VII CORSO del GRUPPO ITALIANO DI EMATOPATOLOGIA

La DIAGNOSTICA EMATOPATOLOGICA nell'ERA della MEDICINA di PRECISIONE

LEUCEMIA LINFATICA CRONICA/LINFOMA A PICCOLI LINFOCITI B: INSIGHTS E PROGRESSIONI

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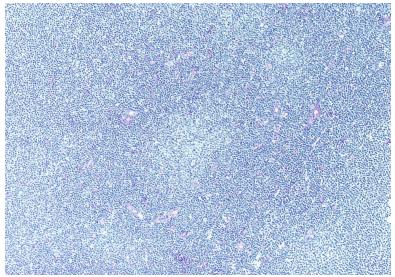
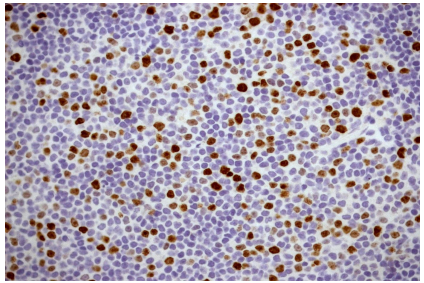
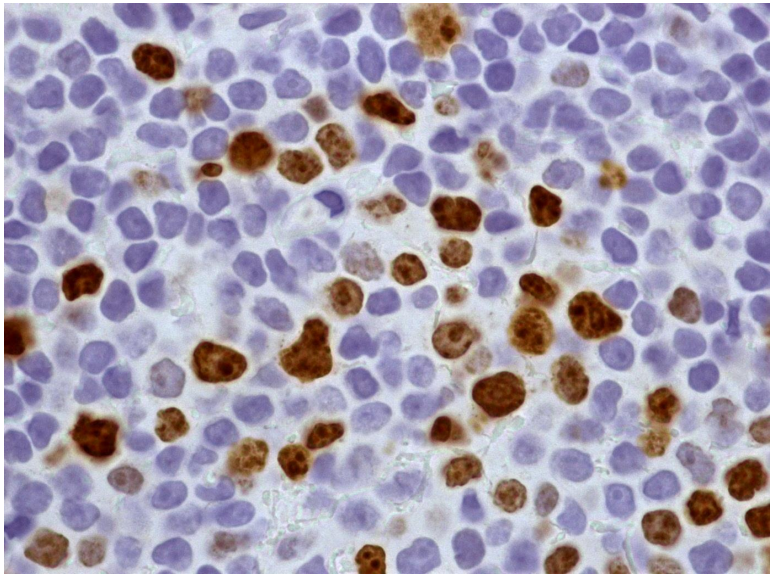
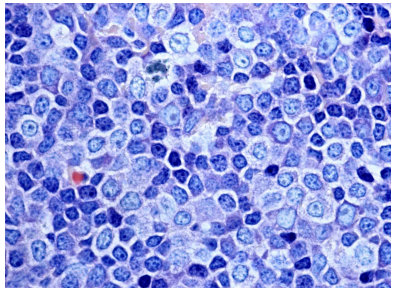
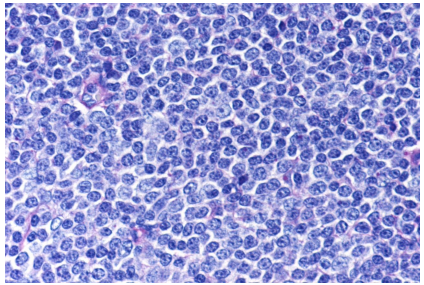
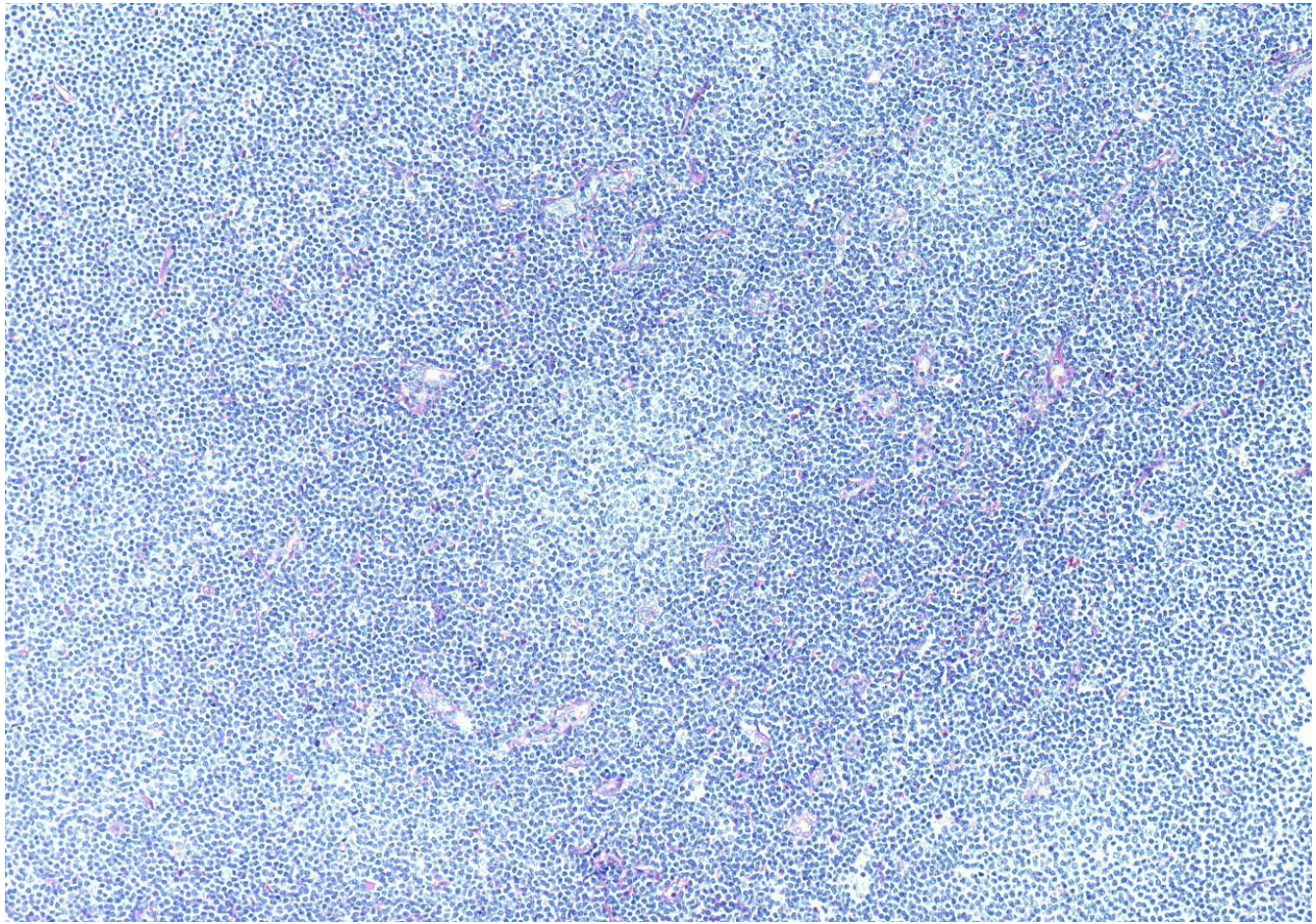
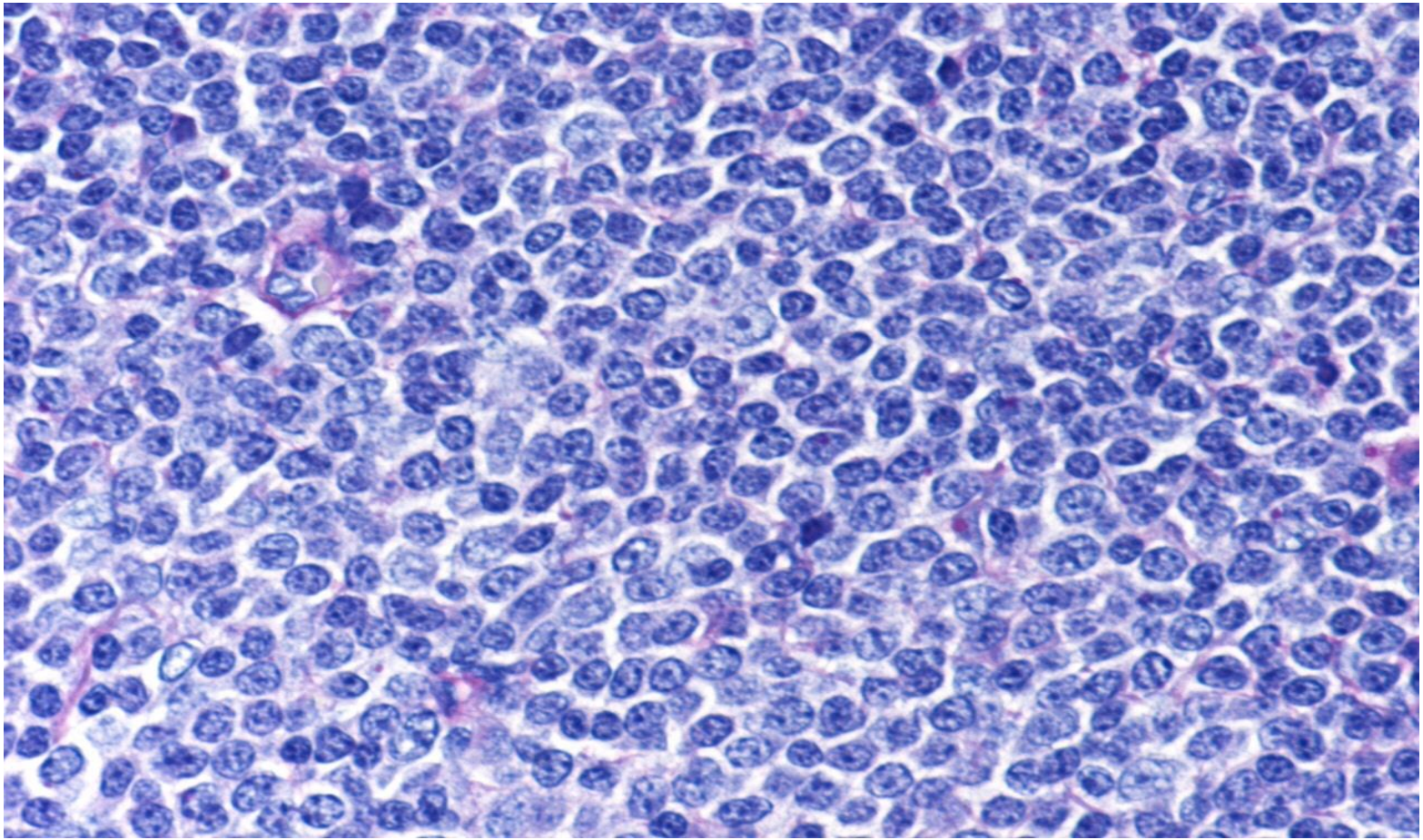
Disclosures of Name Surname

