

Understanding High-Risk CLL in the Era of Targeted Therapies



Learning Objectives

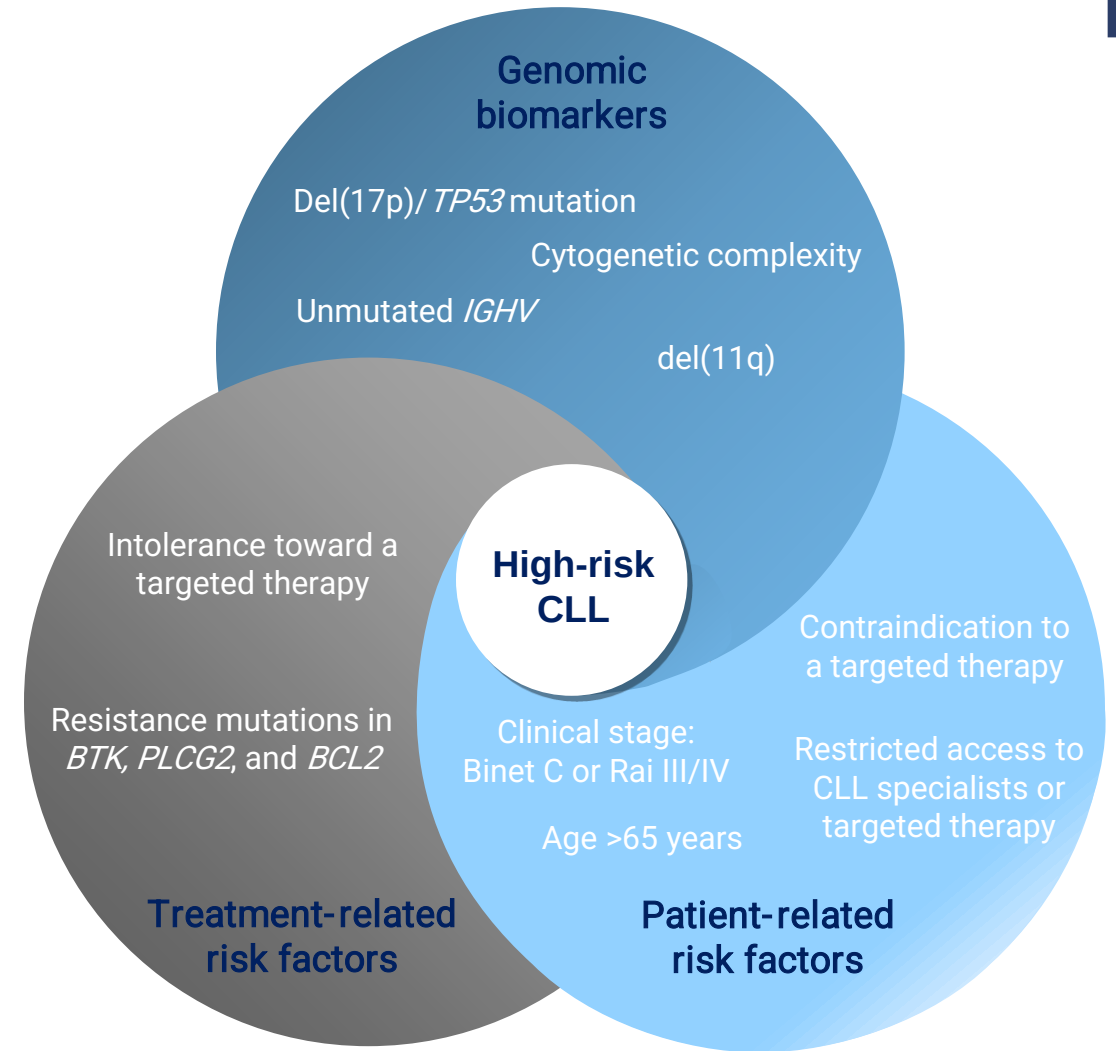
- Describe risk stratification for CLL and prognostic features associated with high-risk disease
- Recognize the clinical application and impact of molecular biomarkers (eg, *TP53* mutations) in defining high-risk CLL in the era of targeted therapies
- Describe common methods used for molecular testing (eg, FISH, NGS) and relevant time points of evaluation
- Understand the role of acquired resistance mutations in drug targets (eg, BTK, BCL-2) for predicting relapse and disease progression

BCL-2, B-cell lymphoma 2; BTK, Bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia; FISH, fluorescence in situ hybridization; NGS, next-generation sequencing; *TP53*, tumor protein 53.

Overview of Factors Associated With High-Risk CLL



- CLL displays **variable clinical behavior**¹
 - Many patients live for years without progressive disease¹
 - Others require early therapeutic interventions often with short lasting responses¹
- Rai and Binet staging systems represent the backbone of risk stratification, but the advent of genetic and biochemical markers have provided additional tools for identifying **high-risk CLL**²
 - **TP53** aberrations are among the strongest markers guiding CLL treatment decisions³
 - In the era of targeted therapies, **patient characteristics** and **treatment history** may also play a role in defining high-risk CLL^{4,5}

