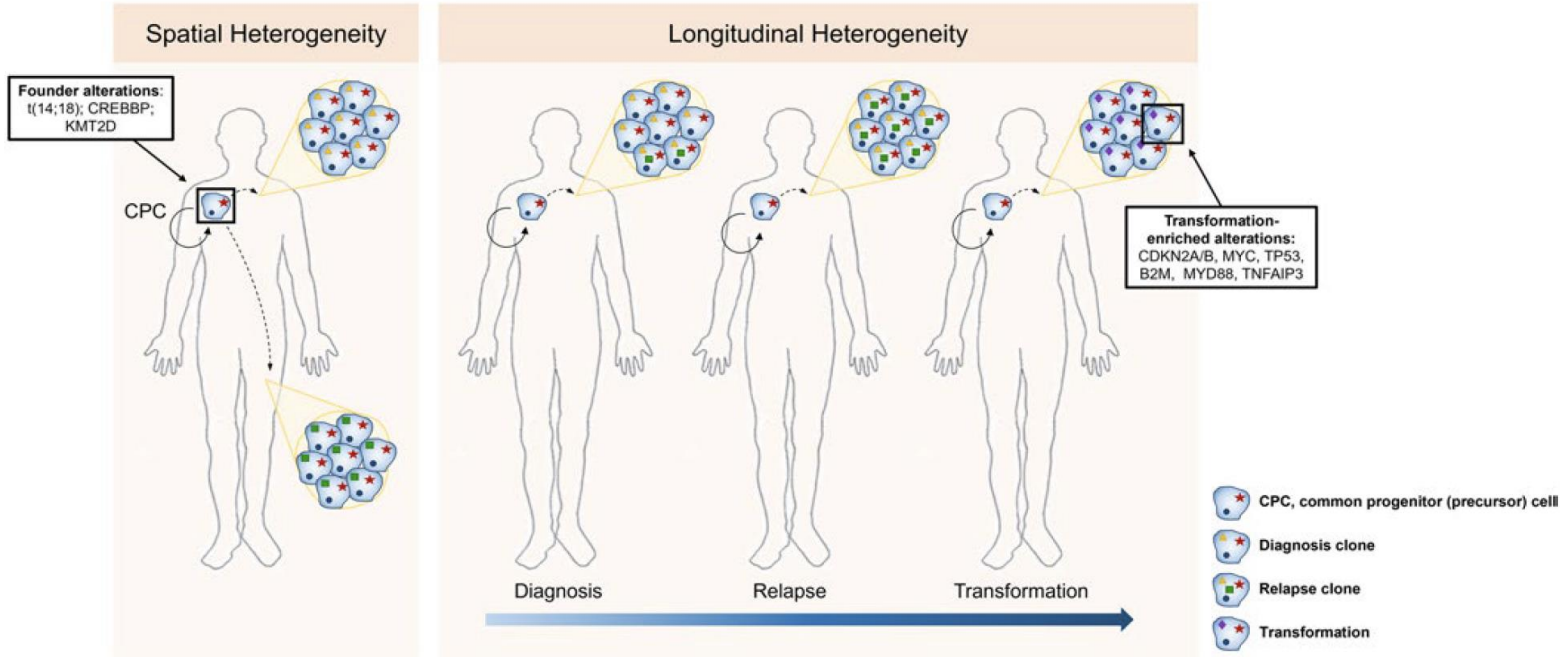
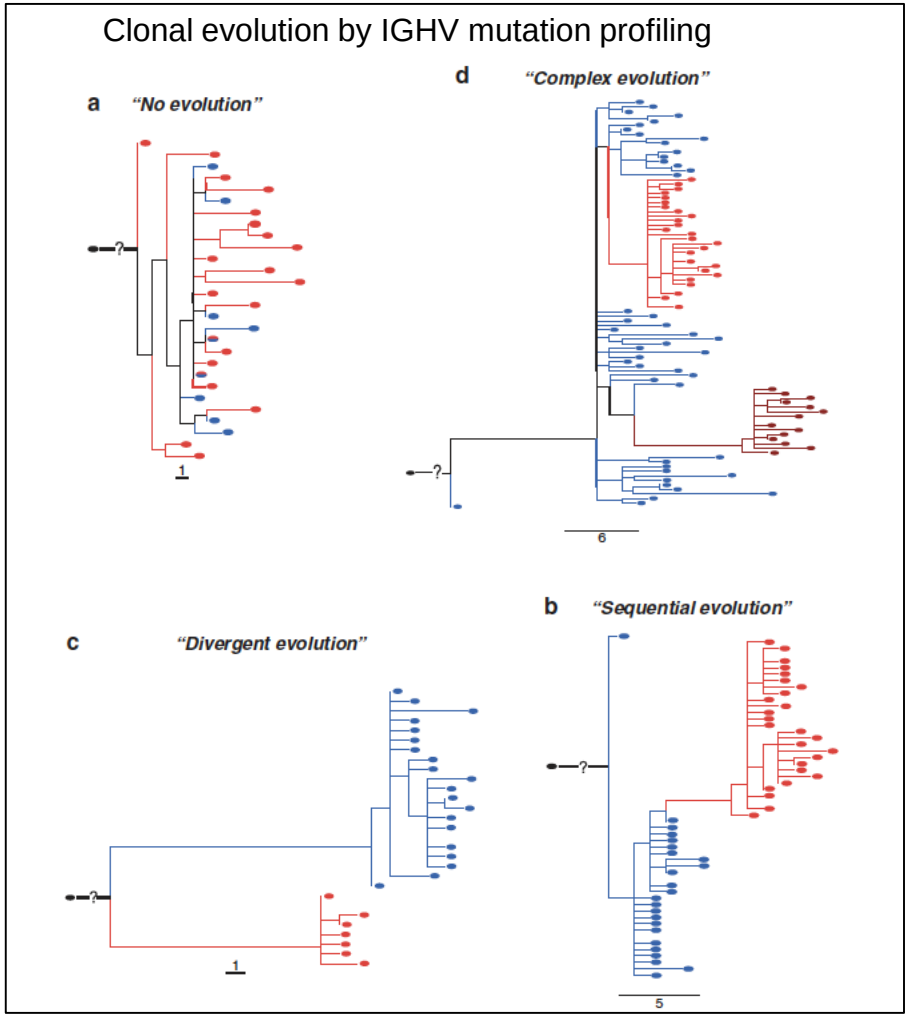



Perrett et al. ASH Hematol Educ Program 2022 (1):688-694

Clonal evolution by IGHV mutation profiling

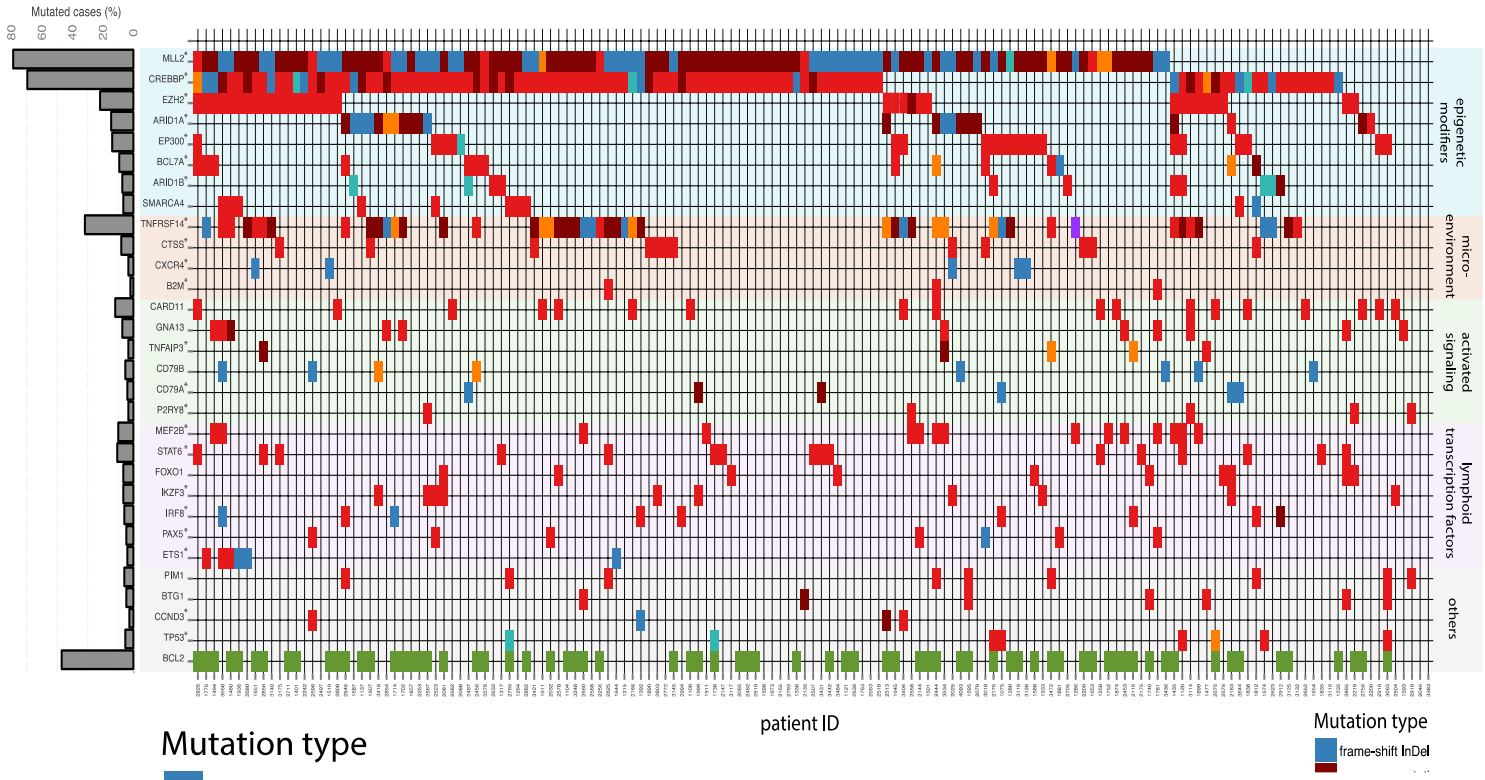



Löffler ..Pott et al Leukemia 2014

# Consortium Project of the GLSG2000 study:

## - Targeted Mutational Landscape of FL -

### Whole Exome Sequencing of 74 target genes




**Mutation type**

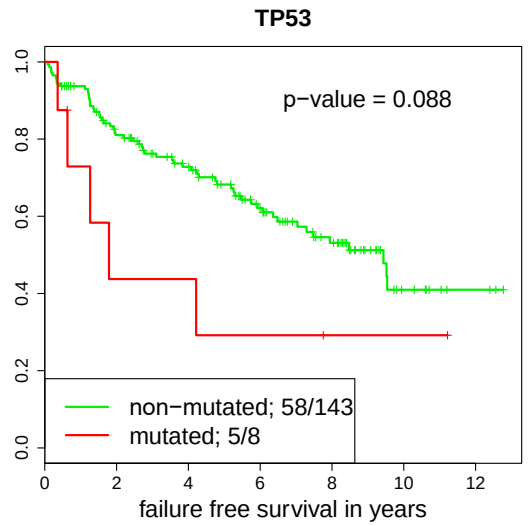
- frame-shift InDel
- nonsense mutation
- missense mutation
- in-frame InDel
- splice site mutation
- translational start site mutation
- BCL2 hypermutation