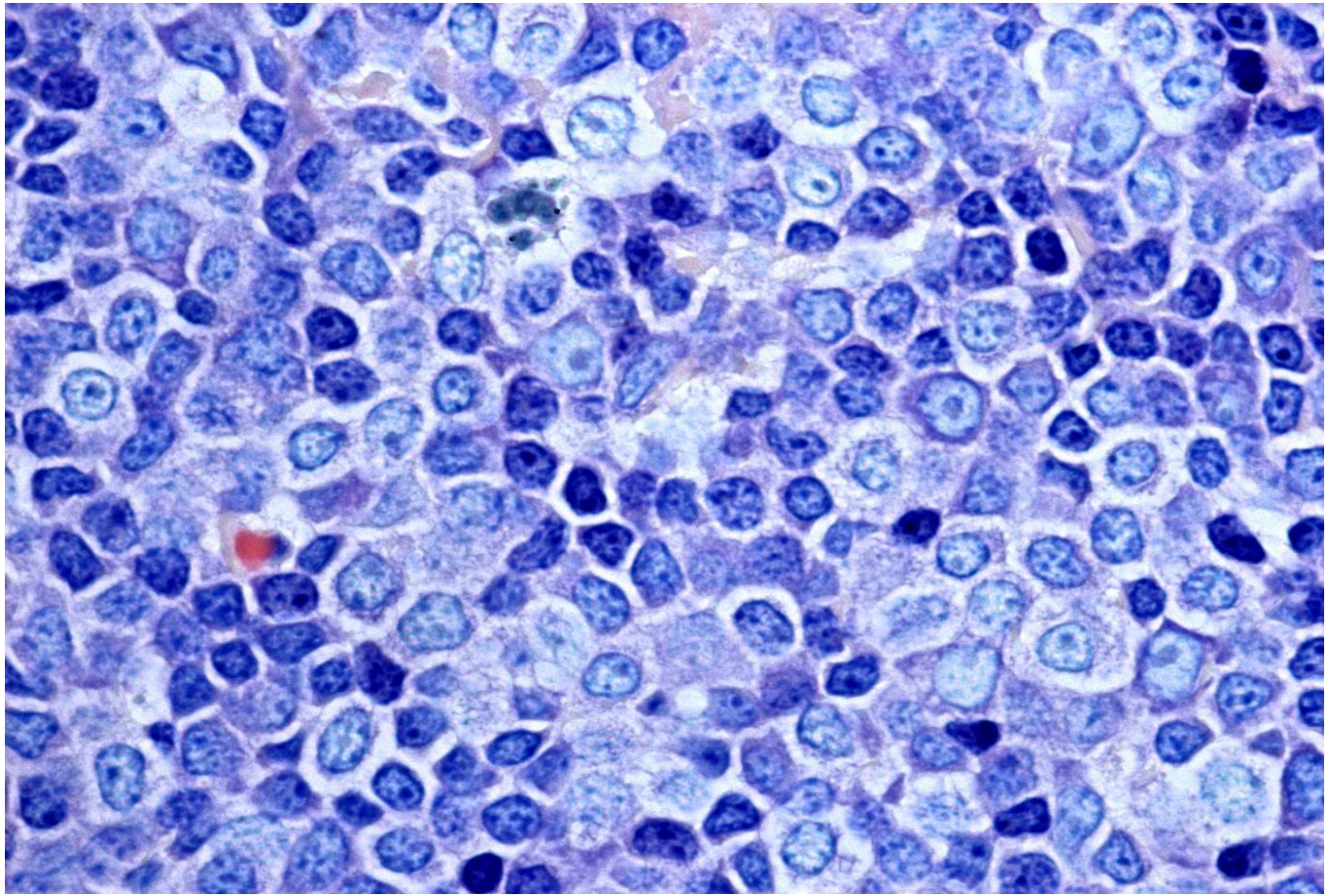
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Ventana Roche			x				
Atra Zeneca						X	

