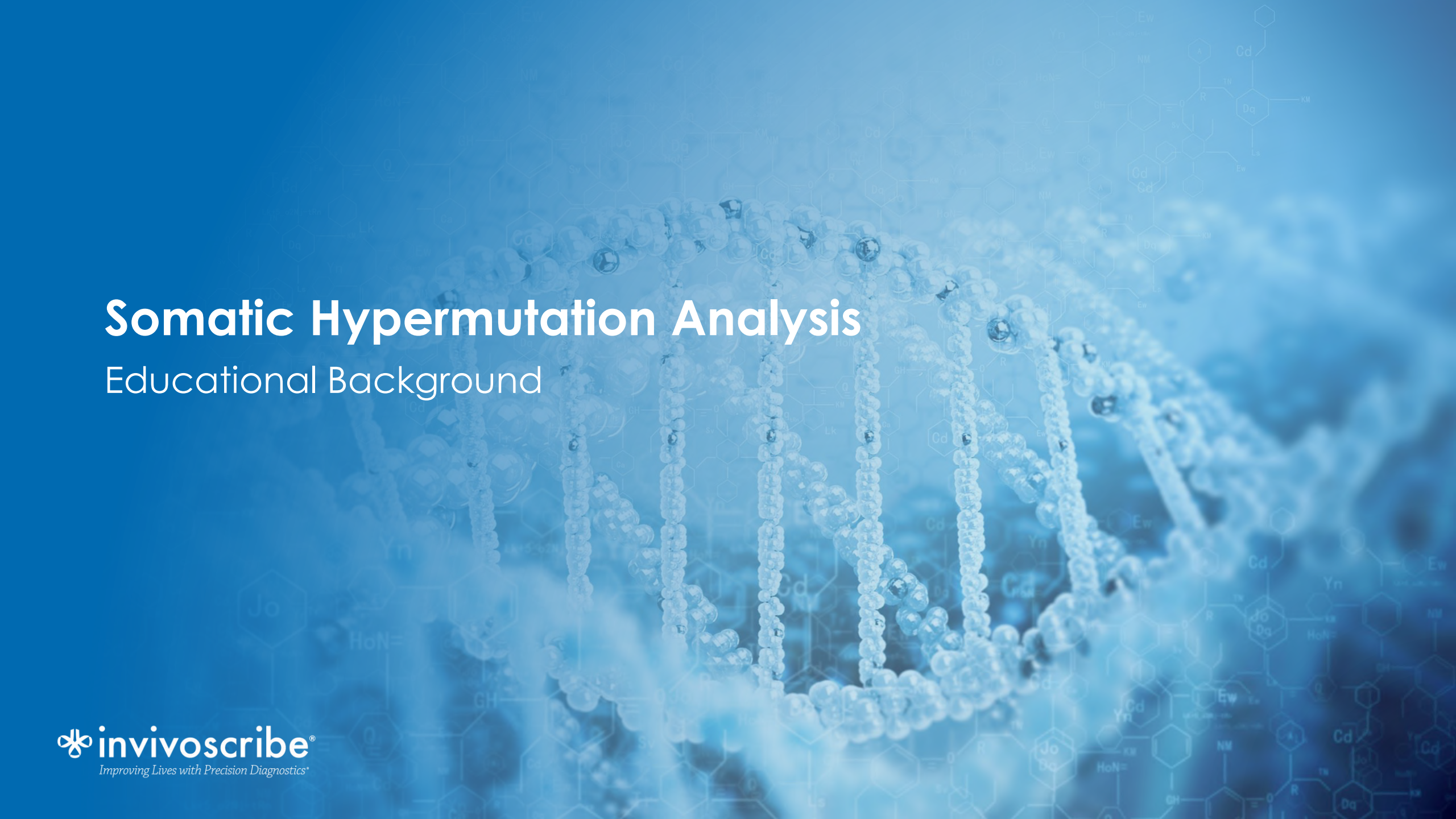
Somatic Hypermutation Analysis

Educational Background



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Somatic Hypermutation (SHM)