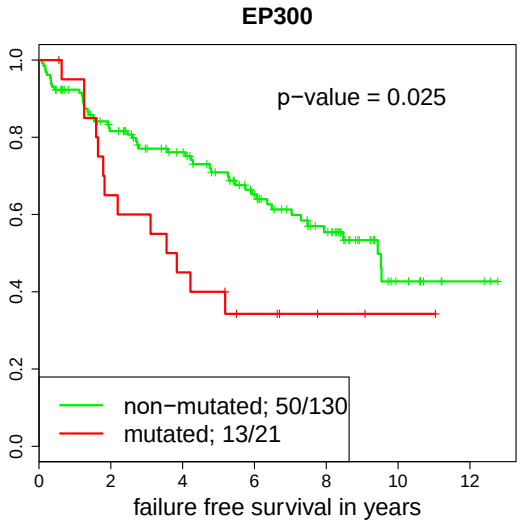
146 (97%) FL harboured non-silent mutations in epigenetic modifiers
*KMT2D, CREBBP, EP300, ARID1A, and BCL7A*

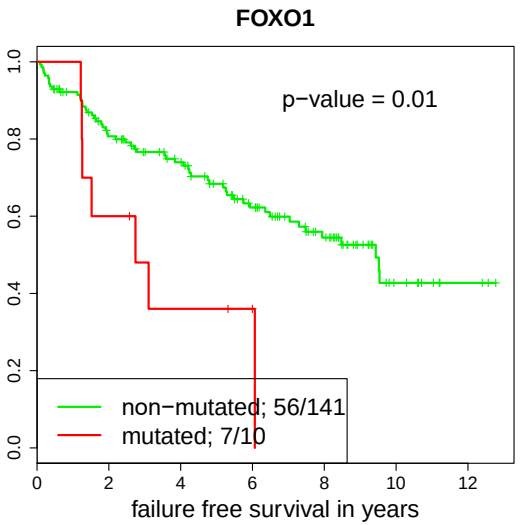
*Pastore A, Lancet Oncology 2015*

# Consortium project of the GLSG2000 study:
## - Correlation of mutations with „Failure free survival“

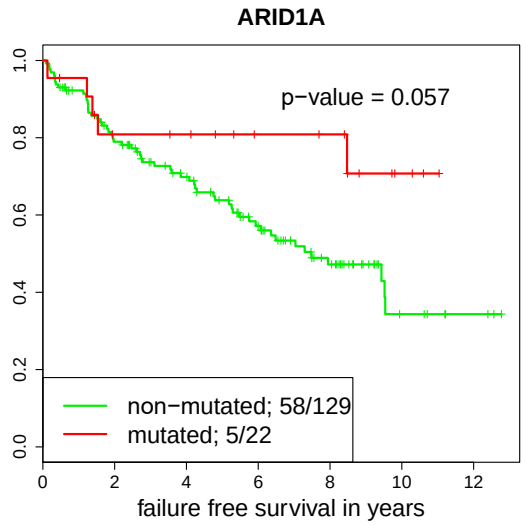
↓ FFS

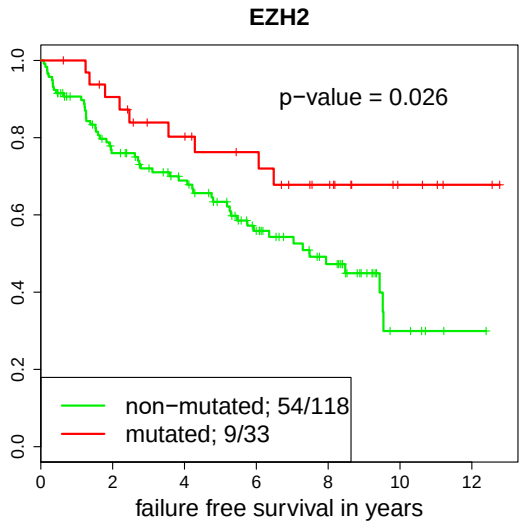


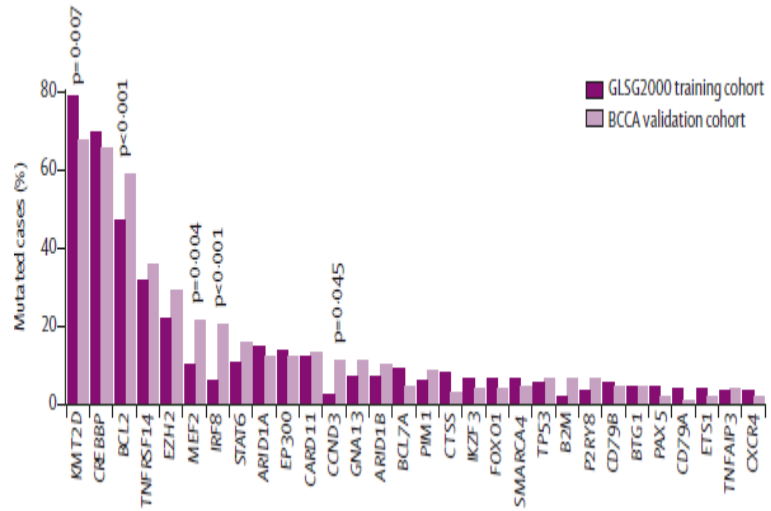

↑ FFS

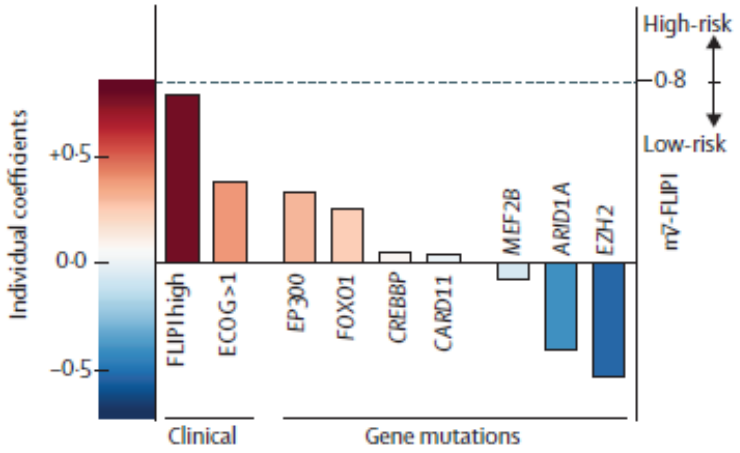


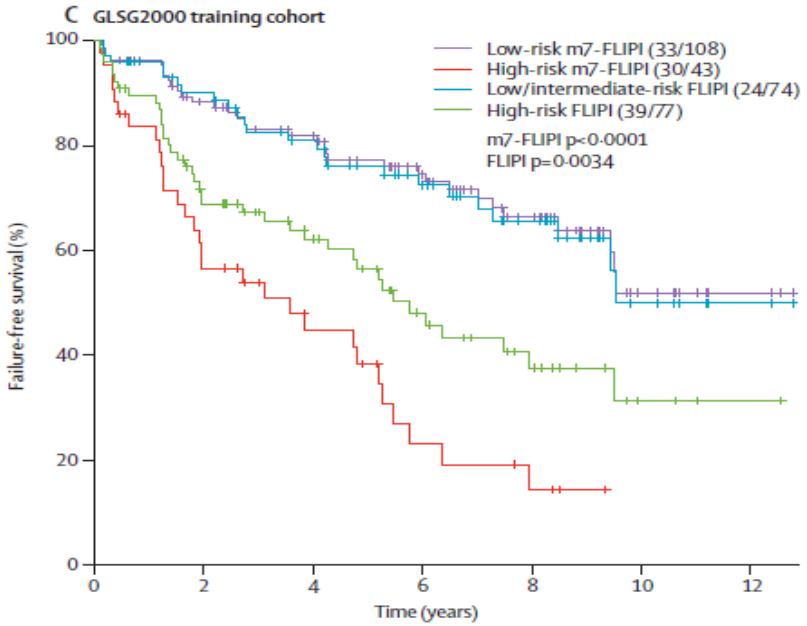



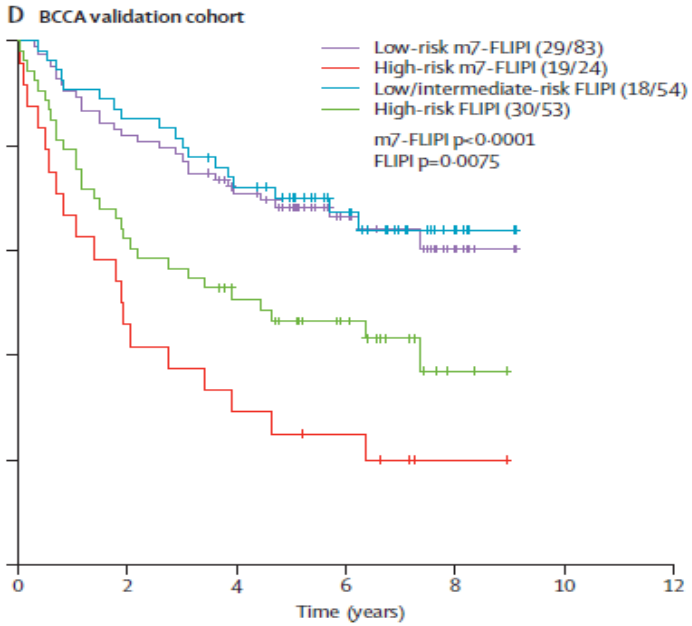

*Pastore A, Lancet Oncology 2015*

# Clinical-genetic risik model: M7-FLIPI
# Prognostic impact



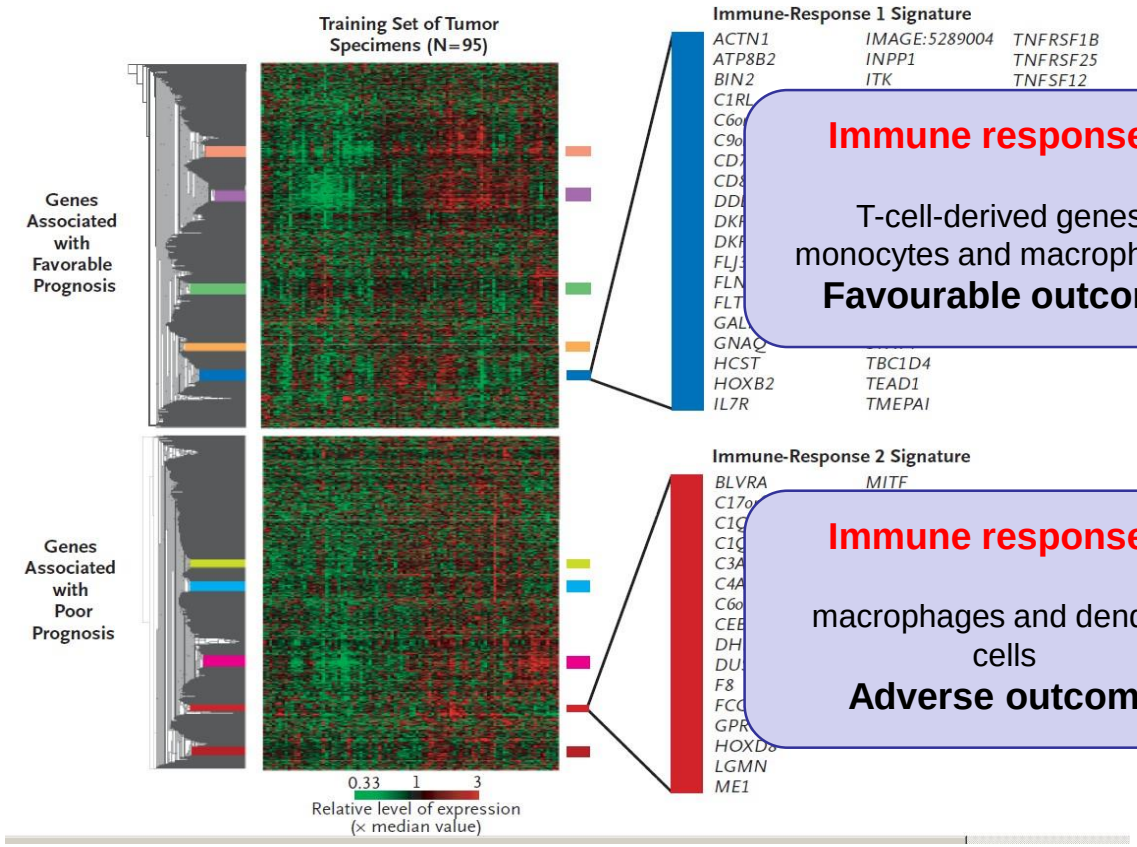

*Pastore A, Lancet Oncology 2015*

# Biology of high risk Follicular lymphoma

- High risk FL is a function of tumor burden
- Genetic alterations
- Microenvironment

# Different TME phenotypes can yield information relevant to a patient's prognosis

## Prediction of Survival in Follicular Lymphoma Based on Molecular Features of Tumor-Infiltrating Immune Cells




**Immune response-1**

T-cell-derived genes, monocytes and macrophages