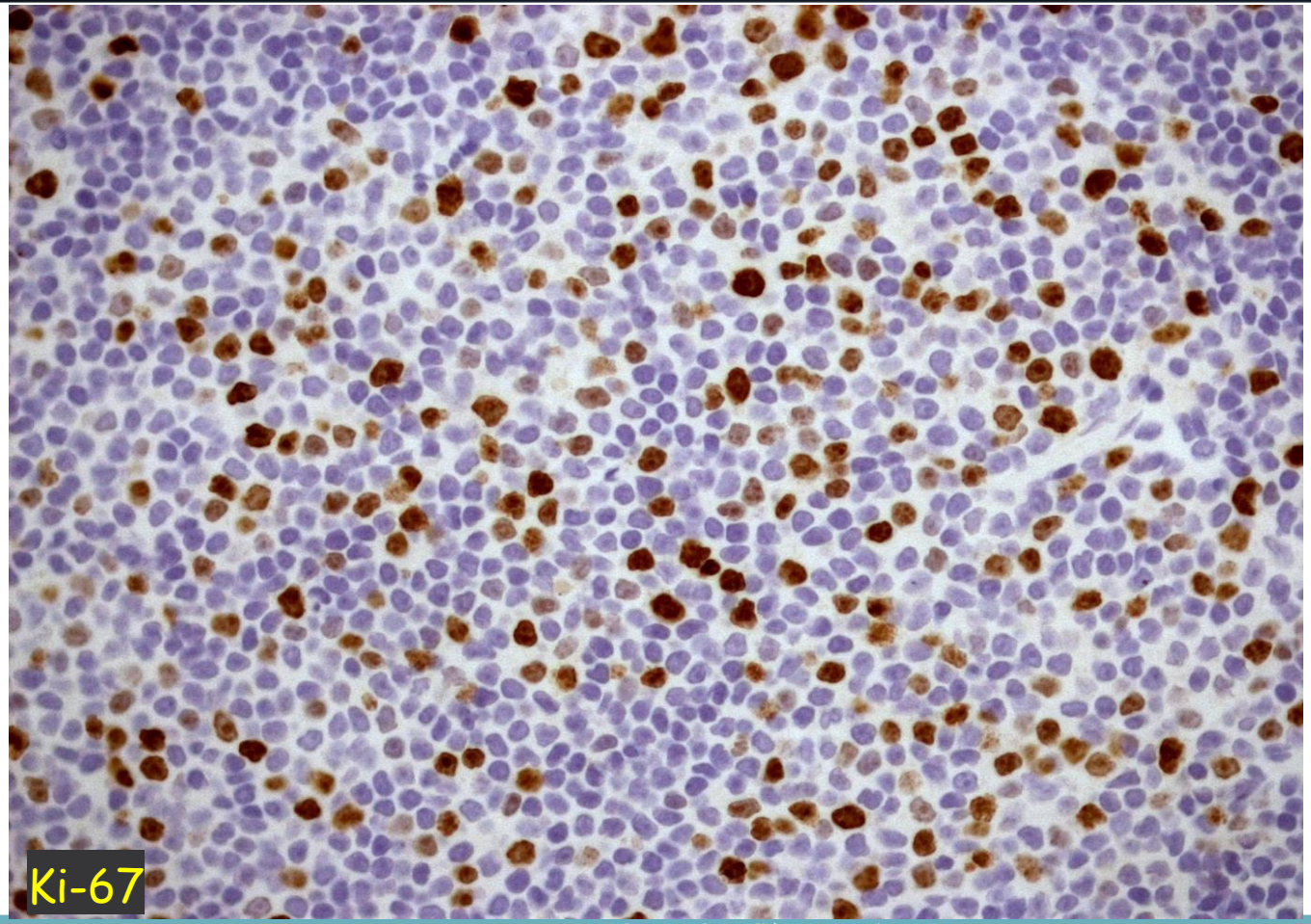
CELLULAR COMPOSITION OF CLL IN THE LYMPH NODE

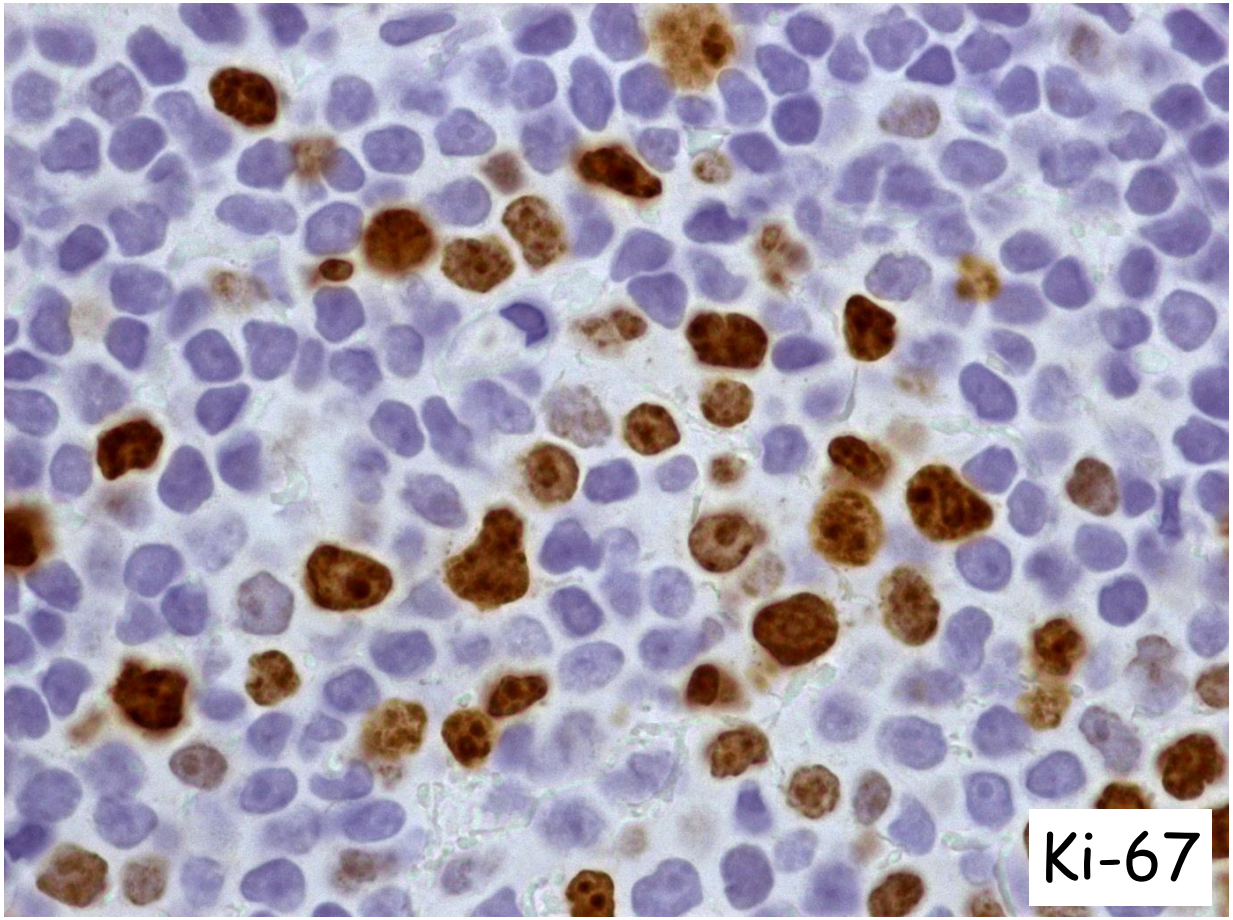













Ki-67


Ki-67

BASIC CLL IMMUNOPHENOTYPICAL MARKERS

- Essential:

CD20 (pay attention to possible 'dim'positivity)
CD5
CD23
Cyclin D1

- Suggested (not mandatory)

LEF1

- Possible occurrences in proliferation centres:

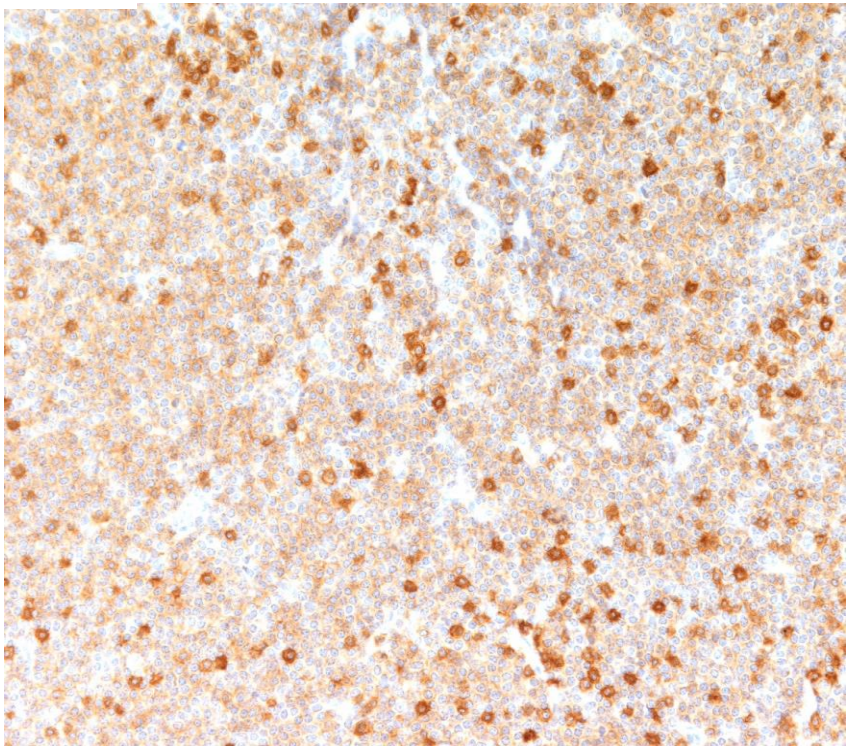
MUM1
Cyclin D1

- The comparison between immunohistochemical and flow cytometric data

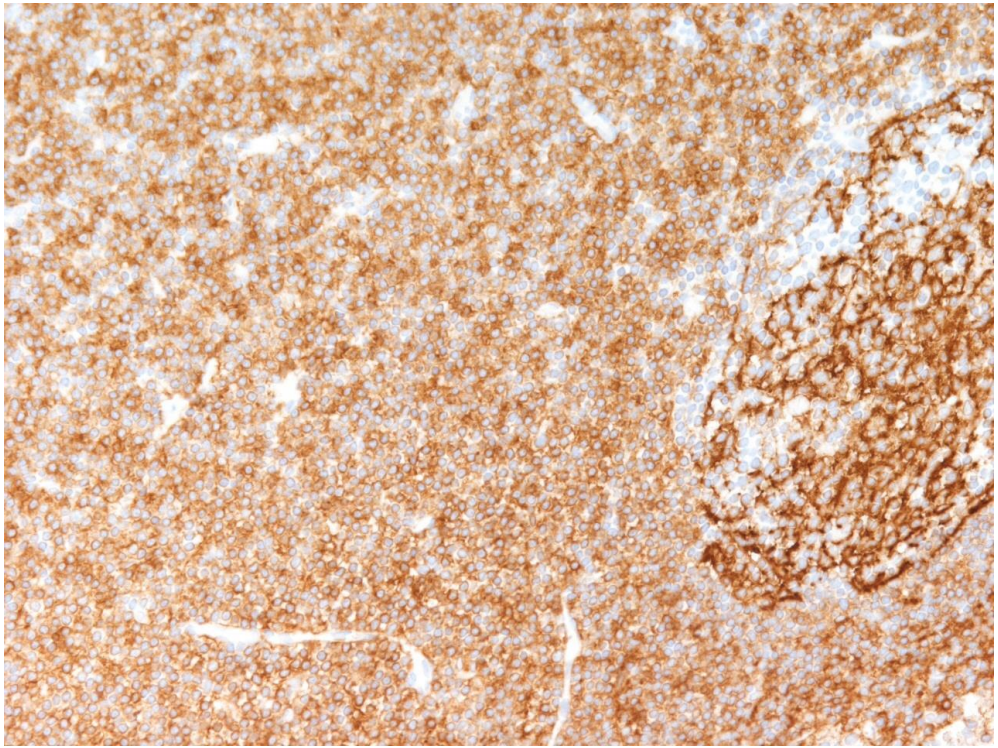
MOST IMPORTANT DIFFERENTIAL DIAGNOSES IN CLL

- Mantle cell lymphoma (watch out CD23!)
- Marginal zone lymphoma (watch out CD5 and CD23!)
- Lymphoplasmacytic lymphoma (watch out CD23 and CD5!)
- Monoclonal B cell lymphocytosis