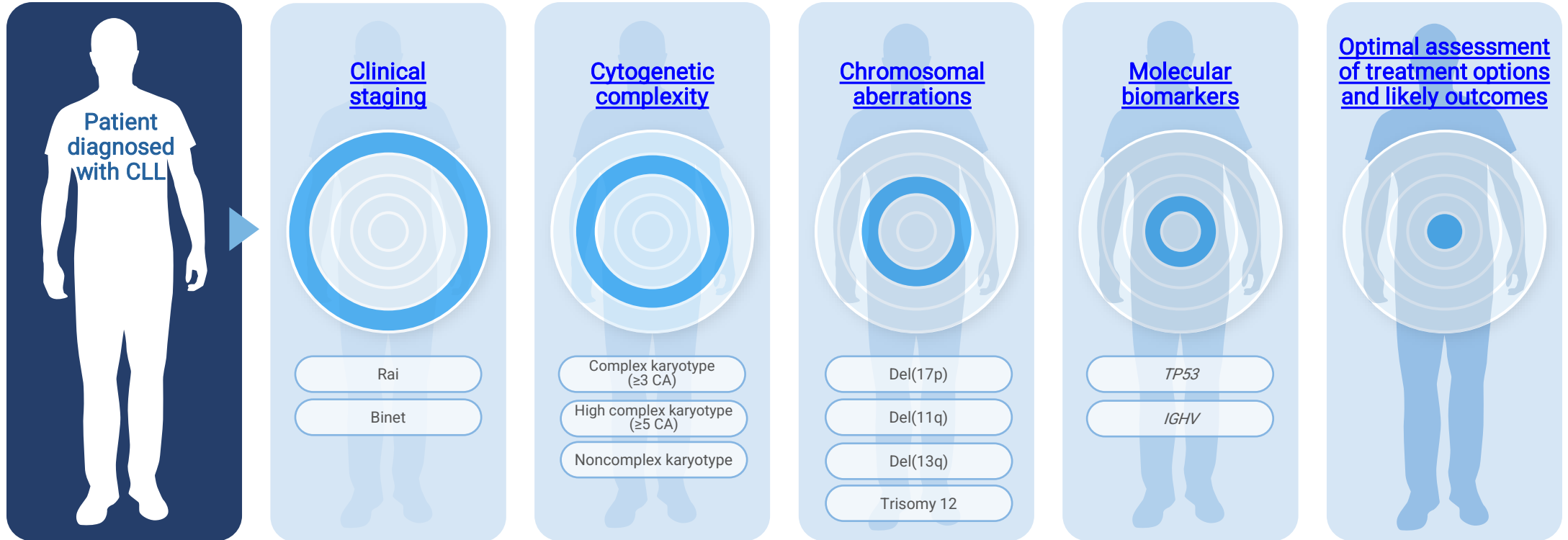


BCL-2, B-cell lymphoma 2; BTK, Bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia; del(11q), deletions of the long arm of chromosome 11; del(17p), deletions of the short arm of chromosome 17; *IGHV*, immunoglobulin heavy chain variable region gene; *PLCG2*, phospholipase C gamma 2; *TP53*, tumor protein 53.

1. Martinelli S, et al. *Mediterr J Hematol Infect Dis*. 2016;8(1):e2016047. 2. Eichhorst B, Hallek M. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):149-155. 3. Campo E, et al. *Haematologica*. 2018;103(12):1956-1968.

4. Edelmann J, et al. *Front Oncol*. 2023;13:1106579. 5. International CLL-IPI Working Group. *Lancet Oncol*. 2016;17(6):779-790.

Identifying High-Risk CLL Using Established Prognostic Factors



Accurate risk stratification is essential in the era of targeted therapies^{1,2}

CA, chromosomal aberration; CLL, chronic lymphocytic leukemia; del(11q), deletions of the long arm of chromosome 11; del(13q), deletions of the long arm of chromosome 13; del(17p), deletions of the short arm of chromosome 17; *IGHV*, immunoglobulin heavy chain variable region gene; *TP53*, tumor protein 53.

1. Edelman J, et al. *Front Oncol.* 2023;13:1106579. 2. Hallek M, Al-Sawaf O. *Am J Hematol.* 2021;96(12):1679-1705. .



- The Rai and Binet clinical staging systems have relied on results of **physical examination** and **blood counts** to provide prognostic information, representing the backbone of prognostication in CLL for decades^{1,2}

High-risk CLL–Binet^{1,3}

- Anemia (hemoglobin <10g/dL)
- Thrombocytopenia (platelets <100 x 10⁹/L)
- Any number of areas of lymphoid tissue enlargement

Referred to as stage C

High-risk CLL–Rai^{1,3}

- Lymphocytosis in blood and/or bone marrow

AND

- Anemia (hemoglobin <11g/dL)

OR

- Thrombocytopenia (platelets <100 x 10⁹/L)

Referred to as high-risk or stage III/IV

With recent advances in CLL therapies, there has been a need to refine risk-stratification with newly established genetic and biochemical prognostic factors^{1,2}

CLL, chronic lymphocytic leukemia.

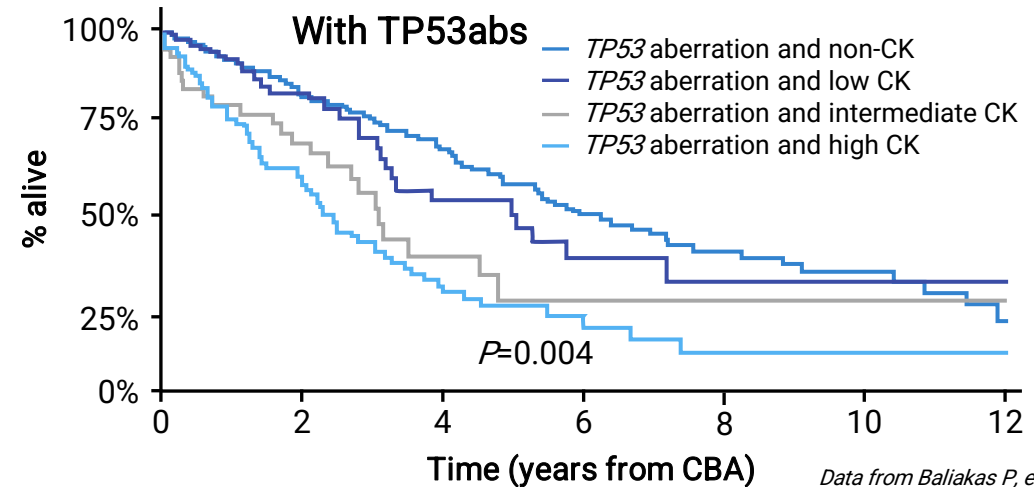
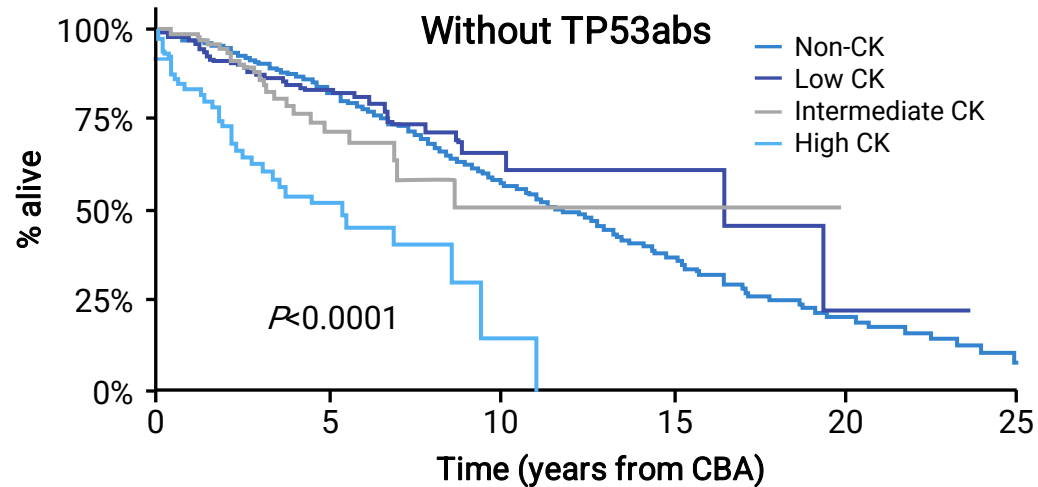
1. Hallek M, Al-Sawaf O. *Am J Hematol*. 2021;96(12):1679-1705. 2. Gonzalez-Rodriguez AP, et al. *J Clin Med*. 2020;9(11):3695. 3. Leukemia & Lymphoma Society. CLL staging. Accessed February 29, 2024. <https://www.lls.org/leukemia/chronic-lymphocytic-leukemia/diagnosis/cll-staging>.