**Favourable outcome**

**Immune response-2**

macrophages and dendritic cells

**Adverse outcome**

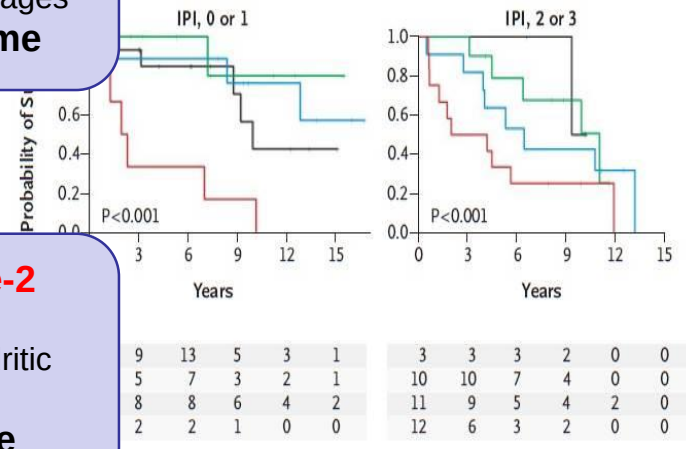



**Table 2. Predictive Power of Gene-Expression Signatures in Follicular Lymphoma.***

| Gene-Expression Signature | P Value for Contribution to Model in Test Set | Relative Risk of Death (95% CI)* | Effect of Increased Gene Expression on Survival |
|---|---|---|---|
| Immune-response 1 | <0.001 | 0.15 (0.05–0.46) | Favorable |
| Immune-response 2 | <0.001 | 9.35 (3.02–28.90) | Unfavorable |

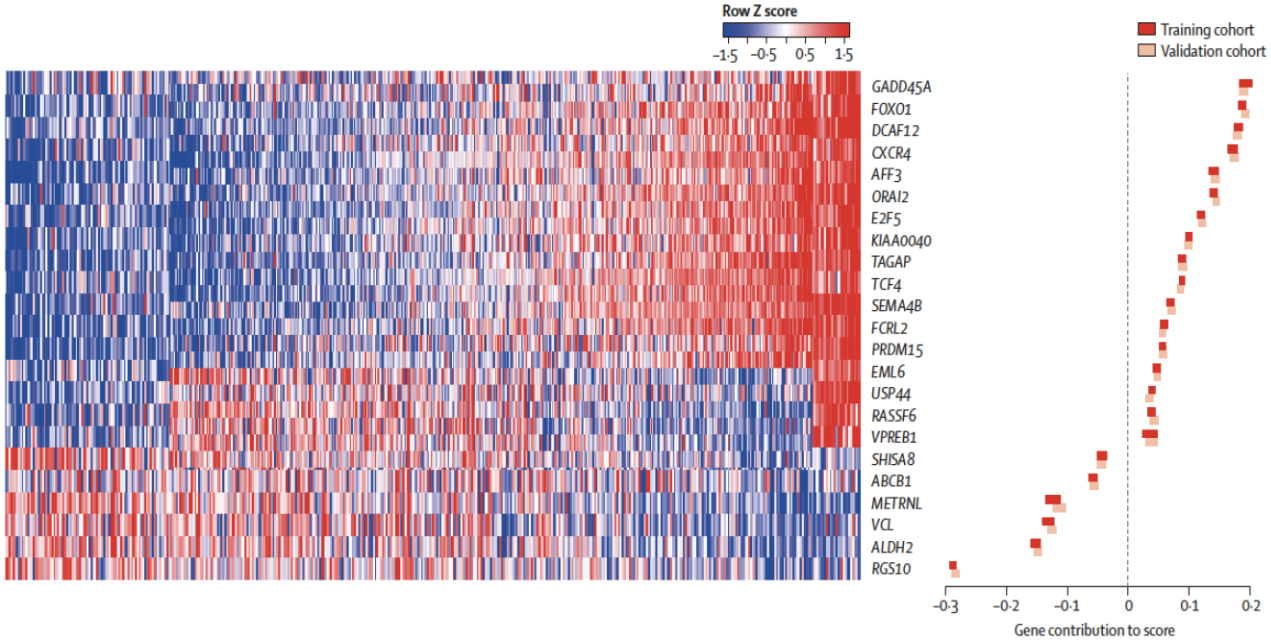
* Relative risks are for patients with specimens in the test set and are based on a doubling of the signature expression as a component in the two-variable survival-predictor model. CI denotes confidence interval.

*Dave S NEJM 2004*

# Gene expression profiling (n=149/488)

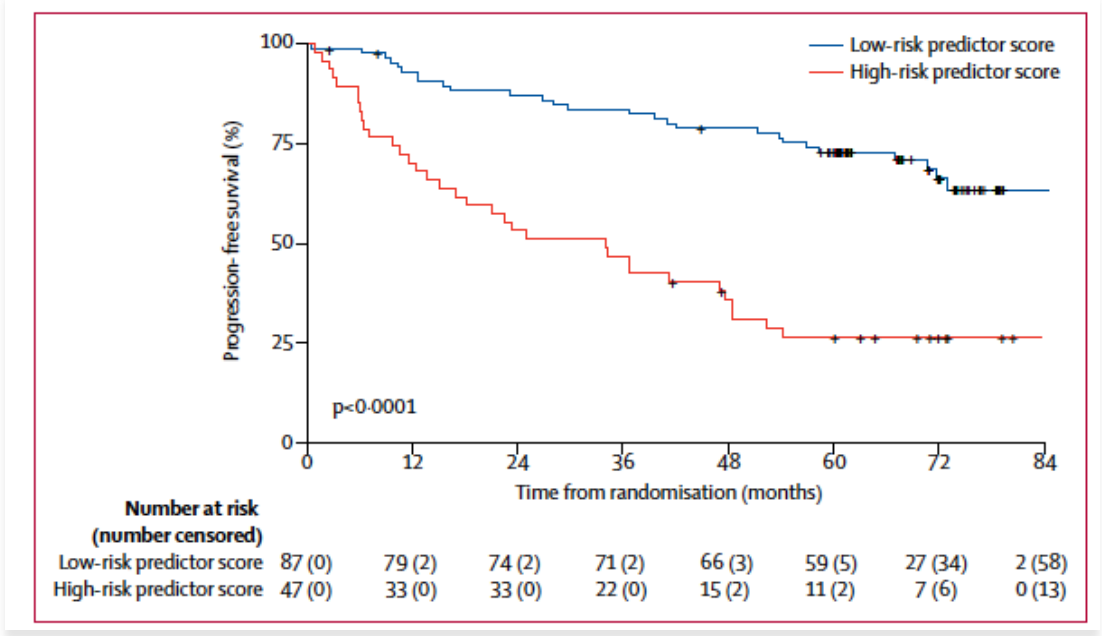
23 gene profile reflecting both B-cell biology and tumour microenvironment