CD5

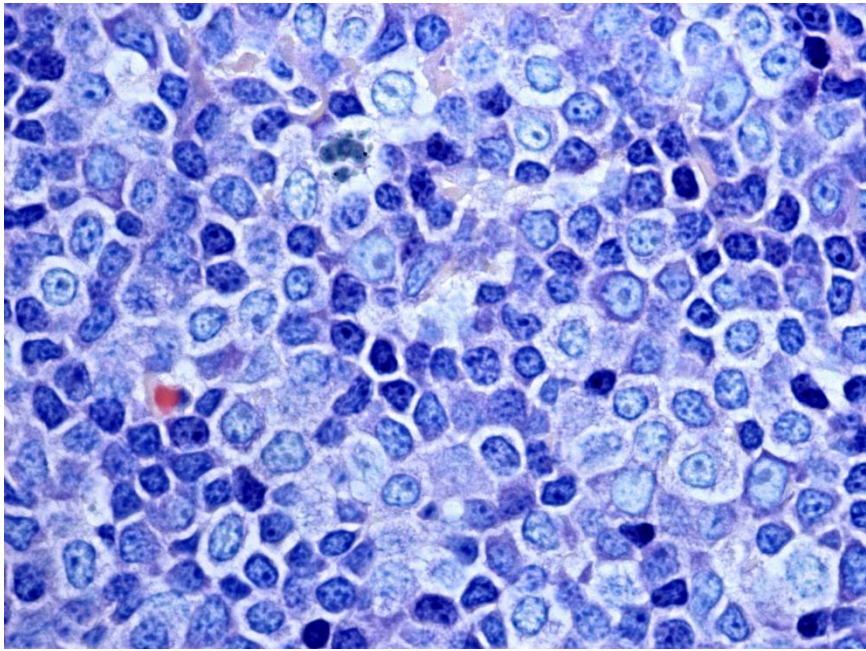


CD23



WHEN PROLIFERATION CENTRES BECOME WORRYSOME?

- Amount?
- Size?
- Occurrence of mitosis?
- Rate of proliferation index?
- what we mean for 'atypical CLL'? Impact of peripheral blood examination



ACCELERATED/HISTOLOGICALLY AGGRESSIVE CLL

Defining criterion; it represents a PROGRESSION, NOT a transformation

DIAGNOSTIC PARAMETERS

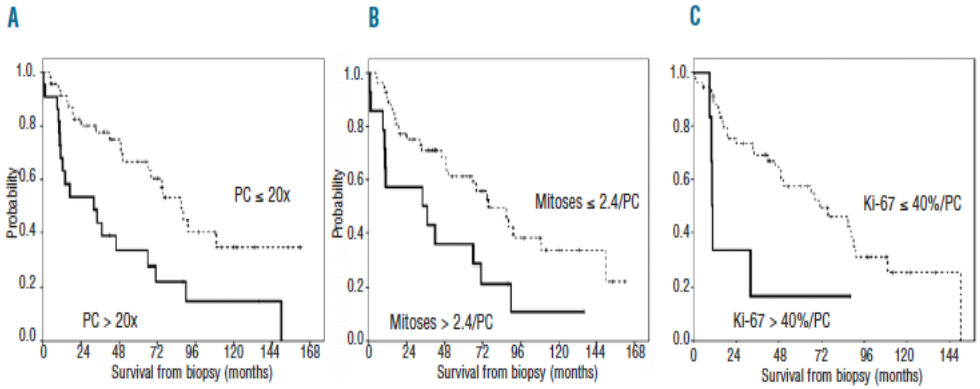
- Confluent and/or broader than 20X field proliferation centres
- Ki-67 >40%
- (?) >2,4% mitoses in proliferation centres

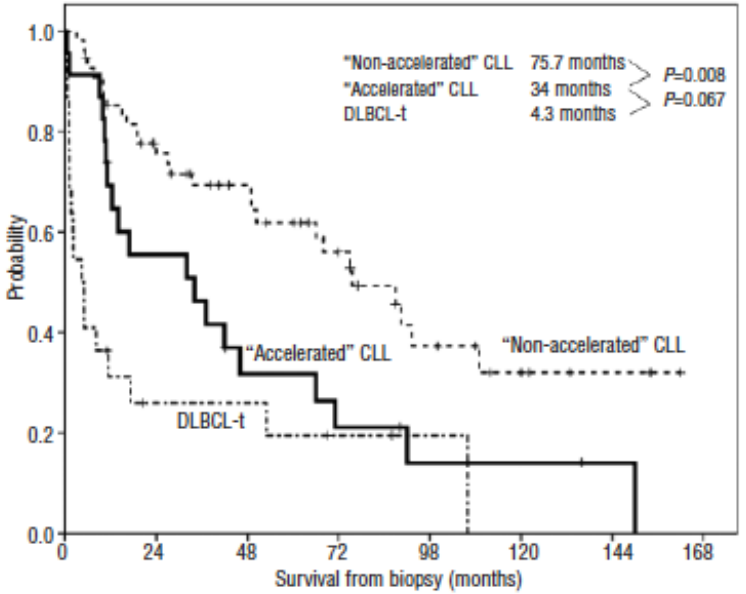
Ginè E at al Haematologica 2010; 95: 1526

PROLIFERATION CENTER-RICH CLL

Ciccone M et al Leukemia 2012; 26: 499

ACCELERATED/HISTOLOGICALLY AGGRESSIVE CLL





Ginè E at al Haematologica 2010; 95: 1526

RICHTER-LIKE TRANSFORMATION

Post-ibrutinib huge accumulation of large B-cells in the tissues (mostly lymph nodes)

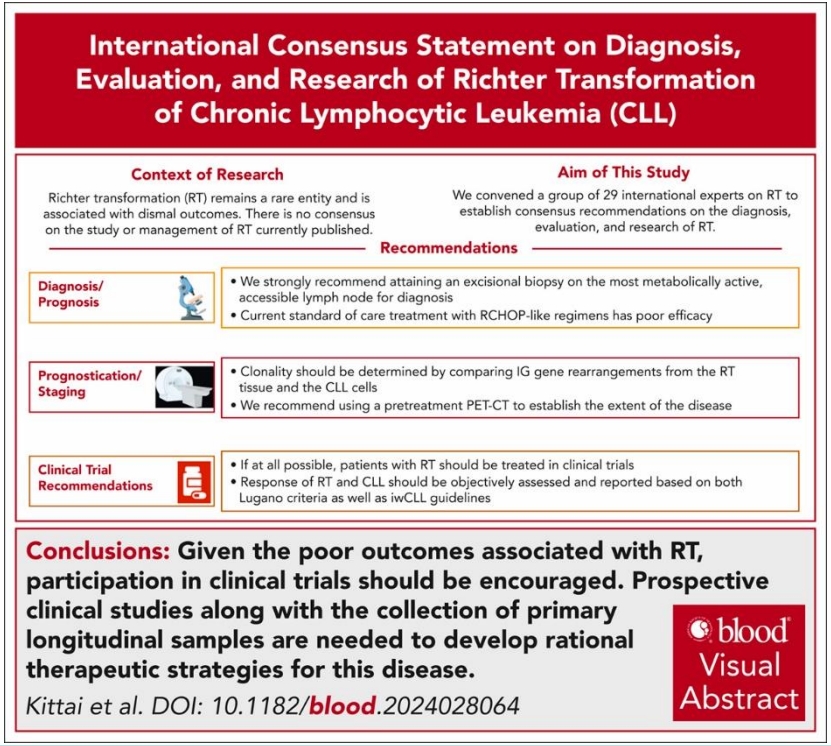
RICHTER SYNDROME

Defining criterion: it represents a TRANSFORMATION, NOT a progression

International consensus statement on diagnosis, evaluation, and research of Richter transformation: the ERIC recommendations

Adam S. Kittai, Monia Marchetti, Othman Al-Sawaf, Ohad Benjamini, Alexey V. Danilov, Matthew S. Davids, Barbara Eichhorst, Toby A. Eyre, Anna Maria Frustaci, Michael Hallek, Paul J. Hampel, Yair Herishanu, Rodney J. Hicks, Arnon P. Kater, Rebecca L. King, Jose I. Martin-Subero, Carolyn Owen, Erin Parry, Maurilio Ponzoni, Davide Rossi, Tanya Siddiqi, Stephan Stilgenbauer, Constantine S. Tam, Elisa ten Hacken, Philip A. Thompson, William Wierda, Gianluca Gaidano, Jennifer A. Woyach, Paolo Ghia

International consensus statement on diagnosis, evaluation, and research of Richter transformation: the ERIC recommendations
Blood, 2025