Important Prognostic Information for

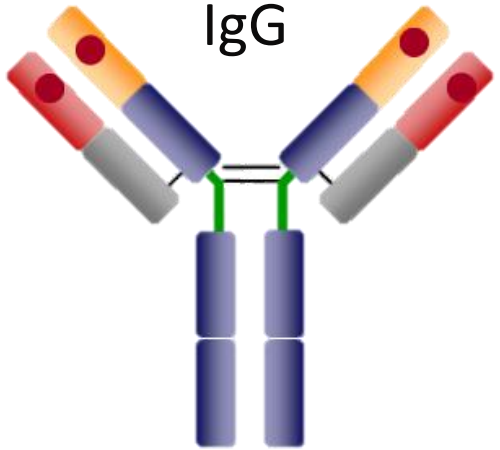
- Chronic Lymphocytic Leukemia (CLL)
- Small Lymphocytic Leukemia (SLL)
- Hairy Cell Leukemia (HCL)

SHM is defined as ≥ 2% Germline Sequence Difference*

- Presence of *IGHV* SHM: **≥ 2%** difference from the germline variable gene sequence correlates with a **favorable prognosis**
- Absence of *IGHV* SHM: **<2%** correlates with a **poor prognosis**

Expression of VH3-21 rearrangement**

- Frequently associated with IGHV Subset#2
- Independent of *IGHV* SHM status
- More aggressive disease



SHM testing is recommended as standard for all newly diagnosed CLL cases

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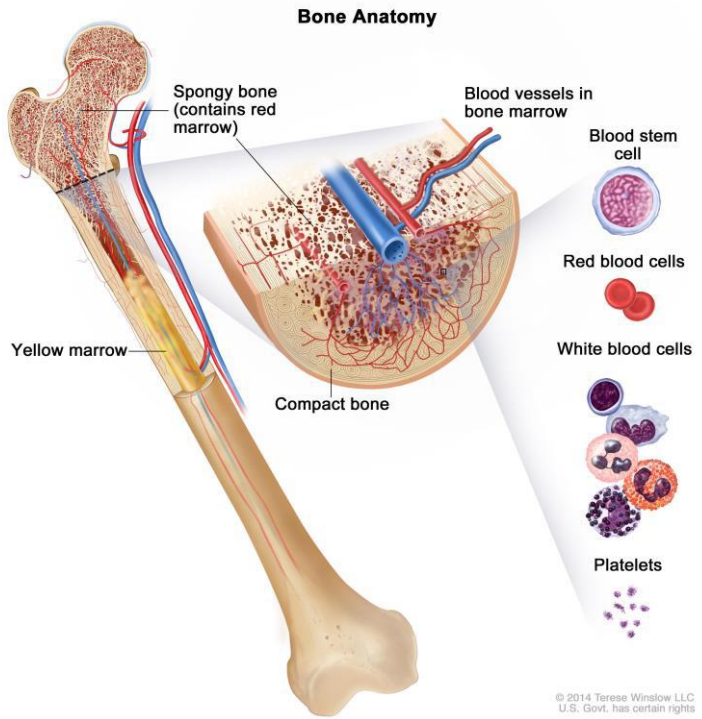
*Gupta et. al "Evaluation of Somatic Hypermutation Status in Chronic Lymphocytic Leukemia (CLL) in the Era of Next Generation Sequencing" Front Cell Dev Biol. 2020; 8: 357.
** Ghia,et. al "Geographic patterns and pathogenetic implications of IGHV gene usage in chronic lymphocytic leukemia: the lesson of the IGHV3-21 gene" Blood 2005 105(4):1678-85

Chronic Lymphocytic Leukemia (CLL)

CLL

- One of the Most Common Form of Leukemias
- Characterized by Accumulation of Mature B Lymphocytes
- Majority of Patients are Asymptomatic at Diagnosis
- Highly Variable Clinical Course
  - Rapid progression with fatal outcome to a relatively indolent behavior



Role of SHM in CLL

- *IGHV* gene mutational status is one of the **most robust prognostic markers in CLL**
- It remains stable over time
- It has a strong predictive value **for response to treatment**[1]

https://www.cancer.org/cancer/chronic-lymphocytic-leukemia/about/what-is-cll.html