## Differentially expressed genes associated with PFS




134 patients from the PRIMA trial (training cohort) with high-tumor burden

## The 23-gene score: PFS according to GEP signature

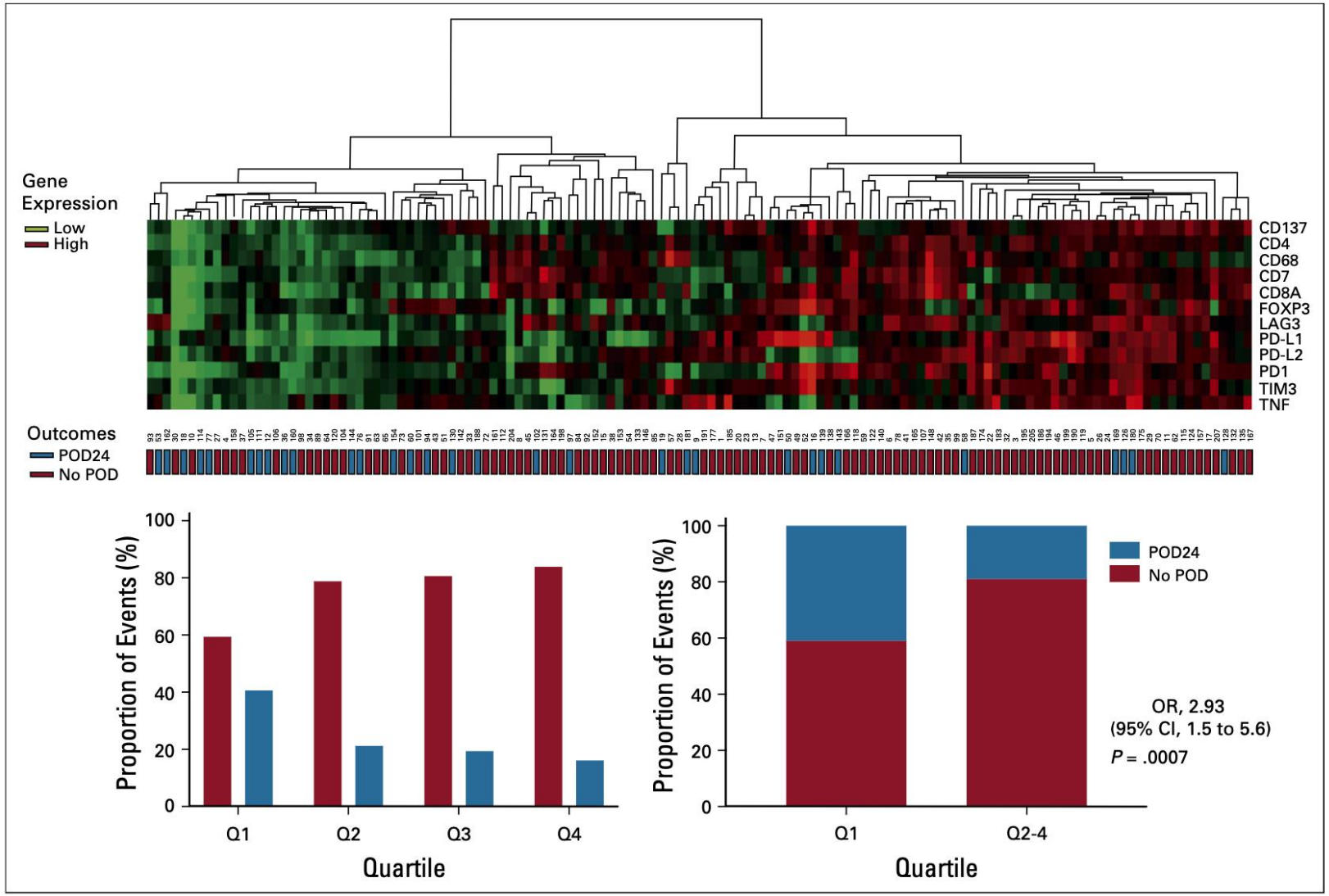



The risk of POD24 was 38% in the high-risk group and 19% in the low-risk group.

***Huet, Lancet Oncol 2018***

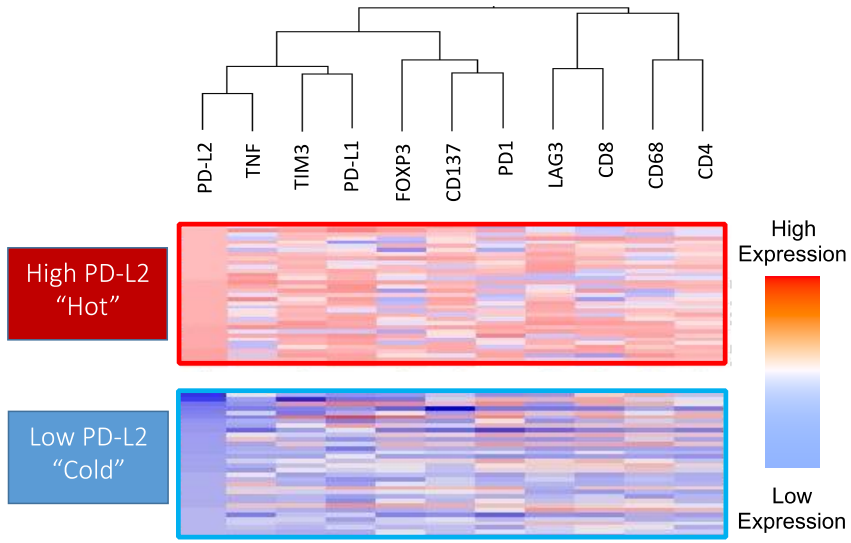
# FL immune milieu is a major contributor to patient outcome

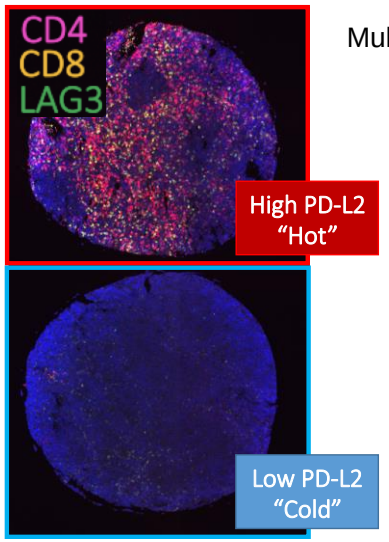
Immune effector, immune checkpoint, and macrophage gene expression FL
Gene expression of 12 genes by NanoString




Tobin JCO 2019

# Intratumoral Immune Infiltration: PD-L2 is Predictive of POD24

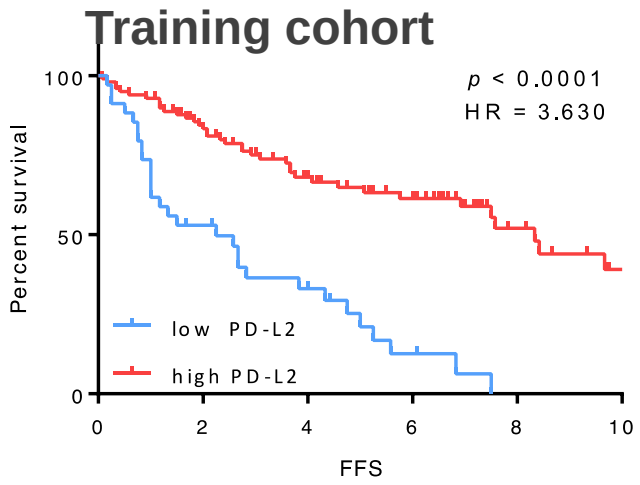


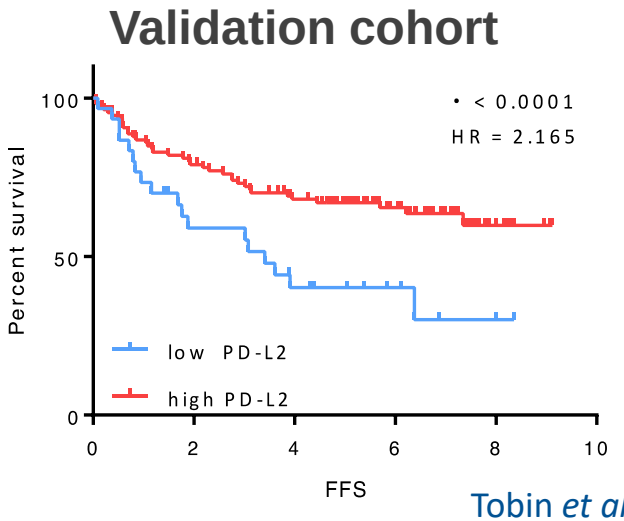




Multispectral immunofluorescence

Journal of Clinical Oncology®
An American Society of Clinical Oncology Journal

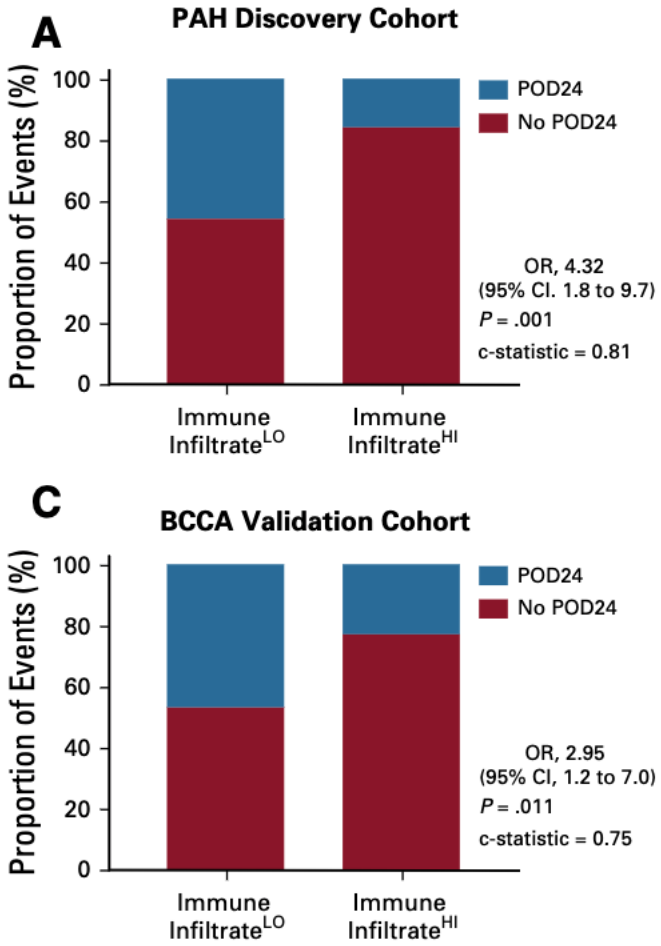
**Progression of Disease Within 24 Months in Follicular Lymphoma Is Associated With Reduced Intratumoral Immune Infiltration**

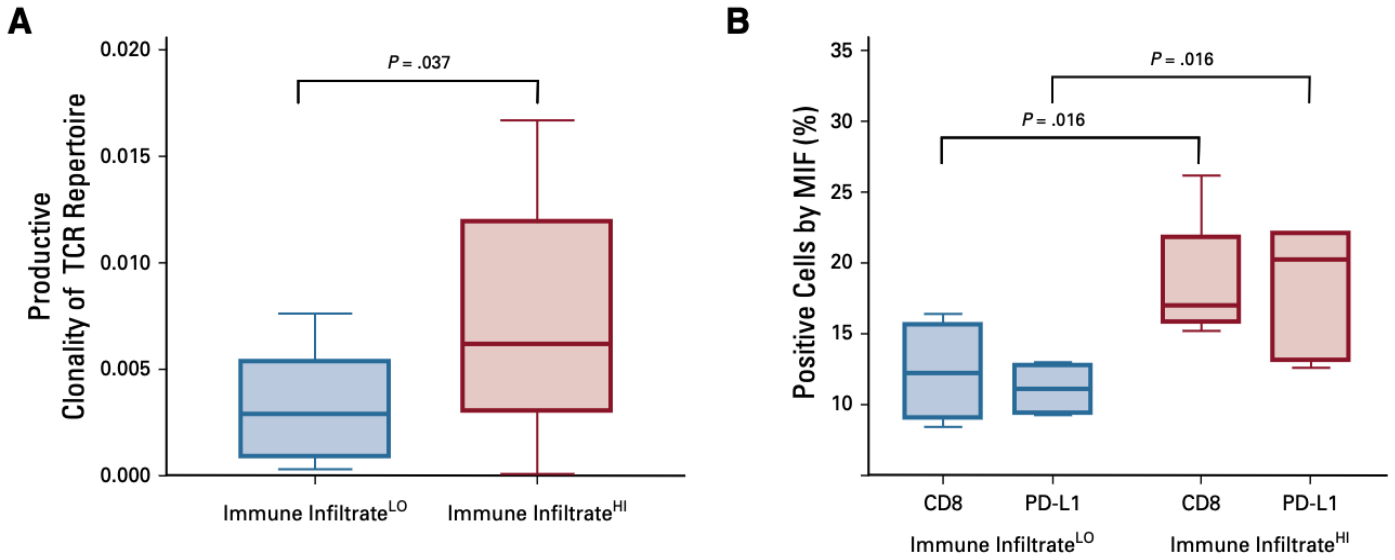



Tobin *et al*, **JCO** 2019
Tobin *et al*, **ASH** 2017 (#728)