RICHTER : POSSIBLE TRANSFORMATIONS

1) DIFFUSE LARGE B CELL LYMPHOMA
- 80% display non-GCB phenotype
- Variable expression of CD5 and CD23
- Clonal relationship (more evident in umutated CLL- poor prognosis- than in mutated CLL) IS NEEDED

RICHTER: POSSIBLE TRANSFORMATIONS

2) HIGH GRADE B CELL LYMPHOMA

FISH

- DOUBLE-HIT (MYC + BCL2 or BCL6 rearrangements)
- TRIPLE HIT (MYC+BCL2+BCL6 rearrangements)

RICHTER : POSSIBLE TRANSFORMATIONS

3) LYMPHOBLASTIC B CELL LYMPHOMA

CD34+, TdT+, CD20-/+

4) PLASMABLASTIC LYMPHOMA

CD138+, MUM1+, CD20-, EBV +/-

5) T-CELL LYMPHOMA

CD3+, CD5+, CD2+, CD7+: possible antigen loss

RICHTER : POSSIBLE TRANSFORMATIONS

6) HODGKIN LYMPHOMA

- Usually EBV+
- Differential diagnosis with: isolated Reed-Sternberg cells and EBV+, Hodgkin-like lymphoproliferative disorders

ACKNOWLEDGEMENTS

- Lucia Bongiovanni
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- Maria Giulia cangi, Lorenza Pecciarini, Gilda Magliacane
- Claudio Tripodo, Stefano Casola

GRAZIE

Foundation for the Institute of Oncology Research
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An institute affiliated to USI

Leucemia linfatica cronica-linfoma a piccoli linfociti: insights e progressioni

Davide Rossi, MD, PhD

Hematology
Institute of Oncology Research
Oncology Institute of Southern Switzerland
Universita' della Svizzera Italiana
Bellinzona, Switzerland

Agenda

1. General aspects
2. Clinico-pathologic variants
3. Molecular biomarkers
4. Acquired resistance

Key Clinical aspects

Most common leukemia in adults: ~5 per 100,000 people per year

Median age at diagnosis: ~70 years

Often asymptomatic:

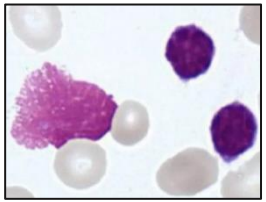
- Lymphocytosis (>5,000/μL clonal B-cells as incidental diagnosis)
- SLL presentation in 10-20% of cases; progression from MBL

Symptoms (if present):

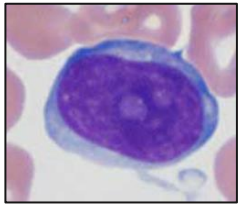
- Fatigue, weight loss, night sweats (B symptoms): rare (5%) at first presentation
- Lymphadenopathy (most common physical finding)
- Splenomegaly
- Recurrent infections (due to immunosuppression)
- Autoimmune complications (hemolytic anemia, thrombocytopenia)

Indolent course but may progress to aggressive disease **(Richter transformation)**