Significance of Complex Karyotype in High-Risk CLL



- Complex karyotype (CK) is defined by the presence of ≥ 3 chromosomal aberrations and is associated with variable clinical behavior in CLL¹
 - Historically, CK has been associated with more aggressive clinical outcomes in CLL^{1,2}
 - Recently published evidence suggests that high CK (≥ 5 abnormalities) is prognostically adverse, independently of clinical stage, *IGHV* status, and *TP53* aberrations¹
 - In contrast, low (3 abnormalities) and intermediate (4 abnormalities) CK are clinically relevant only when coexisting with *TP53* aberrations¹
 - When 0-2 abnormalities are present it is non-complex karyotype¹



Data from Baliakas P, et al.¹

High CK has emerged as prognostically adverse, independently of other biomarkers¹

CBA, chromosome banding analysis; CK, complex karyotype; CLL, chronic lymphocytic leukemia; *IGHV*, immunoglobulin heavy chain variable region gene; *TP53*, tumor protein 53.

1. Baliakas P, et al. *Blood*. 2019;133(11):1205-1216 2. Juliusson G, et al. *N Engl J Med*. 1990;323(11):720-724.



	Description	Frequency	Risk
Del(13q)	Critical region of del(13q14) contains miRNAs that regulate apoptosis and cell cycle progression ^{1,2}	55% of patients with CLL ¹	Favorable prognosis when alone ^{1,2}
Del(11q)	Frequently encompasses 11q23, which harbors the <i>ATM</i> gene; associated with bulky disease and rapid progression ¹	10% of early disease 25% of advanced disease ^{1,a}	Poor prognosis ^{1,2}
Trisomy 12	Role in CLL pathogenesis unclear; may be more common in SLL and cases with Richter transformation ^{1,2}	10%-20% of patients with CLL ¹	Intermediate prognosis ^{1,2}
Del(17p)	17p13 harbors the <i>TP53</i> gene, which encodes tumor suppressor protein P53 ¹	5%-8% of chemotherapy-naïve patients with CLL ¹	Poor prognosis; resistance to genotoxic chemotherapies ¹

^aChemotherapy-naïve.

- CLL appears to have a particular cytogenetic profile with 4 classical abnormalities or recurrent aberrations characterized by karyotyping, FISH, CGH, and SNP arrays³
- Cytogenetic analyses have provided prognoses and predictions for CLL course of disease³

Approximately 80% of patients with CLL carry at least 1 of 4 chromosomal alterations: del(13q), del(11q), trisomy 12, or del(17p)¹

ATM, ataxia telangiectasia mutated; CGH, comparative genomic hybridization; CLL, chronic lymphocytic leukemia; del(11q), deletions of the long arm of chromosome 11; del(13q), deletions of the long arm of chromosome 13; del(17p), deletions of the short arm of chromosome 17; FISH, fluorescence in situ hybridization; SLL, small lymphocytic leukemia; SNP, single nucleotide polymorphism; *TP53*, tumor protein 53.

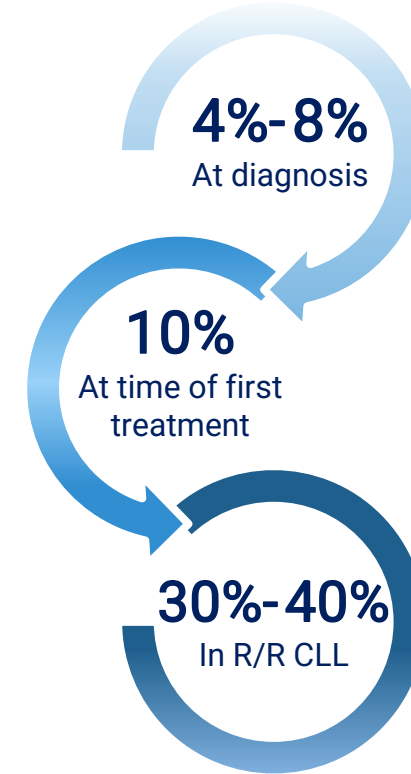
1. Hallek M, Al-Sawaf O. *Am J Hematol*. 2021;96(12):1679-1705. 2. Lee J, Wang YL. *J Mol Diagn*. 2020;22(9):1114-1125 3. Jondreville L, et al. *Am J Hematol*. 2020;95(11):1361-1367.



TP53 Aberrations Can Be Acquired or Selected During the Course of Disease Progression



- *TP53* encodes the tumor suppressor protein p53, which regulates the cell cycle and apoptosis¹
- In CLL, aberrations in *TP53* can arise through deletion of the *TP53* locus on chromosome 17 [eg, del(17p)] or via genetic mutations—most commonly missense mutations in coding region (≈75% of cases)¹



Incidence of *TP53* aberrations observed during course of disease progression²

The presence or absence of del(17p) and/or *TP53* mutations is the single most important prognostic/predictive factor for high-risk CLL and helps directly influence treatment planning^{1,2}

CLL, chronic lymphocytic leukemia; del(17p), deletions of the short arm of chromosome 17; R/R, relapsed/refractory; *TP53*, tumor protein 53.

1. Campo E, et al. *Haematologica*. 2018;103(12):1956-1968. 2. Stefaniuk P, et al. *Cancer Manag Res*. 2021;13:1459-1476.