# The immune infiltration$^{LO}$ subset of patients with FL is enriched for POD24 events

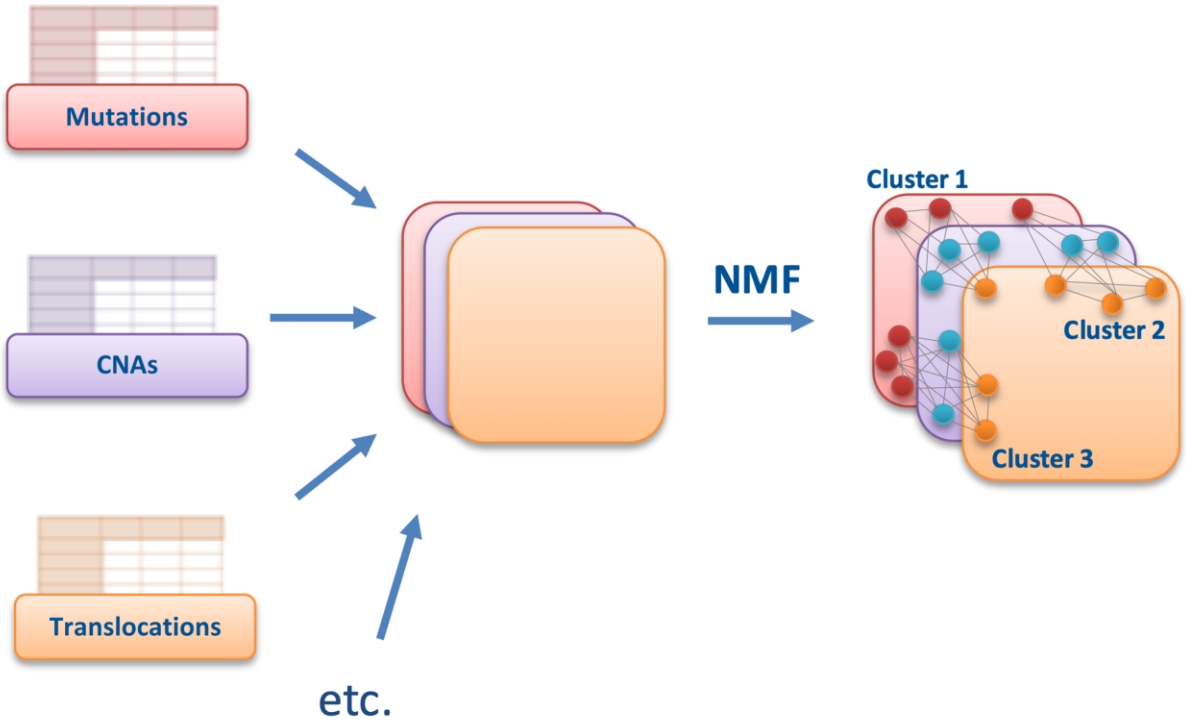


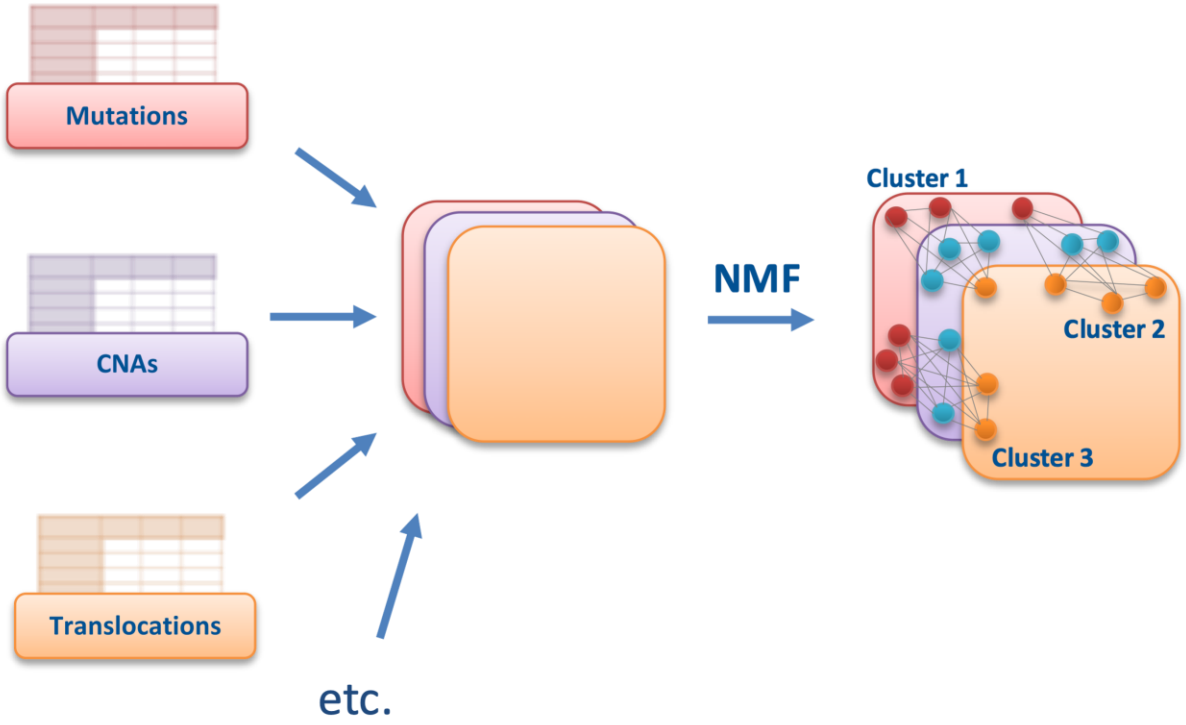

Mutational Profile Is Similar Between Immune Infiltration HI and Immune Infiltration LO Tissue

Tobin JCO 2019

# Integrative Analysis of Omics Data



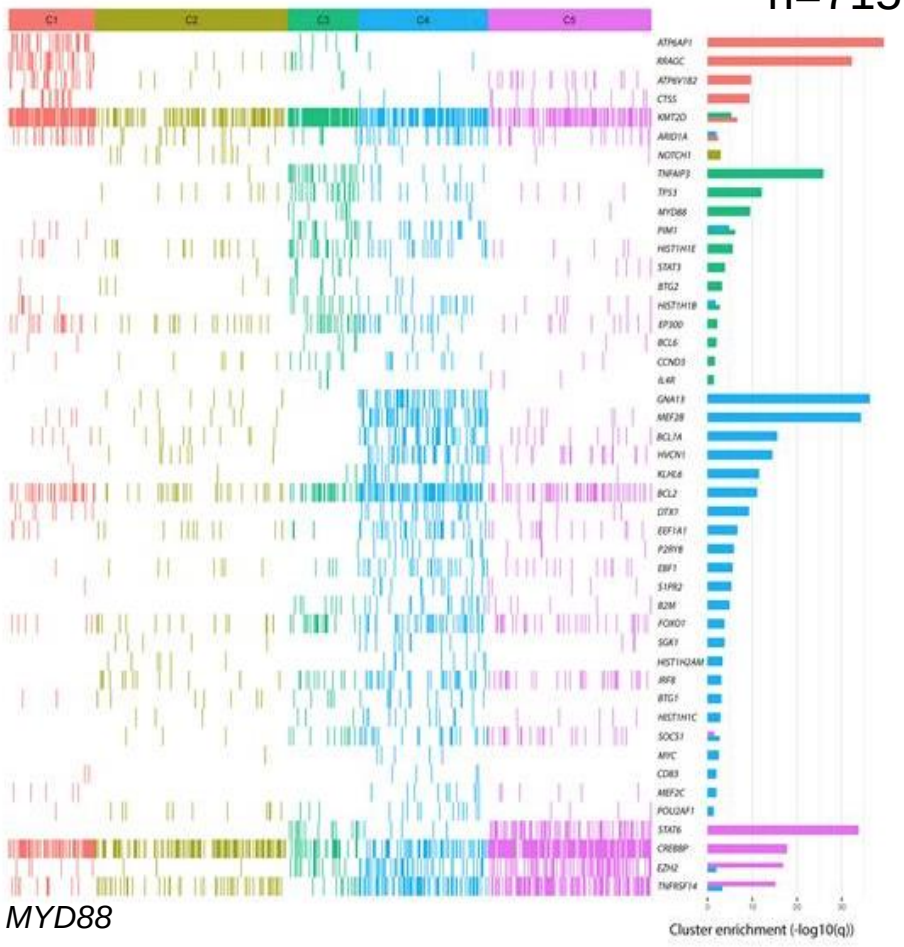
# Integrative Analysis of Omics Data




novel genetic profiles shed light on core pathways

targeted DNA-sequencing of 57 genes

n=715




C1 mTOR signaling pathway

C2 cluster by low mutation burden

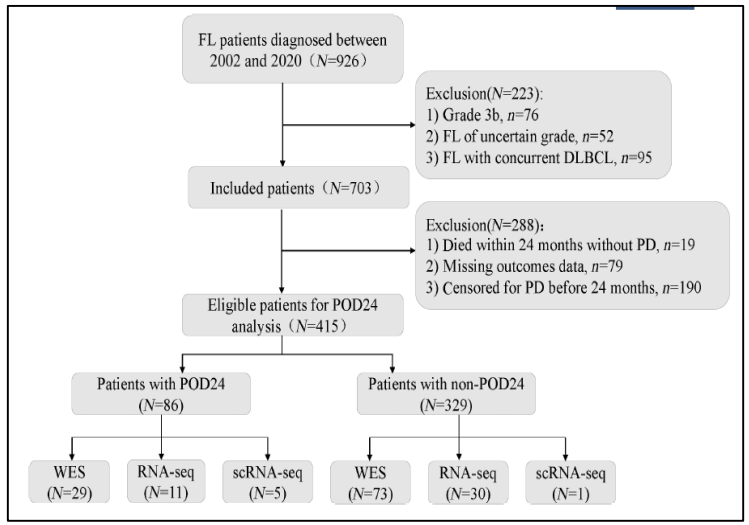
C3 cluster by mutations involving *TNFAIP3, TP53* and *MYD88*