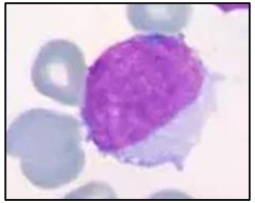
Morphology in peripheral blood



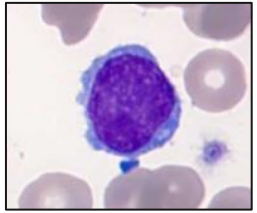
CLL



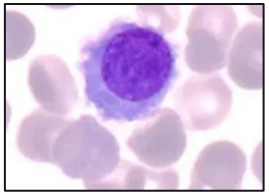
T or B-PLL



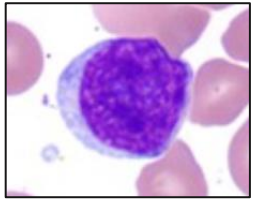
Mantle cell lymphoma



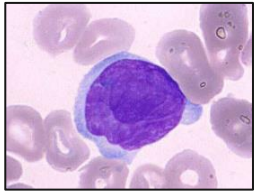
Marginal zone lymphoma



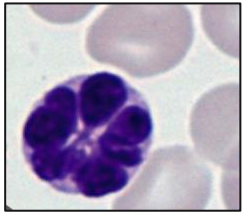
Hairy cell leukemia



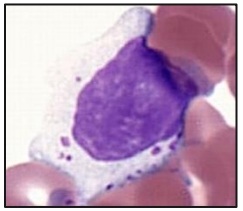
Follicular lymphoma



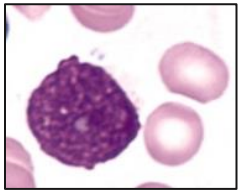
Sezary syndrome



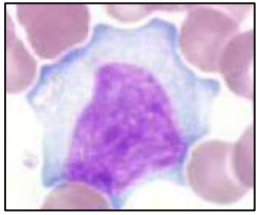
ATLL



T-LGL

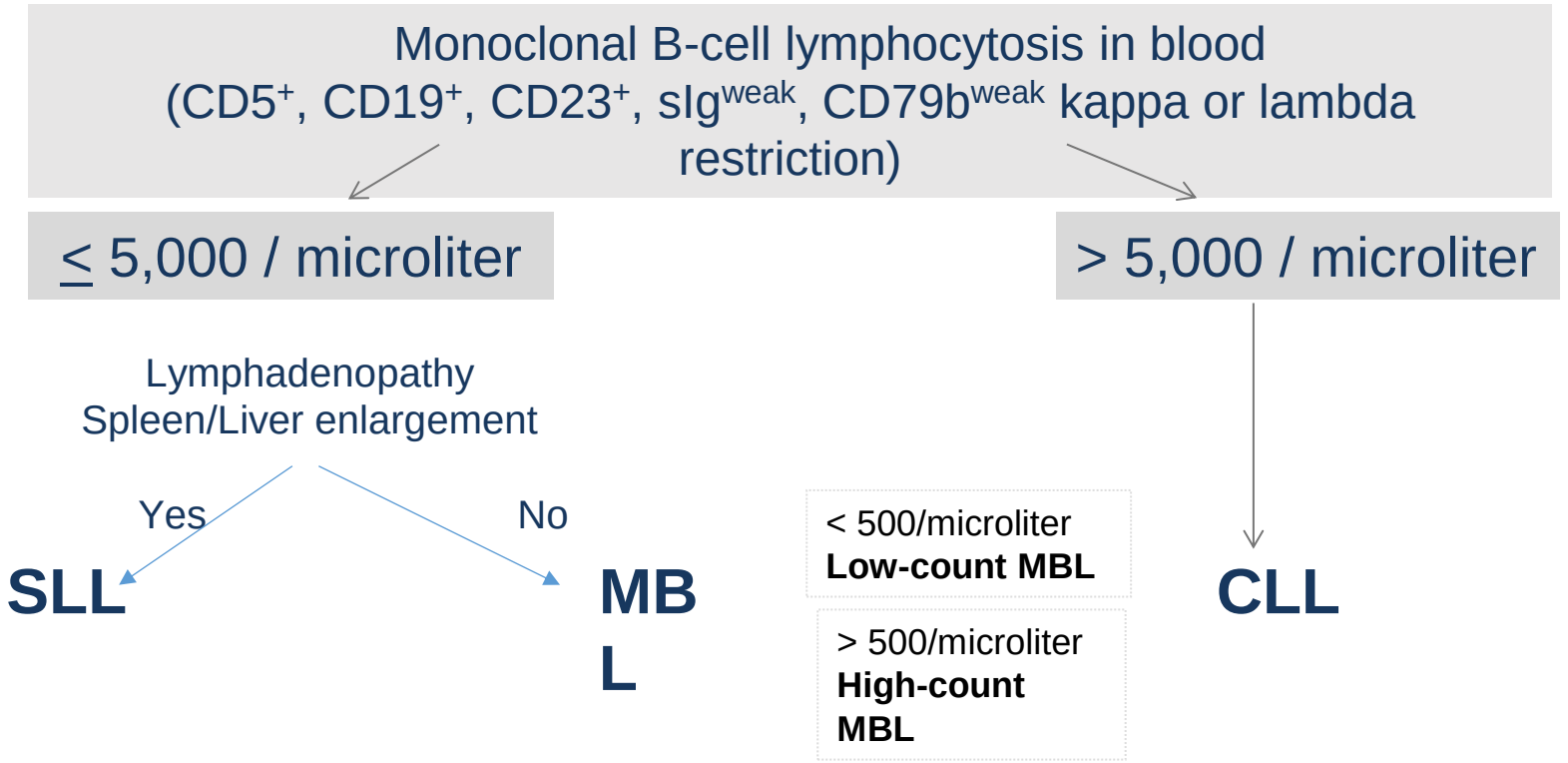


B-ALL

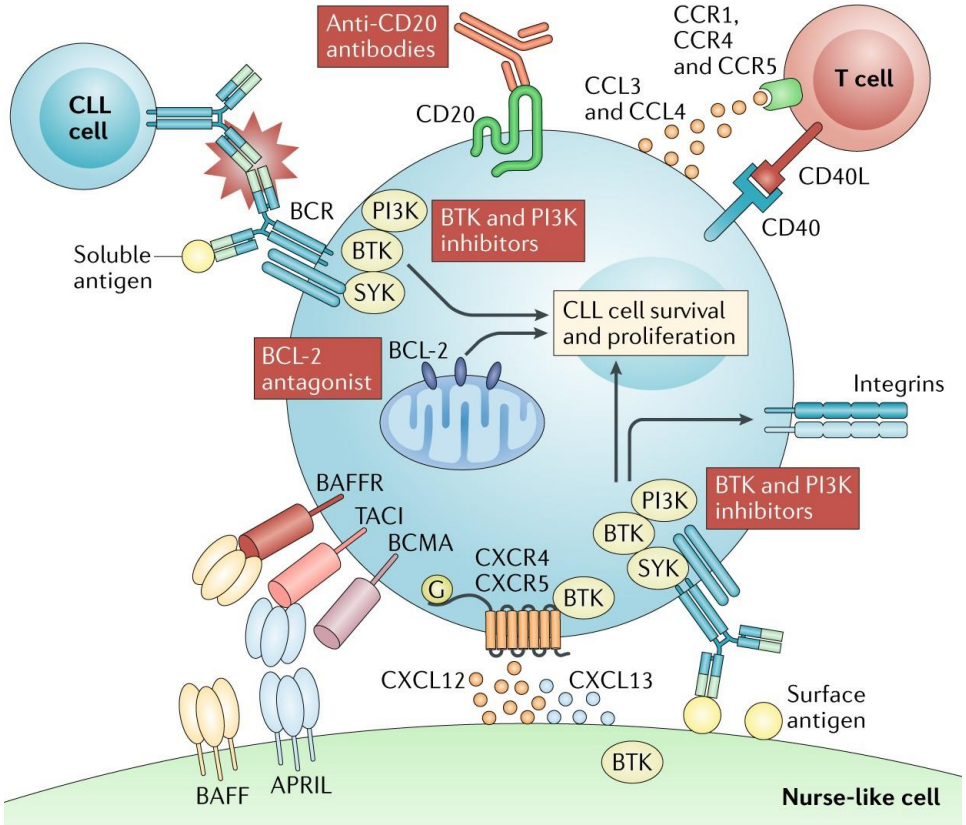


Reactive lymphocytosis: EBV infection

CLL, SLL, MBL diagnosis: iwCLL, ICC and WHO-HAEM5

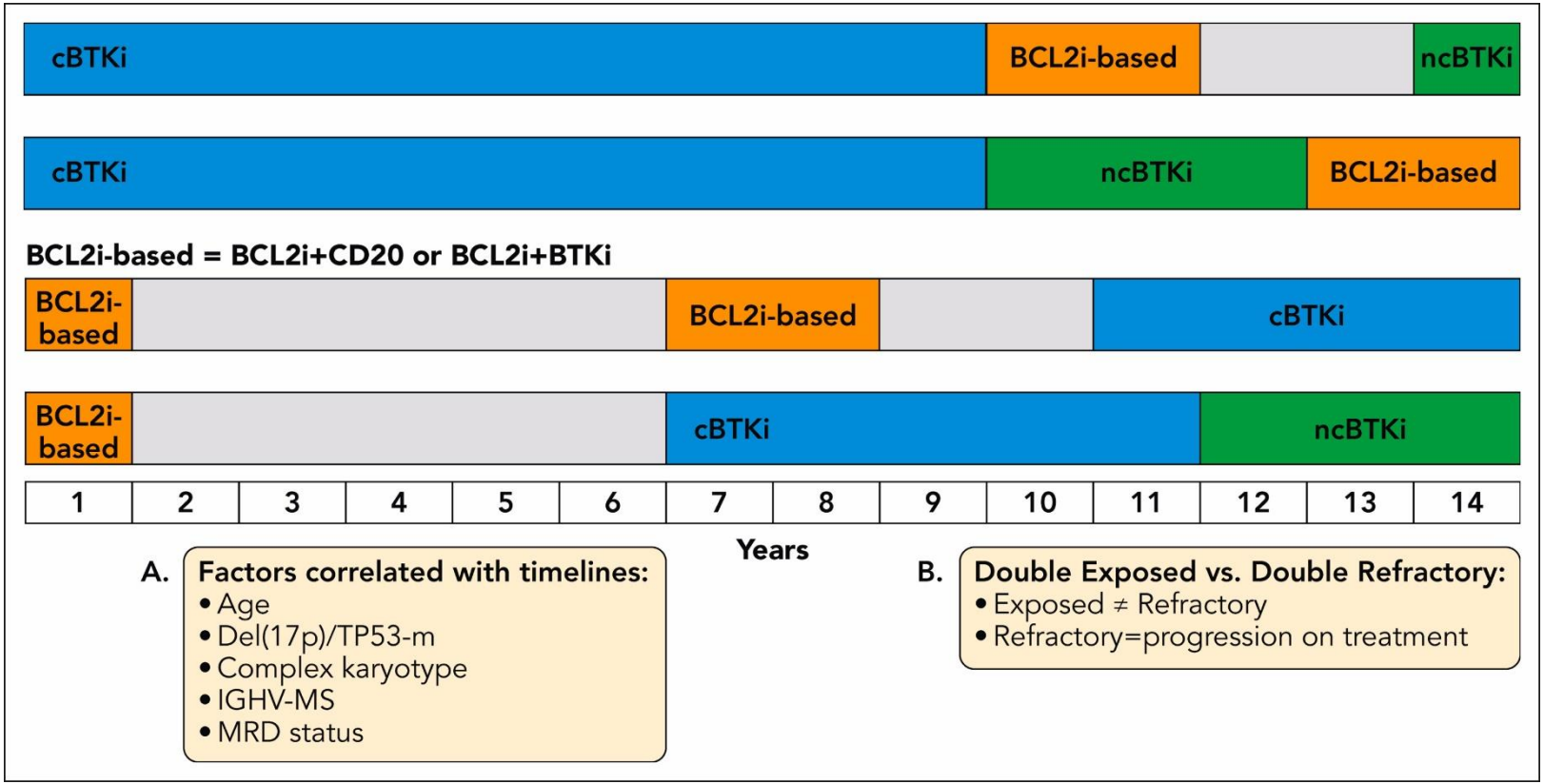


Therapeutic vulnerabilities



Burger JA, O'Brien S. Nat Rev Clin Oncol. 2018

Treatment sequencing



Wierda W, Eichhorst B & Hallek M; Blood 2025; 146 (7): 779–788

Agenda

1. General aspects
2. Clinico-pathologic variants
3. Molecular biomarkers
4. Acquired resistance

Small B-cell neoplasms without defining features

- **Atypical** chronic lymphocytic leukemia
  - **CD5+/CD23+**, bright CD20+/sIg+, CD79b+, FMC7+
- Marginal zone lymphomas
- Lymphoplasmacytic lymphoma **lacking *MYD88* L265P**
- Mantle cell lymphoma **lacking *IGH::CCND1***
- **Splenic** B cell lymphomas:
  - B-cell prolymphocytic leukemia
  - Splenic diffuse red pulp B cell lymphoma

Extended diagnostics

- **Chronic lymphocytic leukemia** → **-13q, *SF3B1* m, *NOTCH1* m**
- Marginal zone lymphomas → **+3, +12, +18, -7, *KLF2* m, *NOTCH2* m**
- Mantle cell lymphoma lacking *IGH::CCND1* → **IHC SOX11**
- B-cell prolymphocytic leukemia → **nucleoli, *TP53* abn, *MYC*-R**
- Lymphoplasmacytic lymphoma → ***MYD88* L265P**
- Splenic diffuse red pulp B-cell lymphoma→ ***MAP2K1* m, *CCND3* m**

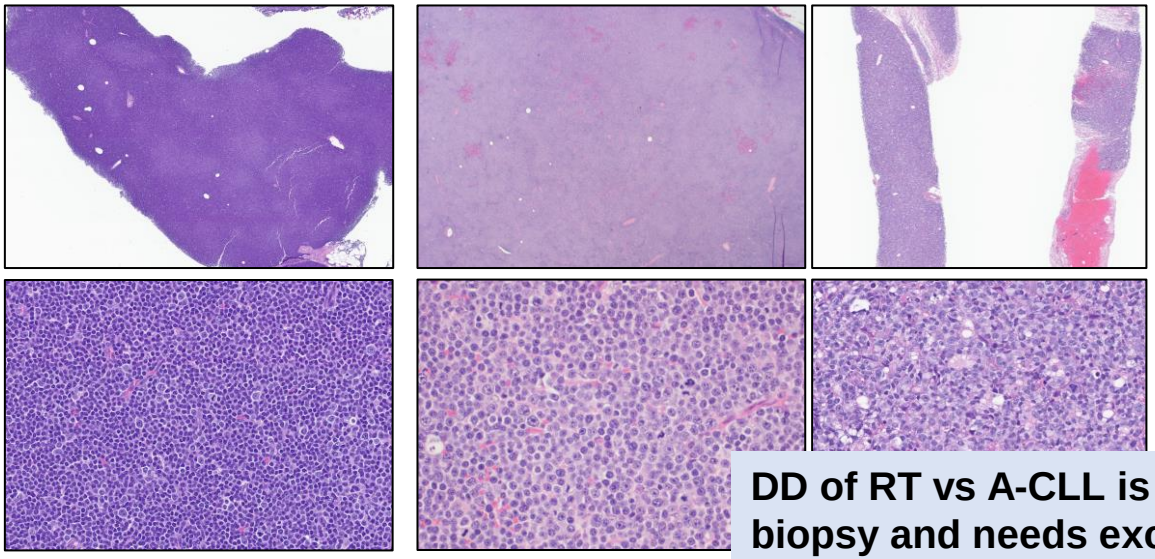
Accelerated CLL

Clinical syndrome

- B symptoms **20%**
- High LDH **70%**
- Fast growing bulky **14%**
- Unusual extranodal sites **0%**
- Hypercalcemia **0%**
- SUVmax >10 **30%**

