Molecular Markers in Transplantable Disease

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Financial Disclosures – None

Learning Objectives

- Review the types of testing for hematologic malignancies
- Increase familiarity with certain disease specific molecular tests
- Summarize clinical trials assessing molecular markers

Molecular markers in transplantable disease - the emerging promise of the personalized medicine revolution….

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Objectives

- Review the types of testing for hematologic malignancies
- Describe the rationale for molecular testing
- Increase familiarity with certain disease specific molecular tests
- AML disease stratification

Testing for Heme Malignancies

- Histology/ Morphology
  - What the cells look like
- Immunohistochemistry (IHC)
  - Staining the cells to identify specific markers
- Flow cytometry
  - Looks at individual cells based on staining for specific markers
- Cytogenetics
  - Chromosome analysis
- FISH
  - Targeting specific chromosomes
- Molecular studies
  - Identifying abnormal gene products

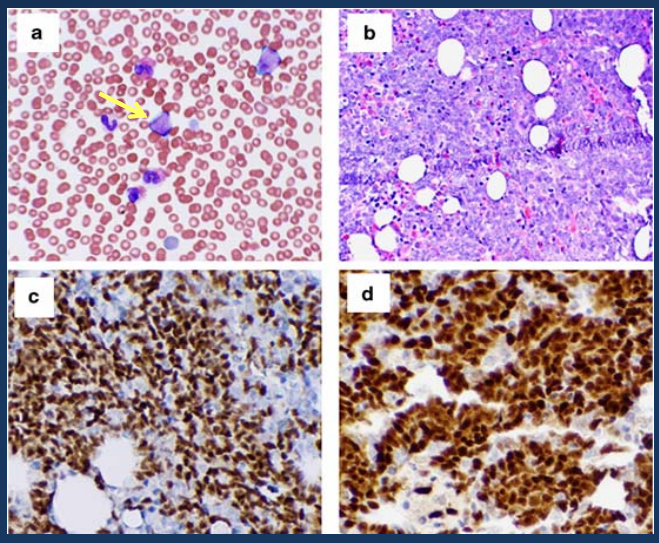
Least sensitive

Most sensitive

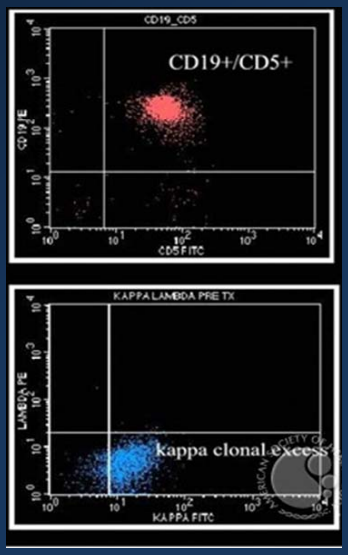
Morphology and IHC

ALL with blasts in the peripheral blood (a) and marrow (b).

IHC documents the blasts are positive for TdT (c) and PAX-5 (d)



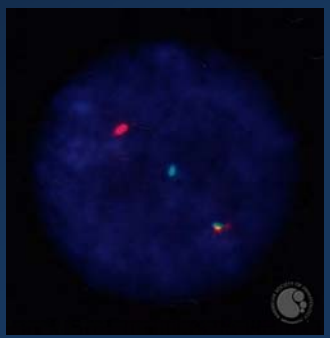
Flow and Cyto



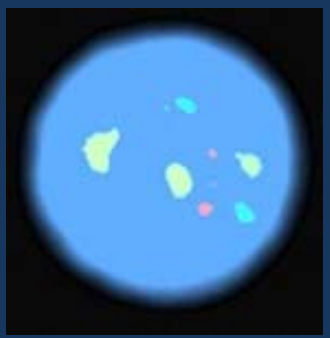
Clonal population of B- cells expressing CD19 and CD5 and kappa restriction

Conventional cytogenetics showing monosomy 7 and t(8;13)(q24.3;q14)

FISH



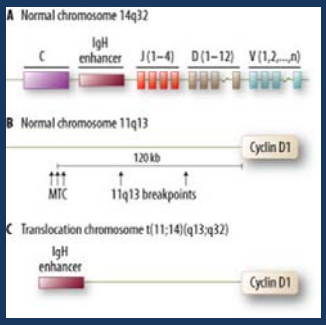
Red signal: ABL gene on a normal chromosome 9
Green signal: BCR on a normal chromosome 22
Yellow (combined): BCR/ABL fusion on the Philadelphia chromosome t(9;22)



