MAYO CLINIC LABORATORIES

IGHV and TP53 Sequencing: Clinical Utility in Chronic Lymphocytic Leukemia (CLL)

Presenter:

Curtis A. Hanson, M.D.

Professor of Laboratory Medicine and Pathology
Chief Medical Officer, Mayo Clinic Laboratories

Department of Laboratory Medicine and Pathology
at Mayo Clinic, Rochester, Minnesota

MAYO CLINIC LABORATORIES

Disclosures

- None

MAYO CLINIC LABORATORIES

Today's Outline

- Understand the use of prognostic markers in CLL patients
- Highlight the importance of molecular analysis for IGHV and TP53 sequencing in CLL patients
- Understand how IGHV analysis provides prognostic information in CLL and helps inform clinicians on treatment decisions
- Recognize that TP53 mutations by sequencing studies are associated with poor outcomes and more likely to be resistant to standard chemotherapy/chemoimmunotherapy regimens

MAYO CLINIC LABORATORIES

Brief Overview of CLL

- CLL is a neoplasm of small mature B-cells
  - Most common leukemia diagnosed in adults
  - Median age at diagnosis is 70 y.o.
    - ~11% are < 55 y.o. and ~2% are < 45 y.o.
  - 5 year survival rate: 84.2%
- Clinical course
  - Varies from indolent and slowly progressive to patients having a more progressive course that requires aggressive treatment
- Prognostic markers
  - Clinical staging systems, serum markers, immunophenotypic markers, and genetic anomalies have been used in predicting outcomes
- Therapies for progressive disease in CLL are expanding with improvements in overall survival

MAYO CLINIC LABORATORIES

CLL Prognostic Markers: Flow-Based

- CD38 and ZAP70: When positive, both are associated with poor outcomes. But both have not stood up as independent markers.
- Discordance with IGHV mutation status occurs in patients with either stable disease as well as with progressive disease.

Conclusion:

- CD38 and ZAP70: Cannot be used as a replacement for IGHV mutation status in determining prognosis and in subsequent treatment decisions
- CD49d: When positive, it is associated with an unfavorable prognosis and shown to be the strongest flow cytometry-based predictor of overall survival and treatment-free survival.

MAYO CLINIC LABORATORIES

CLL Prognostic Markers: FISH

- FISH is not diagnostic of CLL as the following anomalies can be found in other B-cell lymphoproliferative disorders
  - **13q-**: Associated with good prognosis
  - **Trisomy 12**: Associated with intermediate prognosis
  - **11q-**: Associated with poor prognosis
  - **17p- (p53)**: Associated with very poor prognosis;
    - Incidence at diagnosis 5-10%;
    - Incidence following Fludarabine treatment 30-40%

MAYO CLINIC LABORATORIES

CLL Prognostic Markers: IGHV

- Unmutated IGHV
  - Defined as <2% difference from the germline VH gene sequence identity
  - Poorer prognosis: Mean overall survival of 95 months
  - 40% of CLL cases are unmutated IGHV
- Mutated IGHV HV-M
  - Defined as ≥2% difference from the germline VH gene sequence identity
  - Good prognosis: Mean overall survival of 293 months
- IGHV status is now being used to help with treatment decisions and identifies patients who may benefit from BTK inhibitors, such as Ibrutinib