[1] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4760129/

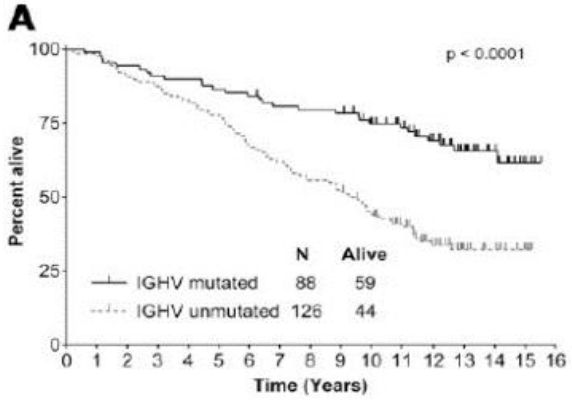
Role of SHM Status in CLL

Prognostic and Predictive

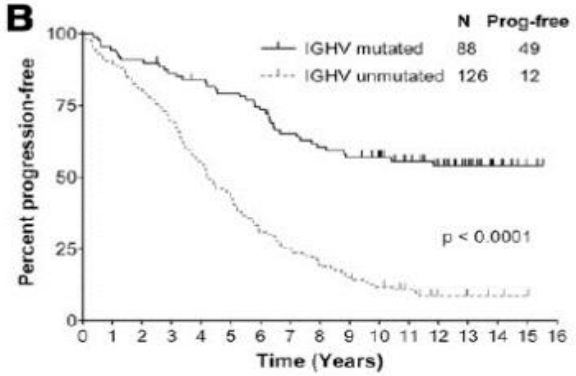
Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in *IGHV*-mutated chronic lymphocytic leukemia

Philip A. Thompson,[1,*] Constantine S. Tam,[2,*] Susan M. O'Brien,[1] William G. Wierda,[1] Francesco Stingo,[3] William Plunkett,[4] Susan C. Smith,[1] Hagop M. Kantarjian,[1] Emil J. Freireich,[1] and Michael J. Keating[1]

(A) OS according to mutation status



(B) PFS according to mutation status



- **Fludarabine, cyclophosphamide, and rituximab (FCR) :** standard treatment for CLL patients requiring therapy.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4760129/

Highlights

	OS (%)	PFS (%)
IGHV-M	65.5	53.9
IGHV-UM	32.2	8.9

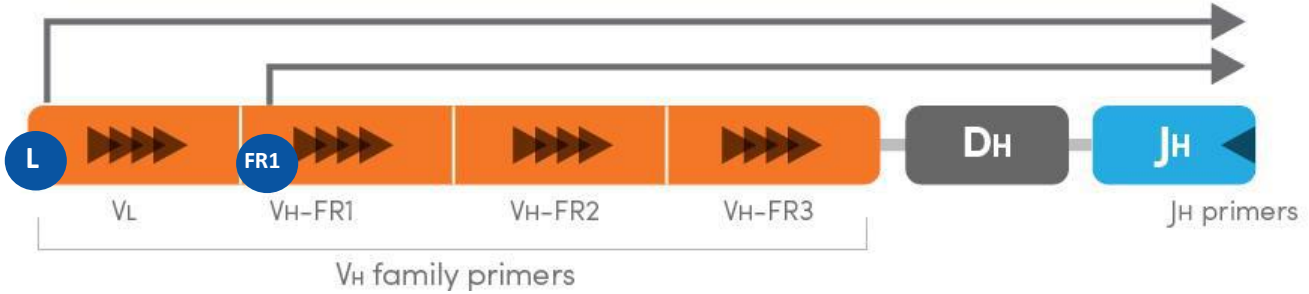
- M-CLL patients compared to UM-CLL patients who received the same treatment :
  - More prolonged response
  - Delayed progression
  - Significant improvement in survival overall

➢ **Determining the SHM status is a not only prognostic, but also a predictive.**

ERIC Guidelines - 2017

These recommendations for genetic testing are widely adopted

- Clear guideline to use Leader primers and to only use FR1 in difficult cases
- Can use either gDNA or cDNA