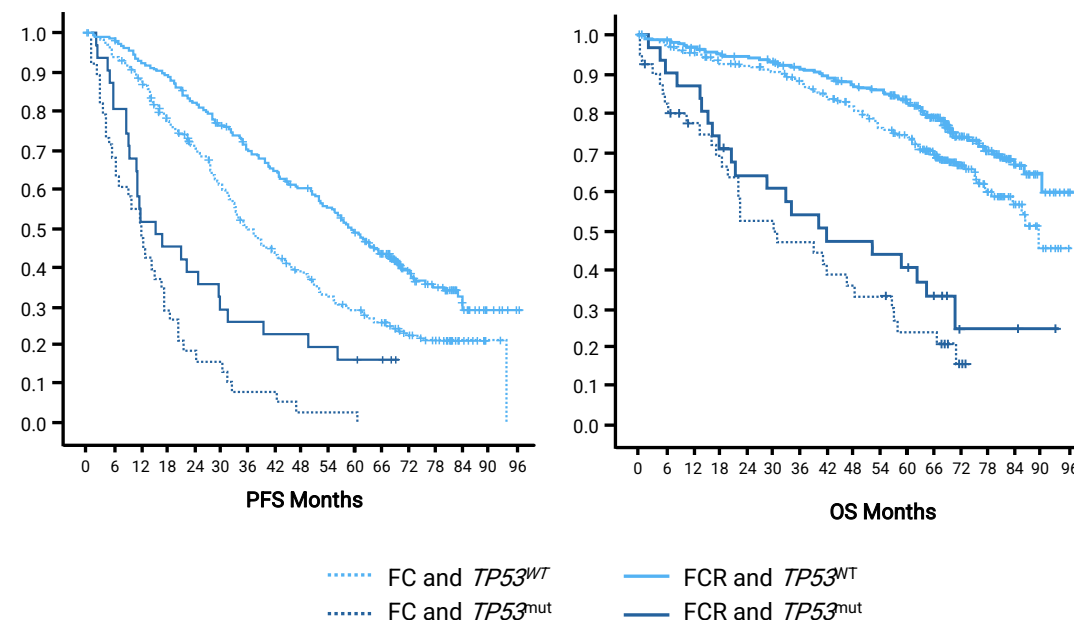


Clinical implications

- TP53* aberrations are associated with high-risk CLL and may result in the following¹
 - Early progression of disease
 - High risk of Richter transformation

Treatment considerations

- TP53* aberrations are not an indication for starting therapy—treatment initiation is still based on disease-related symptoms or progression^{1,2}
- Patients with del(17p) and/or *TP53* mutations respond poorly to CIT and have demonstrated shorter OS and PFS²⁻⁴
- Targeted therapies including BTK and BCL-2 inhibitors have shown improved PFS and OS in *TP53*-deficient patients compared to CIT⁵
 - Limited clinical data suggest second-generation BTK inhibitors may overcome the adverse impacts of *TP53* aberration⁵
 - In contrast, PFS was shorter with fixed-duration BCL-2 inhibition in patients with *TP53* aberrations compared to unaltered *TP53*^{5,6}

PFS and OS by *TP53* mutational statusData from Stilgenbauer S, et al.⁴

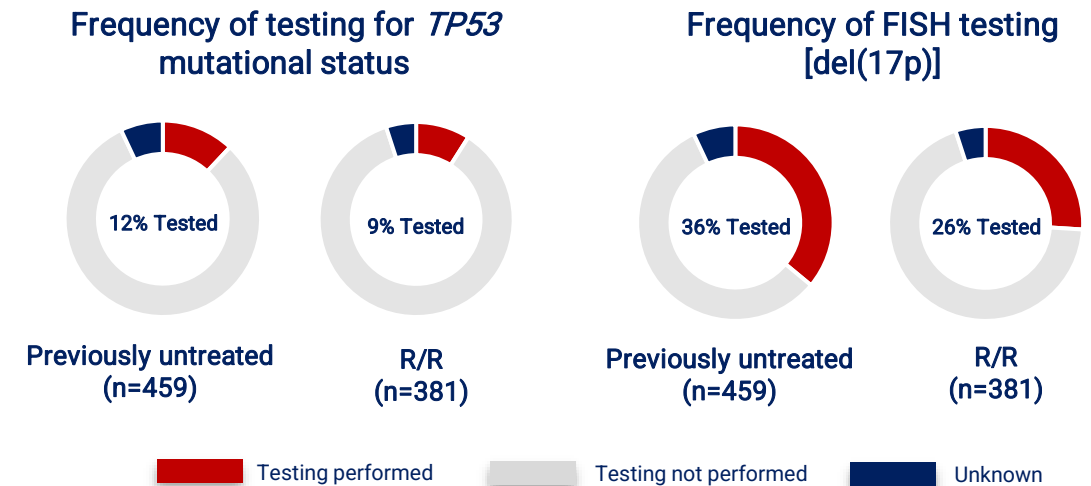
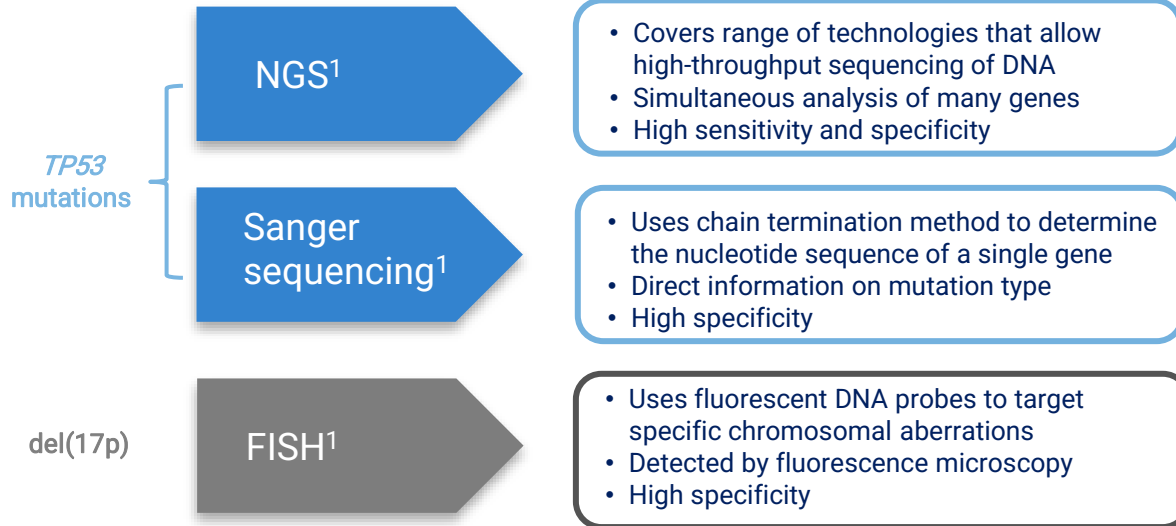
CIT is not considered standard management in patients with *TP53* aberrations and requires drugs that promote cell death independently of *TP53*^{1,5}