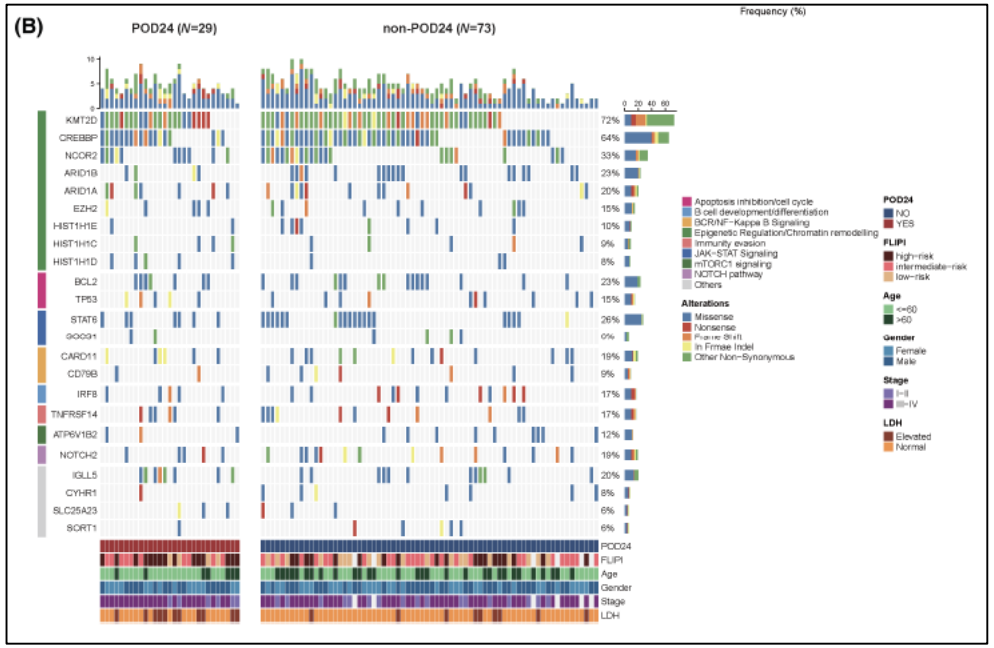
C4 cluster by *GNA13* and *MEF2B* mutations;

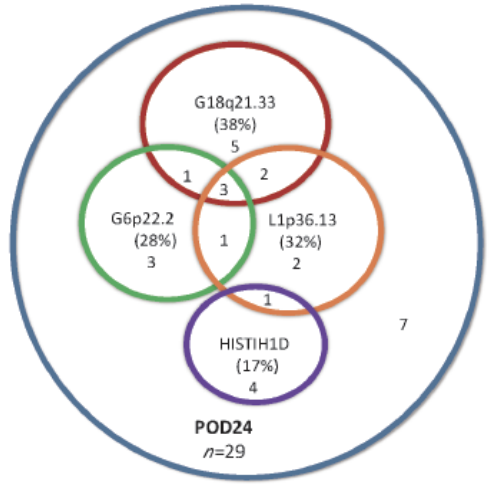
C5 cluster by *STAT6, CREBBP, EZH2* and *TNFRSF14* mutations

Shelton ASH 2022

# Integrative genomic and transcriptomic analysis reveals genetic alterations associated with early progression of follicular lymphoma

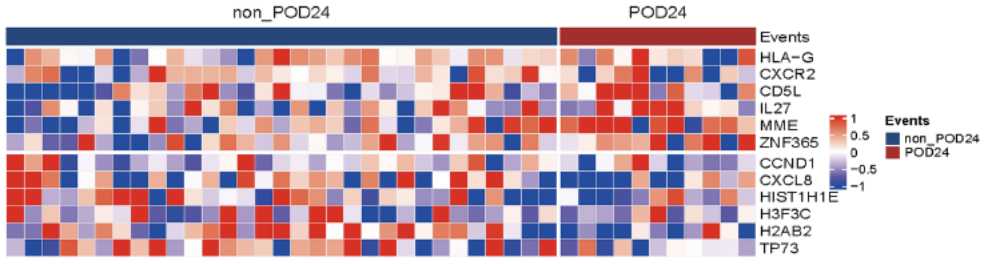


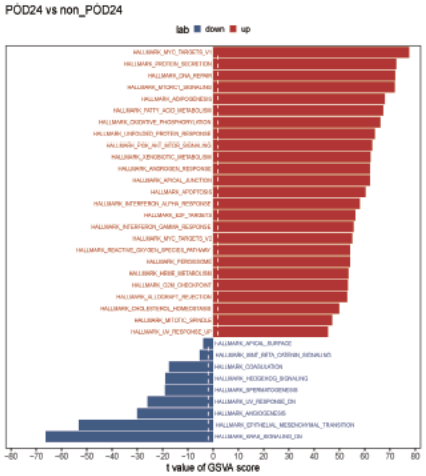

Integration of CNA and driver mutations:
76% with POD24 had a combination of 4 variants
HIST1H1D mutations, gains in 6p22.2,and
18q21.33 and loss in 1p36.13

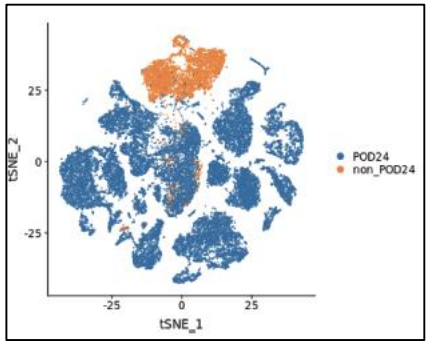
## Different gene expression patterns




POD24: enriched in inflammatory response genes

## Transcriptomic analysis



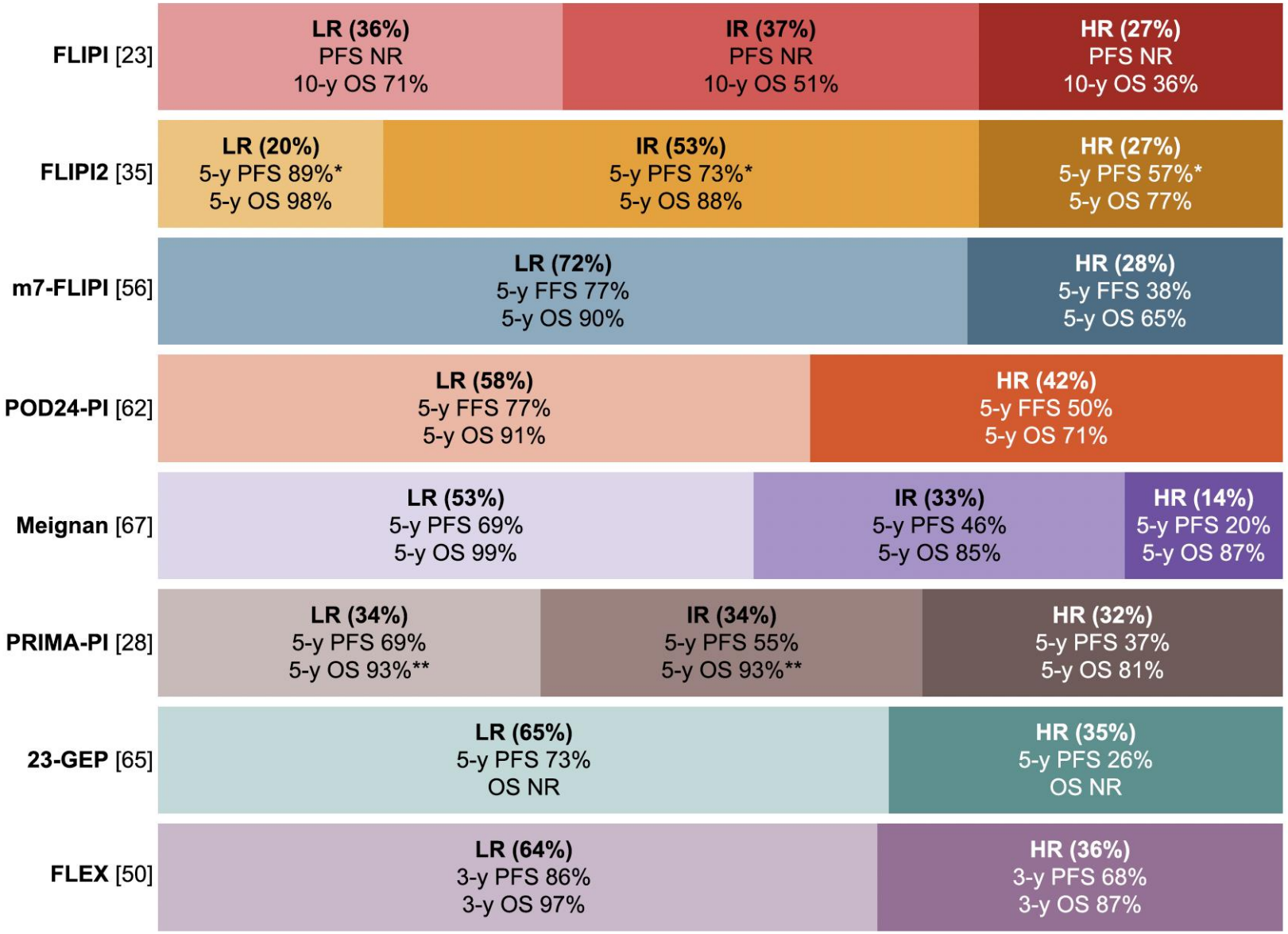




Gao Br J Haematol 2023

# Biology of high risk Follicular lymphoma

- High risk FL is a function of tumor burden
- Genetic alterations
- Microenvironment
- Identification of high risk FL

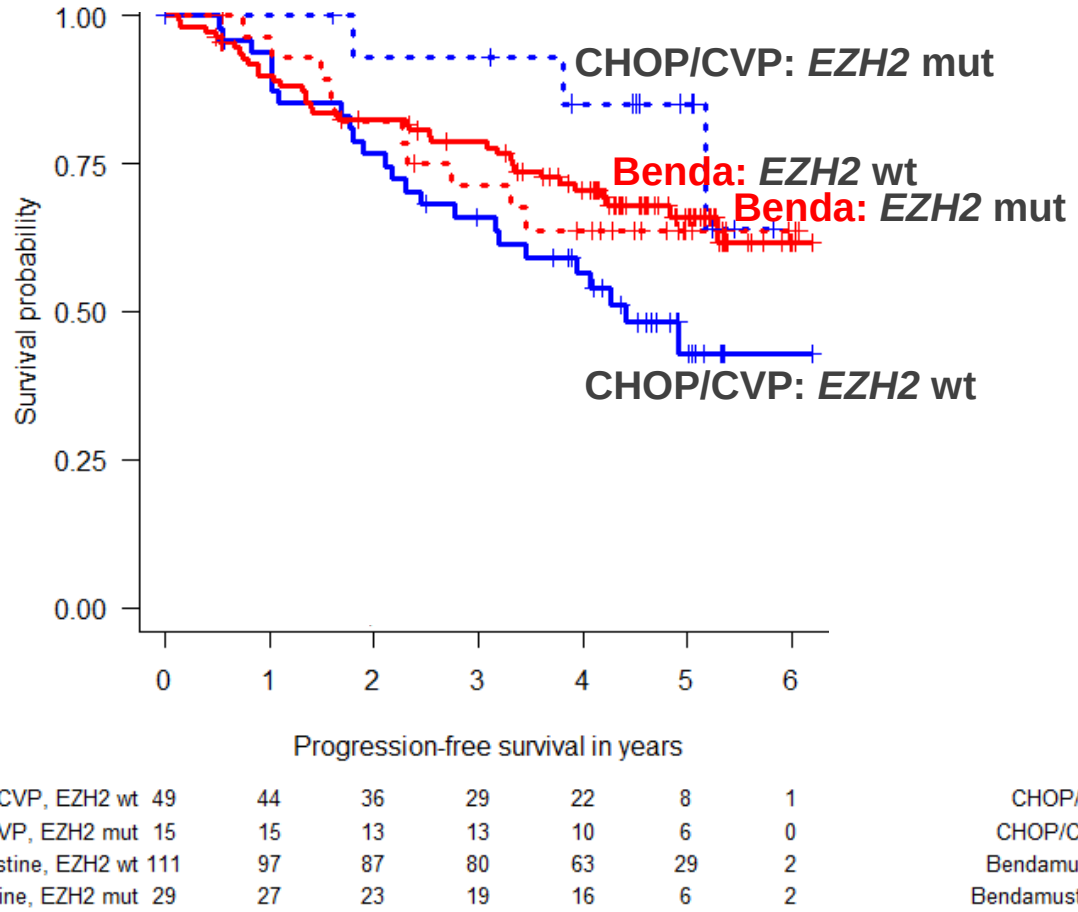
# Distribution of patients into risk categories and outcomes according to the most widely used prognostic indexes