Final sequence analysis and subsetting should be done using IMGT (www.imgt.org)

ERIC Guidelines - 2017

Table 1. Technical considerations for determination of the IGHV somatic hypermutation status of clonotypic IGHV-IGHD-IGHJ gene rearrangements in CLL

Item	*Recommendations*	*Remarks*
Material		
Anticoagulants	EDTA (or CPT)	
Cells/tissue	Blood, bone marrow, tissue biopsy	Purification of B cells usually not necessary unless low fraction of leukemic cells
Nucleic acid	gDNA or cDNA	cDNA useful when mutations within the IGHJ gene impair amplification
Production of template for sequencing		
Primers	5′: leader 3′: IGHJ or IGHC	VH FR1, VH FR2 and VH FR3 primers are not acceptable IGHC primers (on cDNA) useful when mutations within IGHJ gene impair amplification
Amplification	Multiplex PCR	individual PCR reactions (for each 5′ primer) may be useful when more than one rearrangement found
Detection of IGH rearrangement	GeneScan or PAGE electrophoresis	Agarose gel electrophoresis strongly discouraged (lack of resolution)
Cloning	Not necessary	Except in rare circumstances (more than one rearrangement not isolated by simplex PCR)
Sequencing		
Methodology	Direct, both strands	Both strands mandatory for high-quality sequence
Sequence alignment	IMGT/V-QUEST (www.imgt.org)	Adjustable parameters: (1) search for insertions/deletions; (2) number of accepted *D* genes
IGHV identity (%)	Automatic or adjusted	Adjusted: use option 'search for insertions/deletions' when low % identity
Stereotypic subset identification	ARResT/AssignSubsets (bat.infspire.org/arrest/ericll.org/pages/services/tool)	Applicable for the current 19 major BcR stereotyped subsets in CLL[a]

- State whether the identified productive IG gene rearrangement leads to membership in a major stereotyped subset.

Rosenquist R, Ghia P, Hadzidimitriou A, et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: updated ERIC recommendations. *Leukemia*. 2017;31(7):1477-1481. doi:10.1038/leu.2017.125

Reporting *IGHV* SHM status in CLL

- **Subsets** defined by distinctive sequence motifs within the *IGHV* CDR3 region
- Subsets **dictate prognosis** regardless of the mutation status, at least for major subsets (aggressive)
- **ARResT/Assign Subsets** bioinformatics tool enables to determine the CLL stereotyped subset
- LymphoTrack® Dx output can be used for analysis with the ARResT/Assign Subsets tool*

Subset #1	Subset #2	Subset #4	Subset #8
IGHV clan I genes / IGKV1 (D)-39	**IGHV3-21/ IGL3-21**	**IGHV4-34**	**IGHV4-39/IGKV1(D)-39**
Poor prognosis Aggressive clinical course	Poor prognosis	Indolent course	Higher risk of Richter's transformation

*Invivoscribe has not validated use of LymphoTrack Dx data with the ARResT/Assign Subsets tool. However, the data can be analyzed with this tool, should you desire to validate this in your lab.

Rosenquist R, Ghia P, Hadzidimitriou A, et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: updated ERIC recommendations. *Leukemia*. 2017;31(7):1477-1481. doi:10.1038/leu.2017.125

Take Home Message

- *IGHV* somatic hypermutation status: one of the **most robust prognostic markers in CLL**
- **SHM testing recommended as standard** for all newly diagnosed CLL cases
- ERIC guidelines recommend to use Leader primers and to use FR1 in difficult cases
- **Subsets** can be defined by distinctive sequence motifs within the *IGHV* CDR3 region

Quiz

Somatic Hypermutation is defined as < 2% difference from the germline sequence.

- True
- False

Somatic Hypermutations are point mutations affecting the *IGHV* gene of B-Cell Receptors.

- True
- False

Quiz

What are the ERIC recommendations regarding of primers for SHM testing in CLL patients ?

- Use Leader primers only
- Use FR1 primers only
- Use Leader primers and only use FR1 in difficult cases