BCL-2, B-cell lymphoma 2; BTK, Bruton's tyrosine kinase; CIT, chemoimmunotherapy; CLL, chronic lymphocytic leukemia; del(17p), deletions of the short arm of chromosome 17; FC, fludarabine and cyclophosphamide; FCR, fludarabine and cyclophosphamide and rituximab; OS, overall survival; PFS, progression free survival; *TP53*, tumor protein 53.

1. Stefaniuk P, et al. *Cancer Manag Res*. 2021;13:1459-1476. 2. Aitken MJL, et al. *Ther Adv Hematol*. 2019;10:2040620719891356. 3. Campo E, et al. *Haematologica*. 2018;103(12):1956-1968. 4. Stilgenbauer et al. *Blood*. 2014;123(21):3247-3254. 5. Edelmann J, et al. *Front Oncol*. 2023;13:1106579. 6. Al-Sawaf O, et al. *J Clin Oncol*. 2021;39(36):4049-4060.



- Techniques used in clinical practice include Sanger sequencing and NGS for *TP53* mutations and FISH for del(17p)^{1,2}
 - ~30% of all *TP53* defects are mutations lacking del(17p), whereas ~10% are due to del(17p) alone²
 - This highlights the need to evaluate the presence of both²
- In clinical practice, testing for del(17p)/ *TP53* mutations is low, which may lead to suboptimal therapy choices for patients³

Data from Mato AR, et al.³

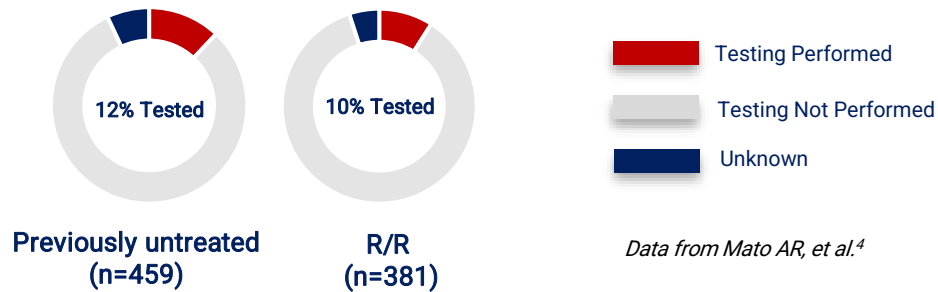
It is recommended to test all patients with CLL for del(17p) and *TP53* mutation before treatment initiation and at every relapse^{1,2}

CLL, chronic lymphocytic leukemia; del(17p), deletions of the short arm of chromosome 17; FISH, fluorescence in situ hybridization; NGS, next-generation sequencing; R/R, relapsed/refractory; *TP53*, tumor protein 53.

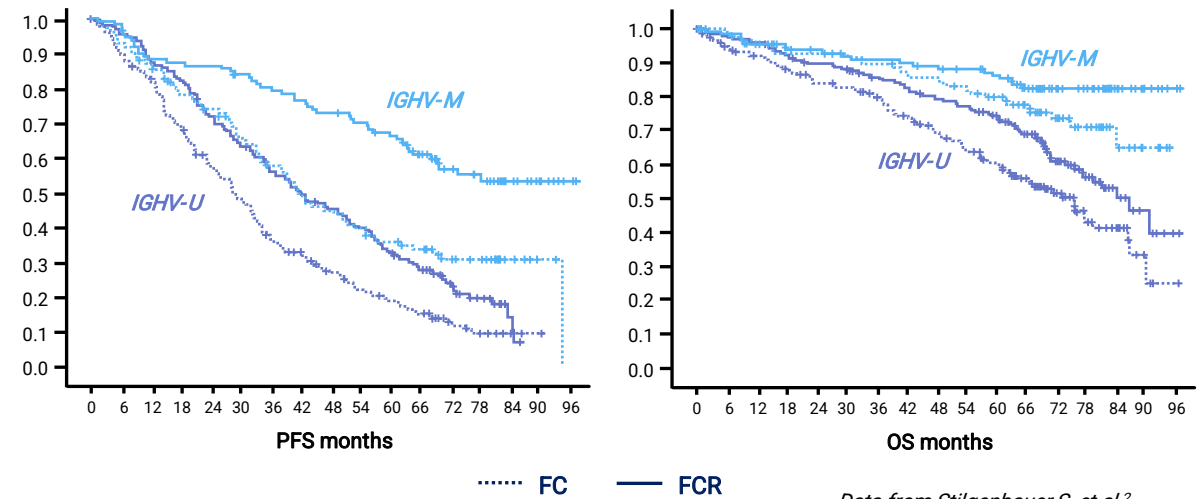
1. Campo E, et al. *Haematologica*. 2018;103(12):1956-1968. 2. Stefaniuk P, et al. *Cancer Manag Res*. 2021;13:1459-1476 3. Mato AR, et al. *Clin Lymphoma Myeloma Leuk*. 2020;20(3):174-183.e3.



- IGHV* mutational status is an established independent prognostic factor for survival outcomes in CLL^{1,2}
- Unmutated *IGHV* (≈40% of untreated CLL)³ is associated with a poor prognosis and predicts a worse response to CIT¹⁻³
- IGHV* status is not affected by disease evolution and remains consistent throughout the disease and treatment course³
- In clinical practice, testing for *IGHV* mutations has been shown to be infrequent⁴:



PFS and OS by *IGHV* mutational status



IGHV mutational status is considered the fingerprint of CLL and determined at the time of diagnosis^{1,5}

CIT, chemoimmunotherapy; CLL, chronic lymphocytic leukemia; FC, fludarabine and cyclophosphamide; FCR, fludarabine and cyclophosphamide and rituximab; *IGHV*, immunoglobulin heavy chain variable region gene; *IGHV-M*, immunoglobulin heavy chain variable region gene mutated; *IGHV-U*, immunoglobulin heavy chain variable region gene unmutated; OS, overall survival; PFS, progression-free survival; R/R, relapsed/refractory.