Mozas Blood Reviews 2021

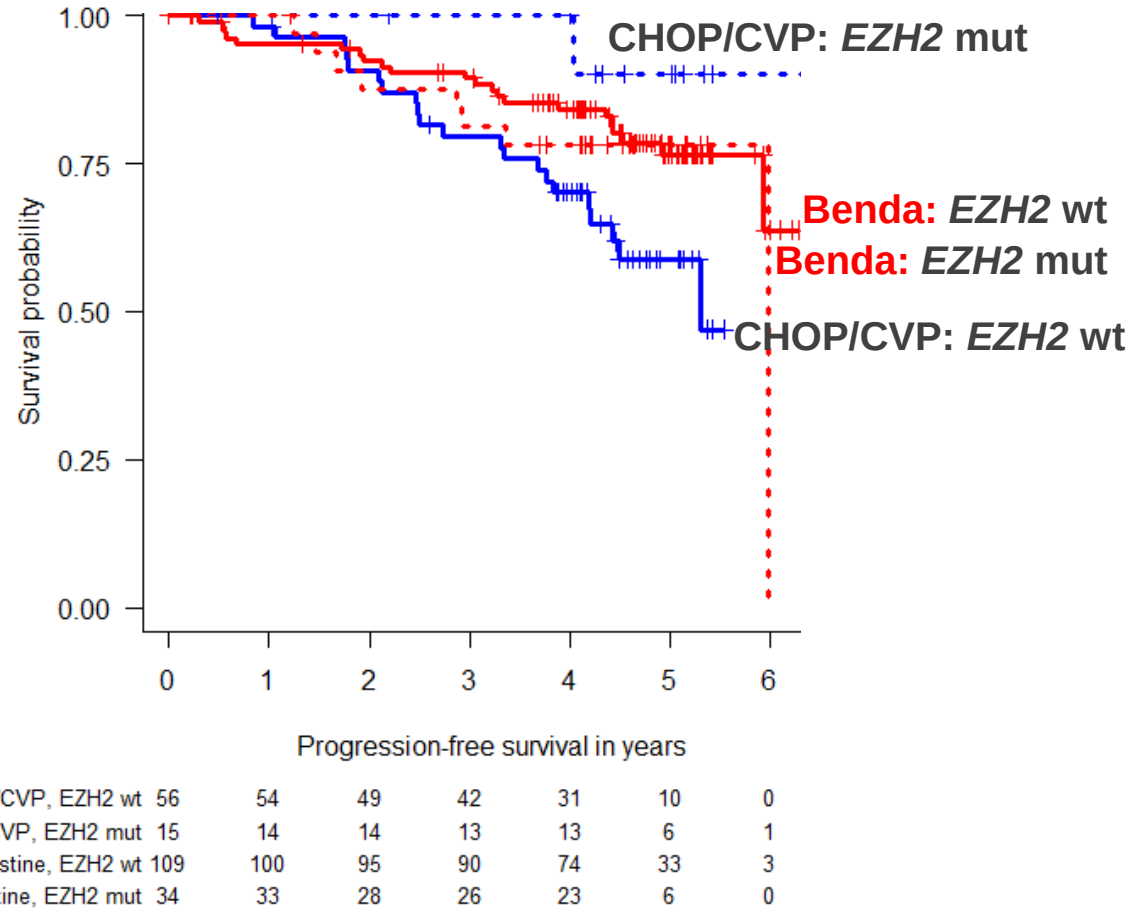
# *EZH2* mutations and chemotherapy

**Rituximab** (R) arm:




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CHOP/CVP, EZH2 wt | 49 | 44 | 36 | 29 | 22 | 8 | 1 |
| CHOP/CVP, EZH2 mut | 15 | 15 | 13 | 13 | 10 | 6 | 0 |
| Bendamustine, EZH2 wt | 111 | 97 | 87 | 80 | 63 | 29 | 2 |
| Bendamustine, EZH2 mut | 29 | 27 | 23 | 19 | 16 | 6 | 2 |

**Obinutuzumab** (G) arm:

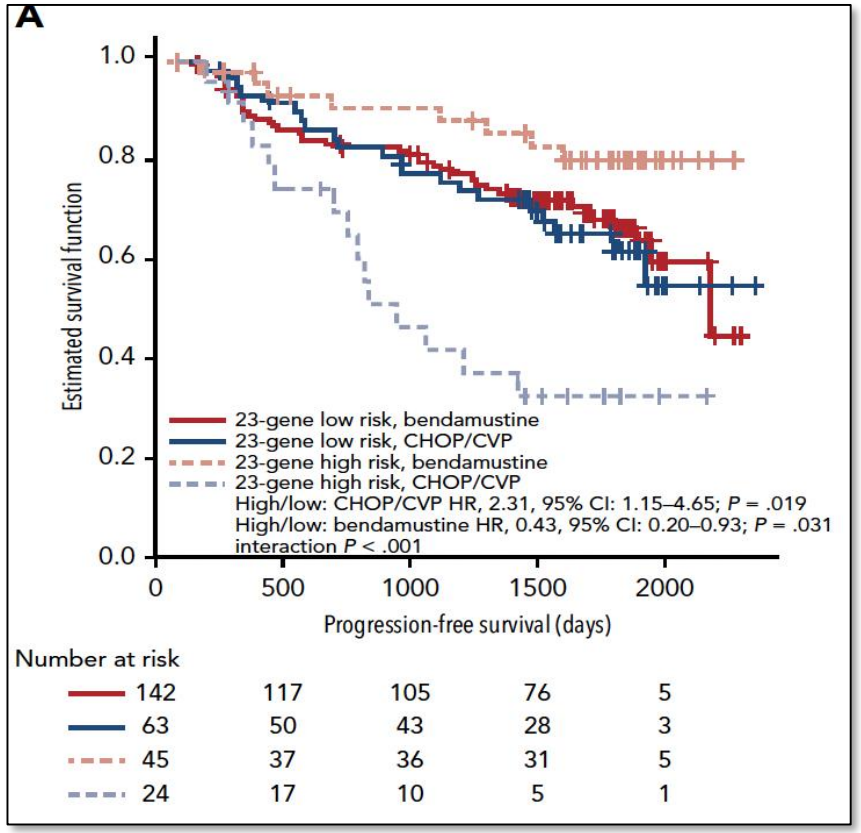


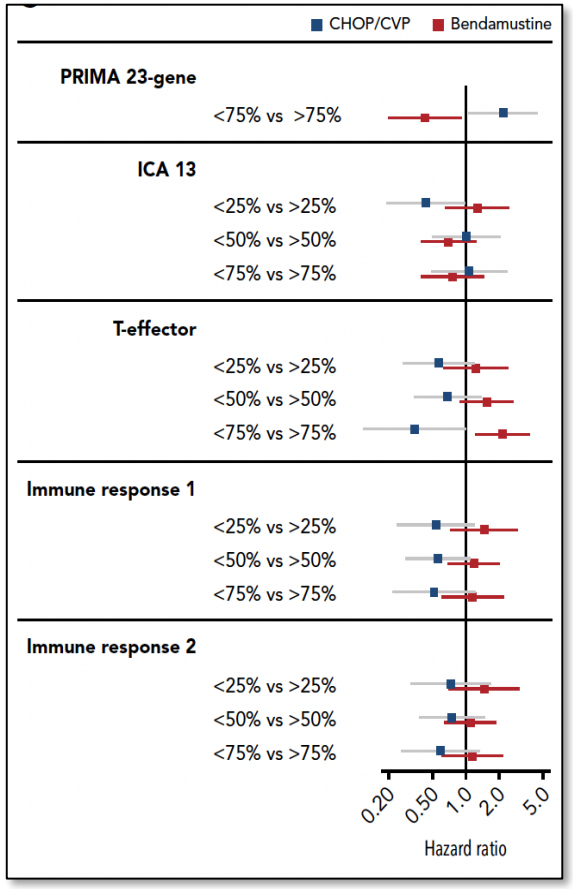

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CHOP/CVP, EZH2 wt | 56 | 54 | 49 | 42 | 31 | 10 | 0 |
| CHOP/CVP, EZH2 mut | 15 | 14 | 14 | 13 | 13 | 6 | 1 |
| Bendamustine, EZH2 wt | 109 | 100 | 95 | 90 | 74 | 33 | 3 |
| Bendamustine, EZH2 mut | 34 | 33 | 28 | 26 | 23 | 6 | 0 |

- Patients receiving **CHOP/CVP**-based regimens: PFS for *EZH2* wt *vs* mut: HR = 0.25, p = 0.0036
- Patients with ***EZH2* wt** FL: PFS for Benda *vs* CHOP/CVP: HR = 0.55, p = 0.0023

# TREATMENT-DEPENDENCE OF HIGH-RISK GENE EXPRESSION SIGNATURES IN *DE NOVO* FOLLICULAR LYMPHOMA (GALLIUM TRIAL)

## 23-gene signature for PFS



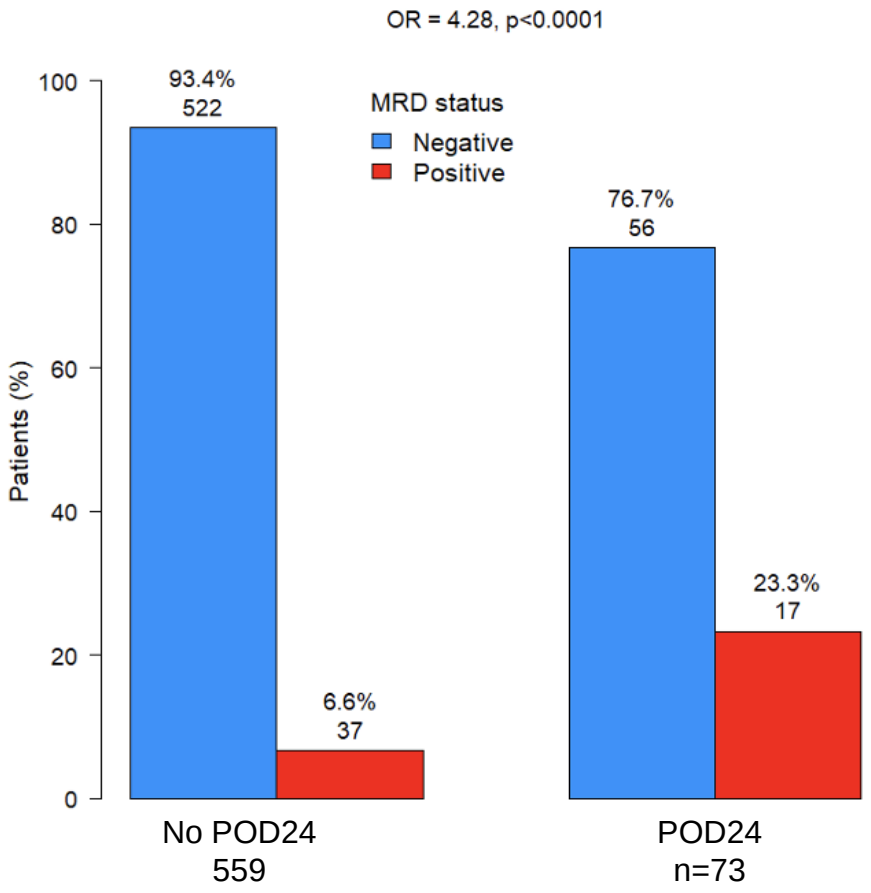




- A differential PFS response was observed according to CHOP/CVP and bendamustine
- Significant interactions between high-risk status and PFS with bendamustine (interaction HR: 0.17, 95% CI: 0.063–0.48; p=0.00074)