Yellow signal: Trisomy 12 in a patient with CLL

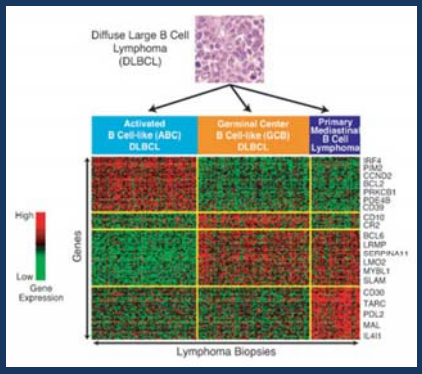
Polymerase Chain Reaction

- Method to rapidly and highly specifically amplify DNA fragments
- Advantages
  - Common, fairly inexpensive
  - Rapid, sensitive and specific
- Disadvantages
  - Requires knowledge of the specific nucleotide sequence
  - Sensitivity may result in false-positive results



Other Techniques

- Gene Expression Profiling
  - Microarray technology to identify a molecular signature of a tumor
- Proteomics
  - Microarray technology to identify protein expression profiles of tissue/cell type



Sensitivity and Specificity

- Sensitivity
  - The ability to detect one malignant cell in many normal cells (the needle in the haystack)
- Specificity
  - The likelihood that the test can discriminate between malignant and normal cells

Maximum Sensitivity

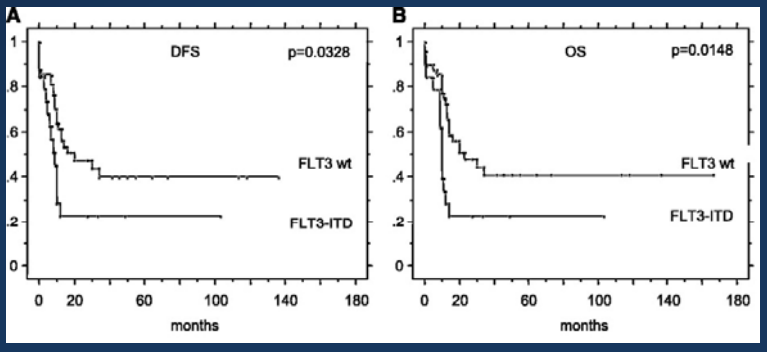
Technique	# of blasts required / 100,000 cells to detect disease
Microscopy Standard Expert	 5000 blasts 1000 blasts
Karyotype analysis	5000 blasts
Flow Cytometry	10 blasts
Polymerase Chain Reaction (PCR)	0.1 blasts

Purpose of Molecular Tests

- Diagnostic accuracy
- Prognostic markers to predict outcomes
- Monitor for minimal residual disease