1. Heerema NA, et al. *Haematologica*. 2021;106(6):1608-1615. 2. Stilgenbauer S, et al. *Blood*. 2014;123(21):3247-3254. 3. Lee J, Wang YL. *J Mol Diagn*. 2020;22(9):1114-1125. 4. Mato AR, et al. *Clin Lymphoma Myeloma Leuk*. 2020;20(3):174-183.e3. 5. Giudice ID, et al. *Haematologica*. 2019;104(2):219-221.



Emerging Molecular Markers for High-Risk CLL



- NGS studies have led to the discovery of additional recurrently mutated genes that may have a potential prognostic impact in CLL¹
- In total, >40 recurrently mutated driver genes have been identified affecting signaling pathways involved in inflammation, DNA damage and cell cycle control, transcription, and ribosomal processing²
 - Mutations currently being evaluated for their prognostic significance in CLL include *ATM*, *BIRC3*, *NOTCH1*, and *SF3B1*³



	Description	Frequency	Risk
ATM	A core regulator of DNA damage response pathway; mutation often co-occurs with <i>SF3B1</i> mutation ³	12% of untreated CLL ³ 22% of R/R CLL ³	Poor prognosis with bi-allelic loss of <i>ATM</i> ⁶
BIRC3	Negative regulator of the noncanonical NF-κB pathway	4% of untreated CLL ³ 11% of R/R CLL ³	High-risk in unselected CLL patients ³
NOTCH1	Supports cell proliferation and survival; mutation leads to constitutive activation and CLL pathogenesis ³	11% of untreated CLL ³ 17% of R/R CLL ³	Poor prognosis ³
SF3B1	DNA damage response, cell proliferation, NOTCH signaling altered by mutation ³	8% of untreated CLL ³ 21% of R/R CLL ³	Shorter OS and TTFT ³

While emerging molecular markers have been signaled for their association with CLL outcomes, their designation as CLL prognostic biomarkers await further validation studies²

ATM, ataxia telangiectasia mutated; *BIRC3*, baculoviral IAP repeat containing 3; CLL, chronic lymphocytic leukemia; NF-κB, nuclear factor kappa B; NGS, next generation sequencing; *NOTCH1*, neurogenic locus notch homolog protein 1; OS, overall survival; R/R, relapsed/refractory; *SF3B1*, splicing factor 3b subunit 1; TTFT, time to first treatment.

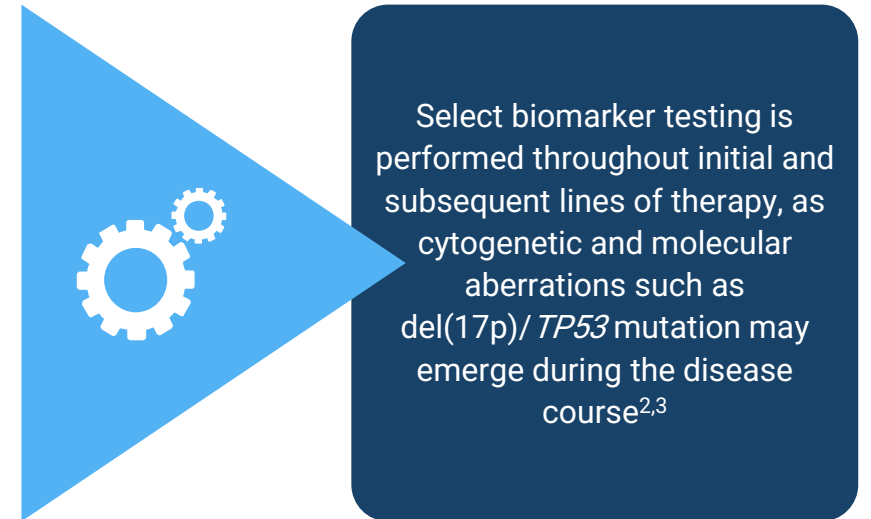
1. Mansouri et al. *Leukemia*. 2023;37(2):339-347 2. Gaidano G, Rossi D. *Hematology Am Soc Hematol Educ Program*. 2017;2017(1):329-337. 3. Lee J, Wang YL. *J Mol Diagn*. 2020;22(9):1114-1125

When to Test? Time Points of Evaluation of Prognostic Factors



Common prognostic factors and points of evaluation^{1,2}

Prognostic factor	Initial diagnosis (asymptomatic)	Initial diagnosis (symptomatic)	Before initiation of first-line therapy	Before initiation of relapse therapy ^e
del(17p)/ <i>TP53</i> mutation	✓ ^a	✓ ^a	✓ ^b	✓ ^b
Del(11q)	✗	✓ ^a	✓ ^c	✓ ^c
<i>IGHV</i>	✓ ^a	✓ ^e	✓ ^e	—
Karyotype	✗	✓ ^a	✓ ^d	✓ ^d



^aIf consequences can be drawn (more/less intensive follow-up or early treatment).

^bUnless *TP53* mutation/deletion was already detected before.

^cIf patient would be suitable for FCR.

^dIf patient can be considered for allogeneic transplantation or clinical trial.