Pathology aspects

- Large and confluent proliferation centers **100%**
- Intermediate cells with nucleoli **100%**
- Ki67 >40% in proliferation centers **100%**
- Sheets of large cells **0%**
- Loss of CD23 **0%**
- Loss of CD5 **0%**



DD of RT vs A-CLL is difficult on core biopsy and needs excisional biopsy

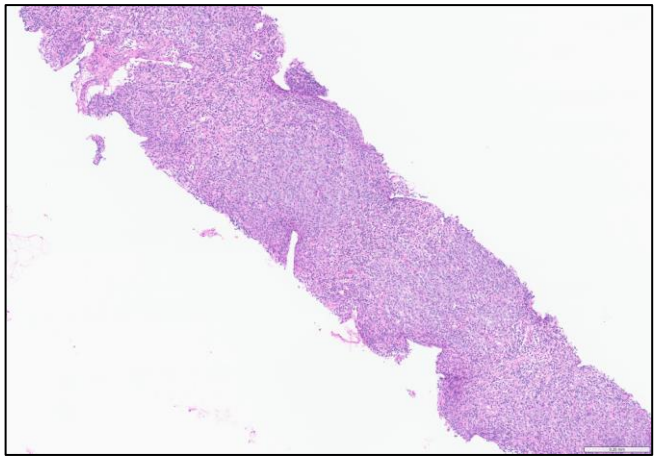
WHOHAEM5, Giné E, et al. Haematologica. 2010, Falchi L, et al. Blood. 2014

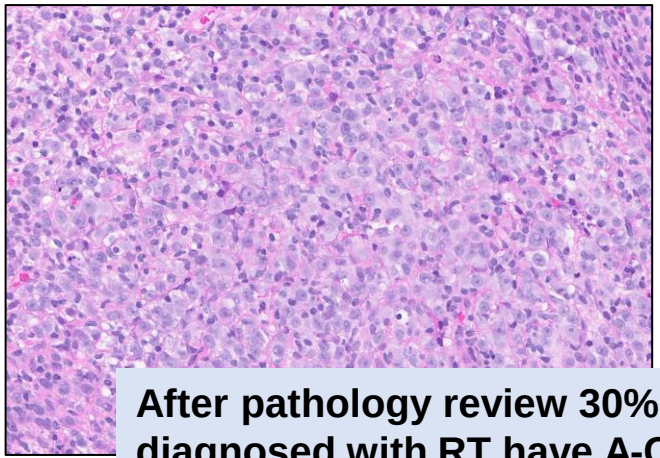
Richter's Transformation (RT)

History of CLL
- Previously treated **70%**
- IGVH unmutated **80%**
- *TP53* abn **50%**
- Complex karyotype **40%**

Clinical syndrome
- B symptoms **50%**
- High LDH **70%**
- Fast growing bulky **60%**
- Unusual extranodal sites **10%**
- Hypercalcemia **10%**
- SUVmax >10 **80%**

Pathology aspects
- Sheets of large cells **100%**
- Centroblasts **90%**
- Non-GCB **90%**
- Loss of CD23 **80%**
- Loss of CD5 **70%**
- EBV + **5%**
- Clonally related: **100%**





After pathology review 30% of cases diagnosed with RT have A-CLL or cHL

WHOHAEM5, Mao Z et al. Am J Surg Pathol 2007, Rossi D, et al. Blood 2011, Kittai AS, et al. Blood Cancer J. 2025, Miller CR, et al. Am J Hematol. 2025, Kittai AS, et al. Am J Hematol. 2023, Hampel PJ, et al. Leukemia. 2025

Clonal relationship between CLL and LBCL

- Tissue biopsy as source of LBCL
- Synchronous/metachronous blood and/or marrow are source of CLL
- Immunoglobulin gene rearrangement PCR comparison of peak sizes
- IGVH gene sequencing
- NGS or FISH to confirm shared mutations and cytogenetic abnormalities

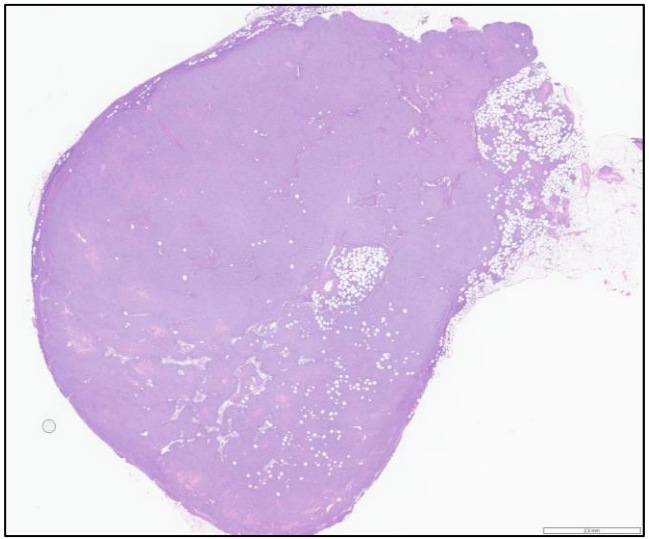
Kittai AS, Blood. 2025

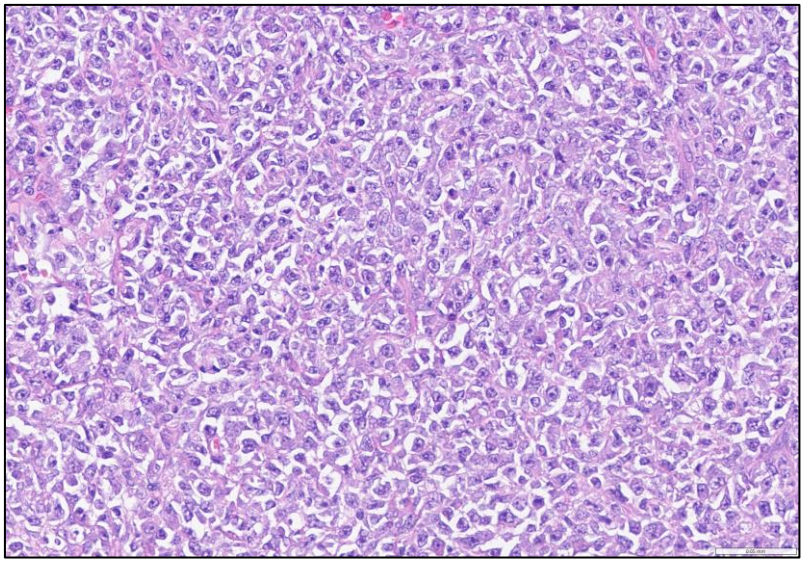
Clonally unrelated RT

	Number of patients (% clonally unrelated)	Median OS in clonally related	Median OS in clonally unrelated	P
Mao et al,[-]2007	23 (22%)	NA	NA	NA
Rossi et al,[-]2011	63 (21%)	14.2 mo	62.5 mo	.017
Abrisqueta et al, 2020	35 (15%)	5.4 mo	74.8 mo	.05
Broséus et al, 2023	58 (25%)	8 mo	35.5 mo	.018
Parry et al, 2023	52 (14%)	5.8 mo	56.4 mo	.0094

Pseudo-Richter

- **3-13** days after BTKi interruption
- From **incidental** finding to **syndrome**
- Ki-67 **>50-90%**
- CD5+ and CD23+ **100%**
- Non-GC **100%**
- Resolution with BTKi reintroduction **100%**