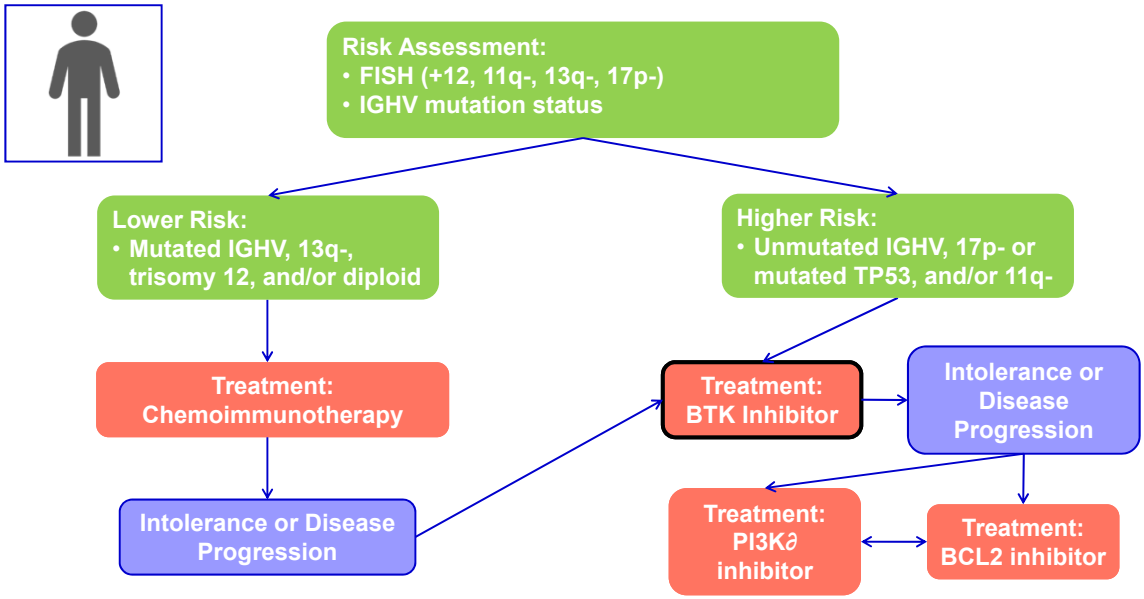
MAYO CLINIC LABORATORIES

CLL Prognostic Markers: TP53

- TP53 point mutations are related, but separate from FISH for 17p-
  - Incidence at diagnosis: 5-10%; incidence s/p Fludarabine treatment: 30-40%
- del(17p) and/or TP53 mutations are associated with poor outcomes and may be relatively resistant to standard chemotherapy / chemoimmunotherapy regimens
- Patients who have 17p- and/or TP53 mutation fare better when treated with small molecule inhibitors of BTK, phosphatidylinositol 3-kinase, or BCL2
- Thus, assessment of both 17p- by FISH and TP53 by sequencing has prognostic value and may help guide therapeutic decisions in routine practice

MAYO CLINIC LABORATORIES

Typical Application of "Risk" in a CLL Patient



International Prognostic Index (CLL-IPI): Study

Study Design	Data
Analysis	3472 treatment naïve patients from 5 countries
External Validation	838 patients from Mayo Clinic
Simple Composite Score: 5 Parameters	17p- FISH, IGHV, Serum B2M, Clinical Stage, Age
4 Risk Groups	Low, intermediate, high, very high
Clinical Application	Improved clinical staging; useful for testing novel therapeutics in high or very high risk groups

MAYO CLINIC LABORATORIES

International CLL-IPI working group: An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. Lancet Oncol. 2016 Jun;17(6):779-790.

IGHV and TP53 Recommended by IWCLL

Diagnostic Lab Tests	Recommendation
At Diagnosis	
CBC and Diff	Always
Blood flow study	Always
Before Treatment	
Bone marrow study	When clinically indicated
Serum chemistries, immunoglobulins, DAT	Always
CLL FISH for 13q-, 11q-,17p-, +12	Always
Chromosome karyotype	Not generally indicated
TP53 mutation	**Always**
IGHV mutation status	**Always**
Serum B2-microglobulin	Desirable

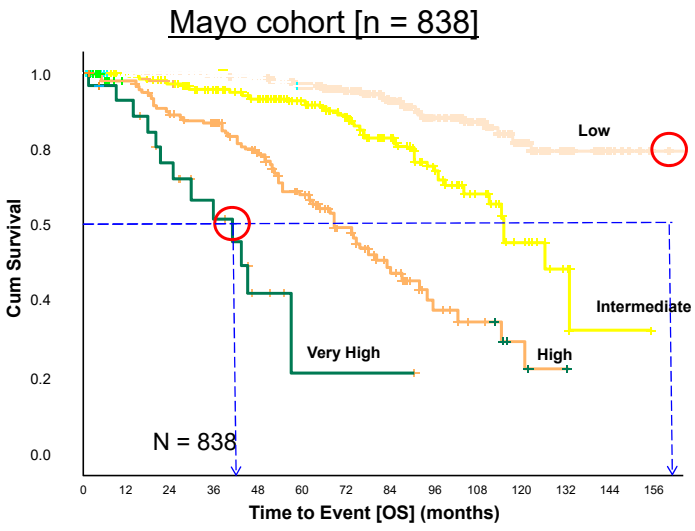
MAYO CLINIC LABORATORIES

CLL-IPI Summary

Variable	Adverse Factor	Grading
TP53 (17p-)	Deleted and/or mutated	4
IGHV	Unmutated	2
Serum B2M (mg/L)	> 3.5	2
Clinical stage	Binet B/C or Rai I-IV	1
Age	> 65	1
Prognostic score		0 – 10

MAYO CLINIC LABORATORIES

CLL-IPI and Overall Survival



MAYO CLINIC LABORATORIES

Kutsch N, Bahlo J, Byrd J, et al: The international prognostic index for patients with CLL (CLL-IPI): an international meta-analysis. J Clin Oncol 2015 33:15, 7002 (abstract)

Simplistic Application of CLL-IPI

Risk Group	General Treatment Direction?
Low	**"Do not touch"**
Intermediate	**"Do not treat"** (Except symptomatic)
High	**"Treat"** (Except asymptomatic)
Very High	**"Consider treatment in experimental protocol or with non-cytotoxic drugs if possible"**

MAYO CLINIC LABORATORIES

Summary

- CLL knowledge has rapidly grown with molecular technologies
- It is critical that the right tests get ordered in patients with CLL
- The CLL-IPI is a relatively simple yet elegant way to stratify patients into distinct prognostic groups that can drive particular therapies
- CLL FISH testing for prognosis is well-established and understood
- IGHV mutation analysis provides critical prognostic information in CLL and also can help inform clinicians on treatment decisions
- Mutations identified by TP53 sequencing are associated with poor outcomes and may be resistant to standard chemotherapy and chemoimmunotherapy regimens

MAYO CLINIC LABORATORIES

Thank You!

MAYO CLINIC LABORATORIES