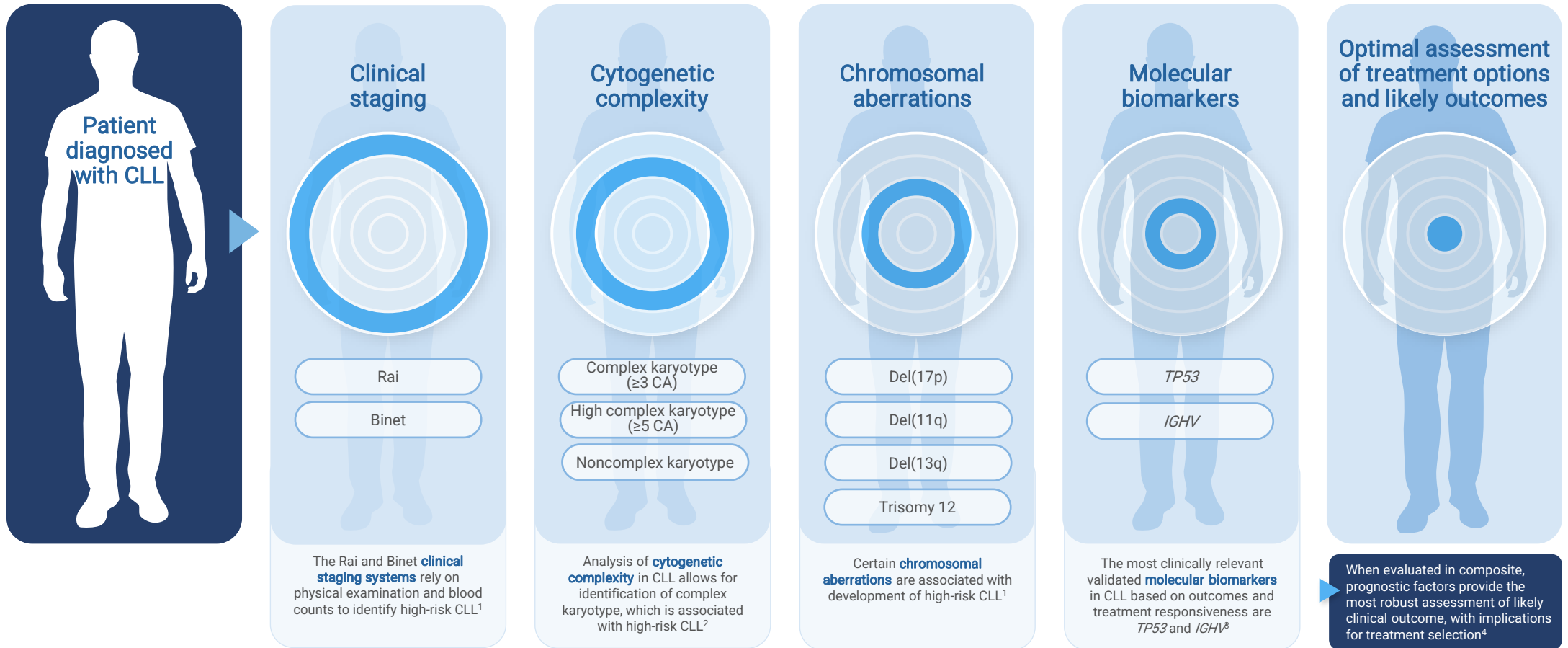
^eIf not done before.

Data from Eichhorst B, Hallek M.¹

CLL, chronic lymphocytic leukemia; del(11q), deletions of the long arm of chromosome 11; del(17p), deletions of the short arm of chromosome 17; FCR, fludarabine and cyclophosphamide and rituximab; *IGHV*, immunoglobulin heavy chain variable region gene; *TP53*, tumor protein 53.

1. Eichhorst B, Hallek M. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):149-155. 2. Cohen JA, et al. *Cancers (Basel)*. 2020;12(4):894. 3. Campo E, et al. *Haematologica*. 2018;103(12):1956-1968.

Collective Impact of Prognostic Factors on Treatment Decisions



***TP53* aberrations by del(17p) and/or *TP53* mutations are the key biomarkers that guide treatment decisions⁴**

CA, chromosomal aberration; CLL, chronic lymphocytic leukemia; del(11q), deletions of the long arm of chromosome 11; del(13q), deletions of the long arm of chromosome 13; del(17p), deletions of the short arm of chromosome 17; *IGHV*, immunoglobulin heavy chain variable region gene; *TP53*, tumor protein 53.

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Predictive Molecular Markers in CLL: Resistance Mutations to Targeted Therapies



Acquired mutations within the drug target

- Common resistance mechanisms associated with targeted therapies include:
 - Mutations in **C481-binding site in *BTK*** and its downstream effector ***PLCG2*** following covalent BTK inhibition¹⁻³
 - Mutations in the **BH3-binding domain of BCL-2** (eg, G101V, D103Y) following BCL-2 inhibition^{1,4}

Strategies to overcome acquired resistance mutations

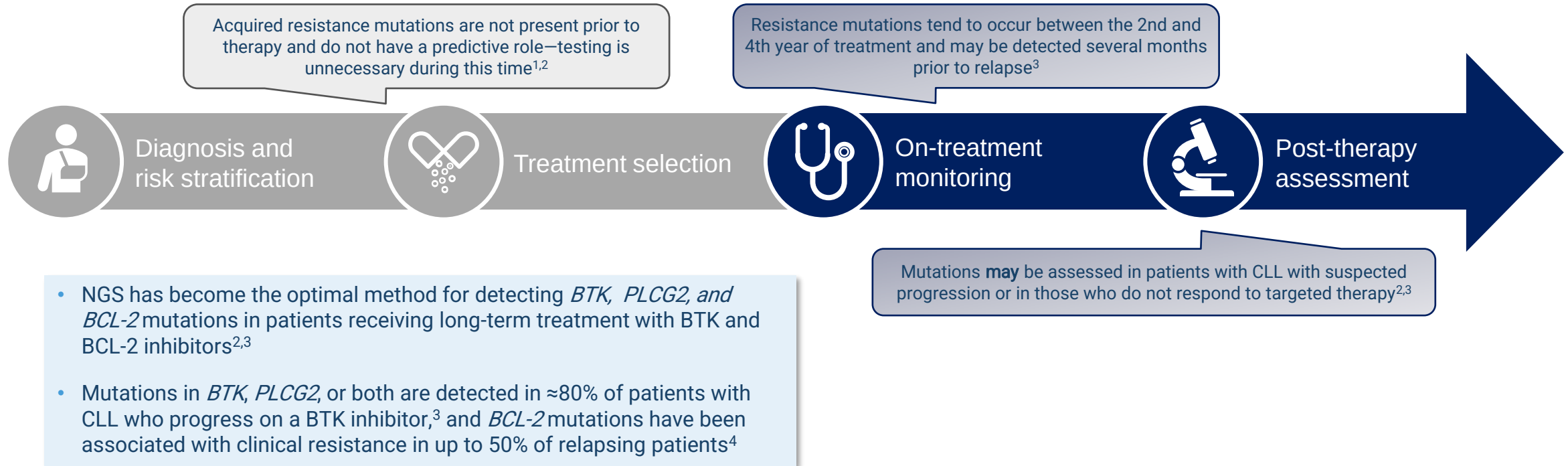
- Optimal sequencing of therapies may help overcome resistance¹
 - BTK inhibitors have been shown to be effective in patients resistant to BCL-2 inhibition and vice versa¹
- Non-covalent (reversible) BTK inhibitors may provide a treatment option for patients resistant to or progressing on or after receiving a covalent BTKi¹
- Novel drugs and drug combinations are also in development to overcome resistance¹

Mechanisms of acquired resistance and disease progression with targeted therapies include the development of secondary mutations within the drug targets (eg, BCL-2, BTK)¹

BCL-2, B-cell lymphoma 2; *BTK*, Bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia; *PLCG2*, phospholipase C gamma 2.