Marco-Ayala J, et a. Clin Lymphoma Myeloma Leuk. 2026

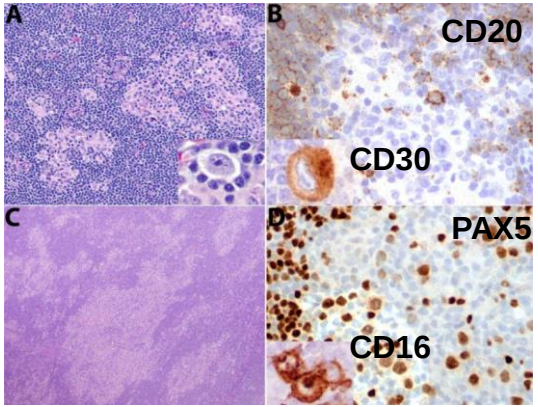
cHL arising in patients with CLL

Clinico-pathology aspects

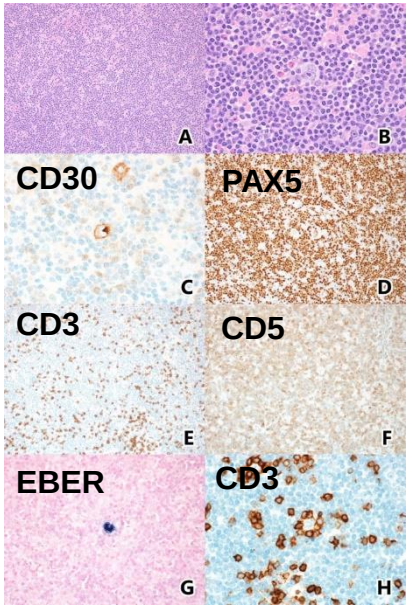
- Limited stage disease **60%**
- B symptoms **50%**
- Rare HRS-cells **100%**
- Inflammatory microenvironment **100%**
- No CLL remaining tin the background **100%**
- EBV+ **50%**
- **Clonally unrelated: 70%**

DD: CLL with HRS cells

cHL in CLL



CLL with HRS cells



Al-Sawaf O et al. Leukemia 2021, Xiao et al, Hum Pathol 2016, King RL, et al. Blood Cancer J 2022, Kantler H, et al. Blood 2000

Agenda

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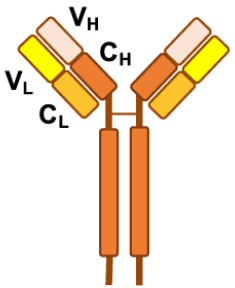
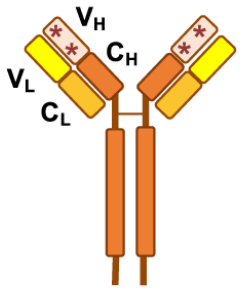
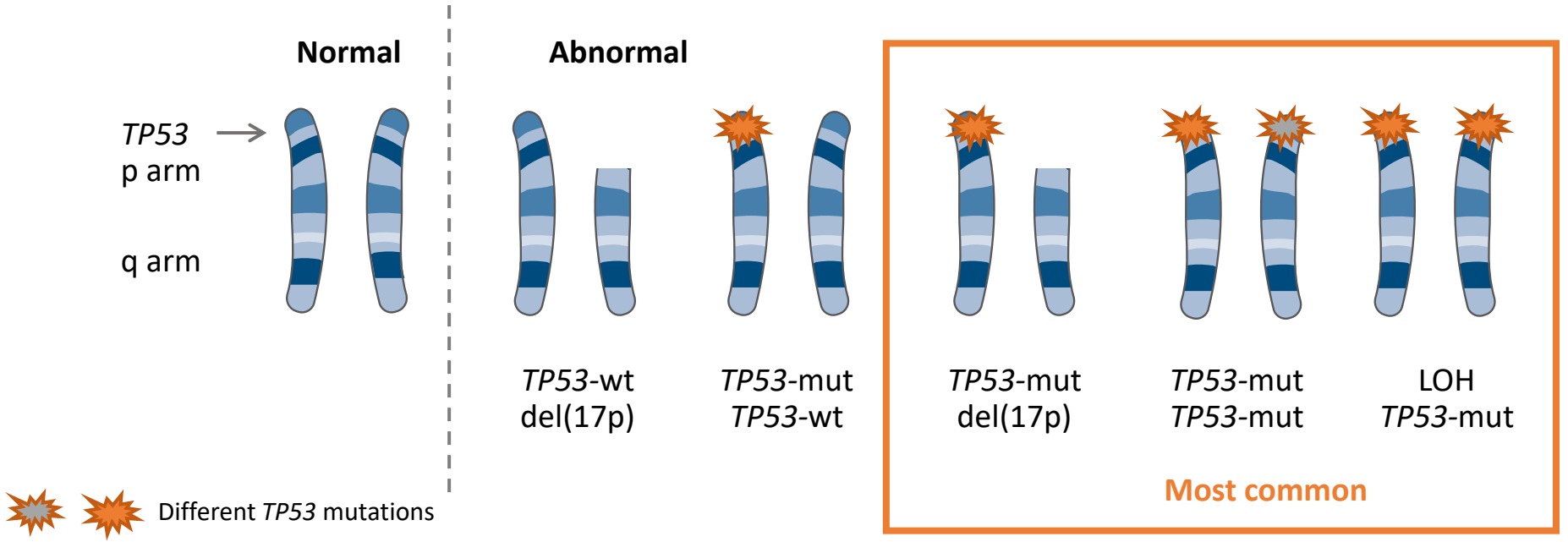
3. Molecular biomarkers
4. Acquired resistance

IGHV mutation status

U-CLL		M-CLL
		
40%	CLL patient frequency	60%
≥98%	Homology with germline IGHV	<98%
High	BCR responsiveness	Low, anergic BCRs
Autoantigens, high poly-reactivity	Antigenic determinants	Microbial/Autoantigens, higher BCR specificity
Dismal	Clinical outcome	Indolent

Ten Hacken E, Leukemia. 2019

TP53 status


Normal
Abnormal
TP53
p arm
q arm
TP53-wt
del(17p)
TP53-mut
TP53-wt
TP53-mut
del(17p)
TP53-mut
TP53-mut
LOH
TP53-mut
Most common
Different *TP53* mutations

Clinical implications

Counseling:

- **IGHV status:** IPS-E for time to first treatment
- **IGHV/*TP53* status**; CLL-IPI for overall survival

Treatment tailoring:

- **mIGHV/*TP53* wt**: Venetoclax-Obinutuzumab
- **uIGHV/*TP53* wt**: Venetoclax-BTK inhibitor
- ***TP53* abn**: BTK inhibitor

Agenda

1. General aspects
2. Clinico-pathologic variants
3. Molecular biomarkers
4. Acquired resistance

Mutations of resistance

***BTK* C481 mutations (I, A, Z)**
- preclude irreversible binding of covalent BTKi to BTK
- result in a greatly reduced drug potency

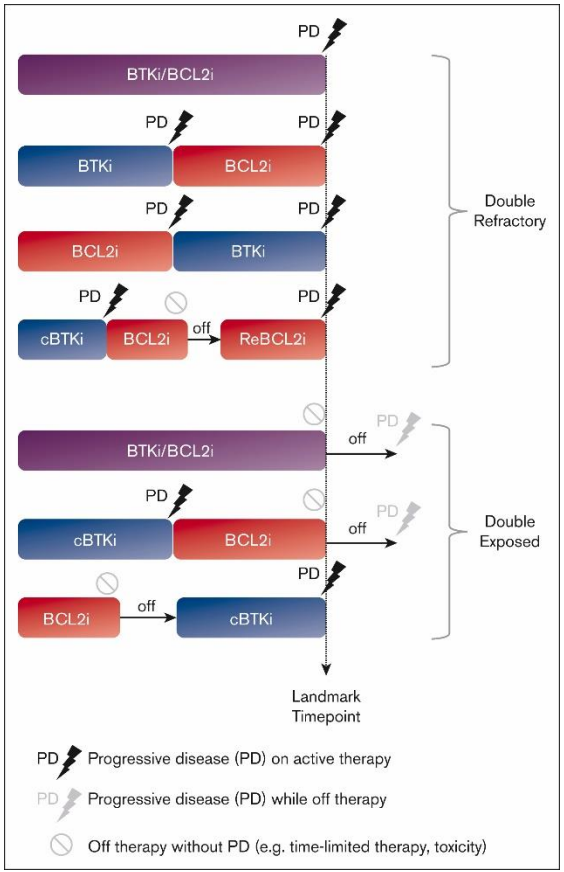
***BTK* T474 gatekeeper (P)**
- interfere with BTKi (both covalent and noncovalent) binding to BTK
- allow for normal B-cell signaling

***BTK* L528W kinase-dead (Z, P)**
- hinder BTK catalytic activity
- B-cell signaling is thought to continue via a BTK scaffolding

***PLCG2* M (I, A, Z, P)**
- co-occur with BTK M
- autonomous signaling

***BCL2* M (V)**
- preclude binding of vnetoclax to BCL2

Incidence of mutations of resistance



- Progression under BTKi: **70%**
- Progression under venetoclax: **50%**
- Progression after time limited therapy: **0%**

- Primary resistance is rare in first line (ORR >90%)
- Discontinuation for intolerance must not be confused with acquired resistance

Yoon JT, t a. Blood Adv 2025

Synergy Between Hematology and Pathology

- Request extended diagnostic work-up in borderline cases
- Assess IGHV and TP53 status on lymph node biopsy in SLL with limited PB/BM involvement
- Discuss aggressive presentations within the Tumor Board
- Communicate ongoing or prior BTK inhibitor treatment when submitting lymph node biopsies