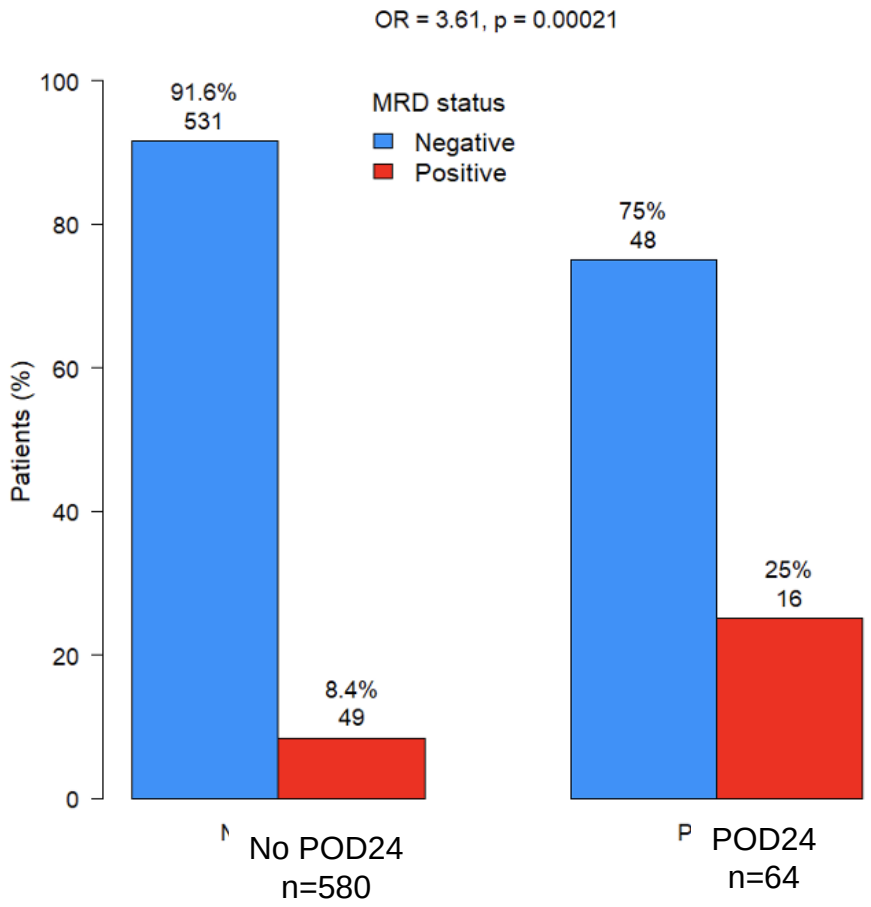
*Bolen Blood 2021*

# Association between POD24 and MRD status during and after induction in Gallium

## mid-induction

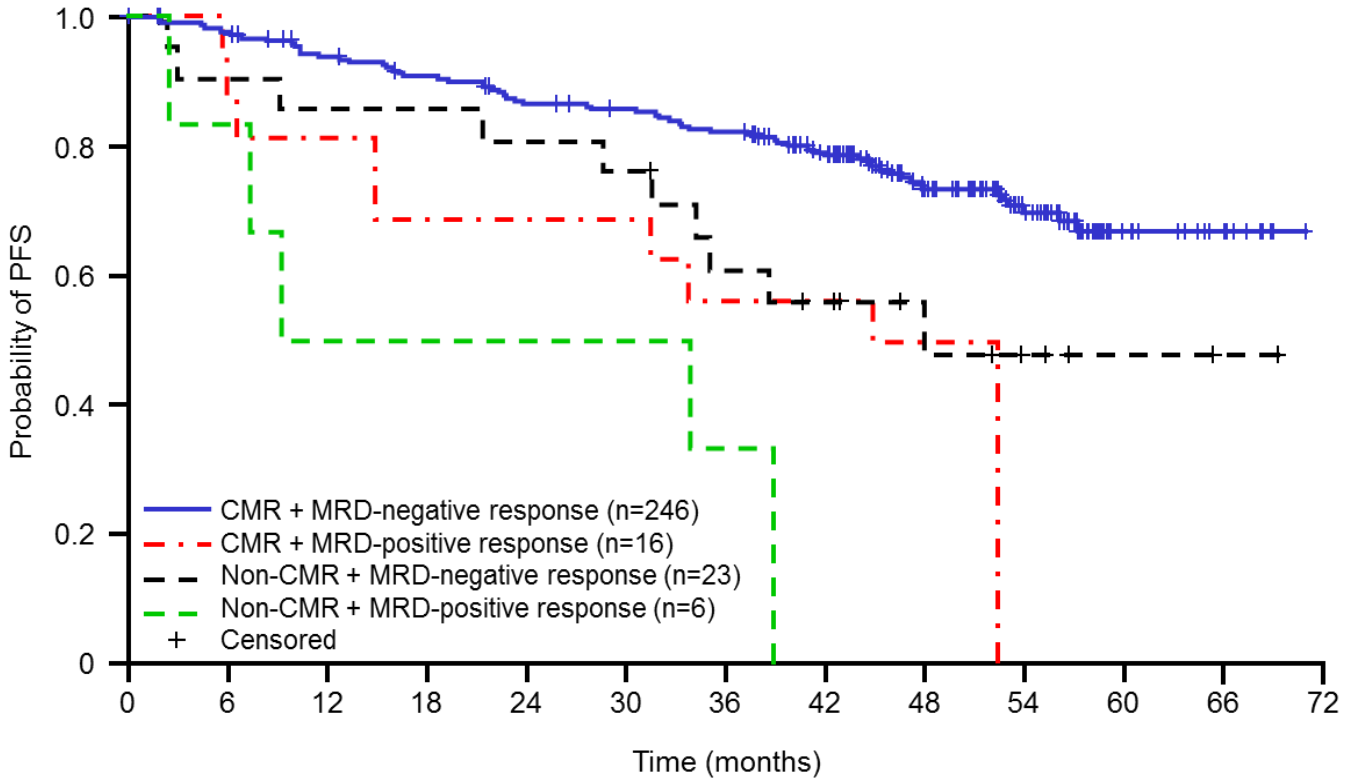



## end-of-induction




Pott et al. JCO 2023 in press

# PFS by PET response and MRD status at EOI




| Population | Comparison | HR | 95% CI | P-value |
|---|---|---|---|---|
| **CMR** | MRD-positive vs MRD-negative | 2.77 | 1.37–5.59 | 0.004 |
| **MRD-negative** | Non-CMR vs CMR | 2.11 | 1.08–4.11 | 0.028 |

- CMR was achieved by 266 (89%) evaluable patients, 250 (94%) of which were MRD-negative
- In patients with CMR + MRD-negative response vs CMR + MRD-positive response,‡ 2.5-year PFS (from EOI) was 85% (95% CI: 80–99) vs 69% (95% CI: 40–86)

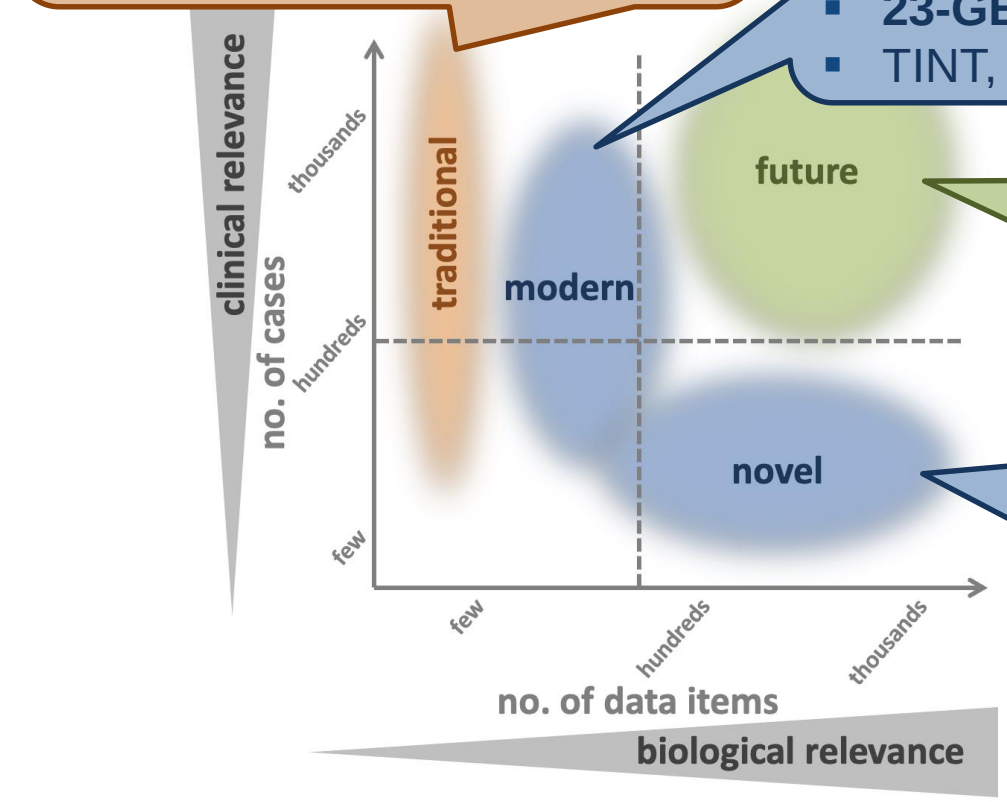
*7 patients who progressed prior to their MRD assessment were excluded; †Using Cox proportional modelling; ‡Median follow-up was 44 months.

Pott et al. EHA 2018 abstract PF436

# Summary

**“Established”**
- ***TP53*** mutation status
- ***EZH2*** mutation status
- MRD (CTC, ctDNA, …), **…**

**“in Evaluation“**
- **m7-FLIPI**
- **23-GEP Score**
- TINT, **…**




**“Perspective“**
- **From Risk-Adapted Treatment**
- **Towards Biology-Guided Therapies**

**“in Development“**
- **Molecular Subtyping**
- **CITI, …**

Adapted from O. Weigert

German Lymphoma Alliance

# Thank you very much


Study meeting 2021 Leipzig