1. Skånland SS, Mato AR. *Blood Adv.* 2021;5(1):334-343. 2. Lee J, Wang YL. *J Mol Diagn.* 2020;22(9):1114-1125 3. Chirino A, et al. *Genes (Basel).* 2023;14(12):2182. 4. Kotmayer L, et al. *Int J Mol Sci.* 2023;24(6):5802.

Testing for Acquired Resistance Mutations in CLL



While testing for resistance mutations may be performed at suspected progression, the presence of mutations alone without clinical relapse has not yet become a trigger for changing treatment^{2,3}

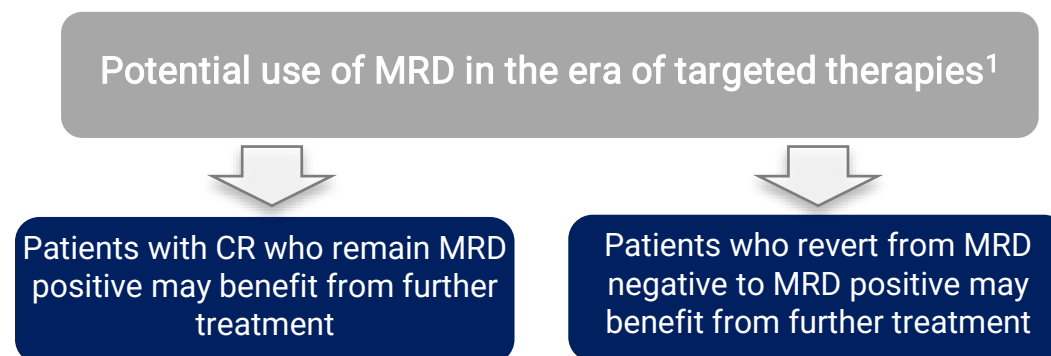
BCL-2, B-cell lymphoma 2; *BTK*, Bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia; NGS, next generation sequencing; *PLCG2*, phospholipase C gamma 2.

1. Eichhorst B, Hallek M. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):149-155. 2. Lee J, Wang YL. *J Mol Diagn*. 2020;22(9):1114-1125. 3. Sedlarikova L, et al. *Front Oncol*. 2020;10:894. 4. Kotmayer L, et al. *Int J Mol Sci*. 2023;24(6):5802.

Future Directions: Clinical Significance of MRD in the Era of Targeted Therapies



- MRD is defined by the presence of residual cancer cells after therapy in patients with clinically undetectable disease and is measured in either PB or BM by flow cytometry, PCR, or NGS¹
 - Roles in CLL currently include end point for survival and a prognostic marker in **clinical trials**¹
- EOT MRD status is a strong prognostic feature in the **context of combination therapy**, including both CIT and targeted combination therapies²
 - The **prognostic value of MRD is limited with continuous BTK inhibitor monotherapy**, as effective disease control can be achieved with maintenance therapy despite detectable²
 - » The disease-modulating effects of continuous BTK inhibitor may work independently of MRD depletion, limiting its clinical implications in this setting²



Data from Benintende G, et al.¹

In CLL, MRD is **not yet recommended** by current guidelines or used in routine clinical practice to evaluate response to treatment or guide clinical decision making and has limited prognostic value in the context of continuous BTK inhibition^{1,2}

BM, bone marrow; BTK, Bruton tyrosine kinase; CLL, chronic lymphocytic leukemia; CIT, chemoimmunotherapy; CR, complete response; EOT, end of treatment; MRD, minimal residual disease; NGS, next-generation sequencing; PB, peripheral blood; PCR, polymerase chain reaction.

1. Benintende G, et al. *Front Oncol.* 2023;13:1112616. 2. Al-Sawaf O. *Acta Haematol.* 2024;147(1):22-32.

Future Directions: CLL International Prognostic Index



CLL-IPI risk group criteria and scoring^{1,2}

Criteria	Scoring basis	Points
Age	≤65 years	0
	>65 years	1
Clinical stage	Binet A or Rai 0	0
	Binet B-C or Rai I-IV	1
Serum β2M (mg/L or μ/mL)	≤3.5	0
	>3.5	2
IGHV mutational status	Mutated	0
	Unmutated	2
TP53 status	No abnormality	0
	Deletion 17p (FISH) and/or TP53 mutation (sequencing)	4

5-year survival by CLL-IPI risk group^{1,2}

CLL-IPI score	Risk	5-year survival
0-1	Low	93.2%
2-3	Intermediate	79.3%
4-6	High	63.3%
7-10	Very high	23.3%

- While guidelines have not yet incorporated CLL-IPI into treatment management algorithms, the CLL-IPI provides additional prognostic information regarding overall survival compared with conventional clinical staging¹

The CLL-IPI combines genetic, biochemical, and clinical parameters into a prognostic model that categorizes patients into 4 subgroups: low, intermediate, high, and very high risk each with different survival at 5 years^{1,2}

CLL-IPI, International Prognostic Index for Chronic Lymphocytic Leukemia; del(17p), deletions of the short arm of chromosome 17; FISH, fluorescence in situ hybridization; IGHV, immunoglobulin heavy chain variable region gene; TP53, tumor protein 53.

1. International CLL-IPI Working Group. *Lancet Oncol.* 2016;17(6):779-790. 2. Hallek M, Al-Sawaf O. *Am J Hematol.* 2021;96(12):1679-1705.

Summary



- Prognostic factors are important for identifying high-risk CLL and can help guide clinical management decisions
- The presence or absence of del(17p) and/or *TP53* mutations is the single most important prognostic/predictive factor for high-risk CLL and helps directly influence treatment selection
- In the era of targeted therapies, genetic mutations in drug targets are a mechanism of acquired resistance and may serve as predictive molecular markers of relapse and progression

CLL, chronic lymphocytic leukemia; del(17p), deletions of the short arm of chromosome 17; *TP53*, tumor protein 53.