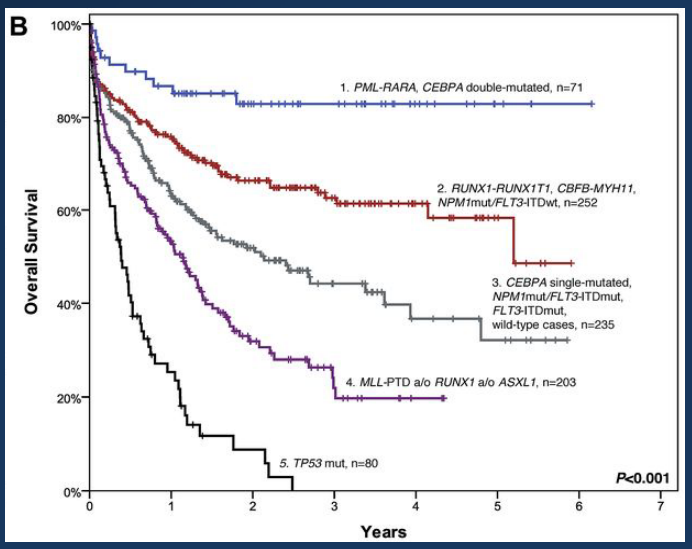
Prognostication

- Normal karyotype AML with or without Flt3-ITD mutation



Bienz M et al. Clin Cancer Res 2005;11:1416-1424

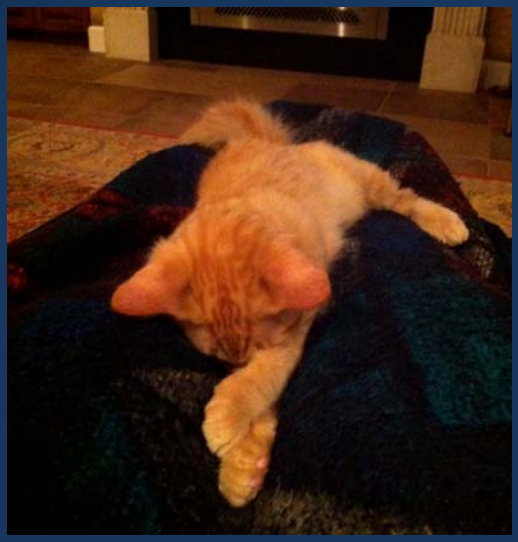
AML Model based on molecular mutations



Grossmann V et al. Blood 2012;120:2963-2972

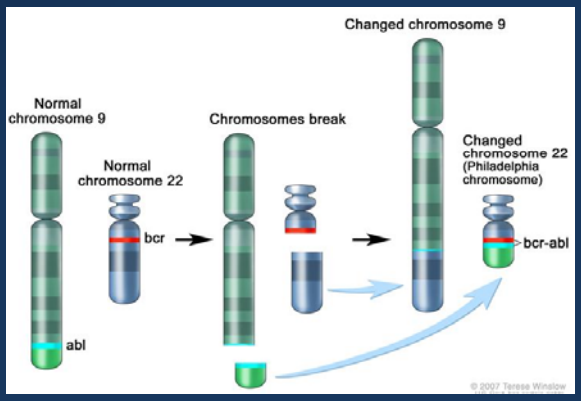
For those still awake…..

It's time to bury your head!



BCR / Abl

- Fusion protein that results in increased activity of a tyrosine kinase
- Present in CML, ALL (30 -35% adult B-cell), and some AML



- Can be followed quantitatively with a Major Molecular Response (MMR) determined as ≤ 0.1% BCR-ABL (ratio of BCR-ABL/BCR)

IgH and T-cell Receptor Gene Rearrangements

- Diverse gene product to allow for wide immunity
- Mutations result in clonal population
- May have false positives due to recovery post-transplant or ongoing infection

CEBP-α

- On chromosome 19q
- Normal function: Transcription factor for maturation of granulocytes
- Mutated in 15 – 20% of patients with AML
- Improved outcomes for patients with this mutation, independent of other mutations

Flt3

- Chromosome 13q
- Normal function: tyrosine kinase that is important for proliferation and differentiation of hematopoietic progenitor cells
- Mutated in 30 – 40% of AML patients
  - ITD, D835 point mutation, overexpression without mutation
- Uncontrolled proliferation leads to inferior overall and disease-free survival

NPM1

- On chromosome 5q
- Normal function: controls genomic stability
- Mutation in 50 – 60% AML
  - Either insertion or deletion
  - Increased in women
- Sole mutation present, improved outcomes
  - Outweighed by other negative mutations like FLT3

MLL

- On chromosome 11q
- Normal function: encodes enzyme that regulates homeostasis
- Mutation in 7 – 8% of AML patients as a partial tandem duplication
- Decreases overall survival

IDH1 and IDH2

- IDH1 on Chromosome 2q
- IDH2 on Chromosome 15q
- Normal function: critical to the Krebs cycle
- Mutations in 15 – 30% AML patients
- Results in increased expansion of HSCs and impaired differentiation

BCL-1 (CCND1)

- On chromosome 11q
- Normal function: cell cycle regulation
- In Mantle cell lymphoma t (11;14)
  - Moved upstream of IgH gene (chromosome 14)
- Mutation leads to dysregulated cell cycle and proliferation

BCL-2

- On chromosome 18q
- Normal function: inhibit apoptosis and modulates cell cycle progression
- In Burkitt's lymphoma, moves upstream of IgH t(14;18)
- Overexpression leads to prolonged cell survival

BCL-6

- On chromosome 3q
- Normal function: represses transcription
- Often overexpressed in DLCL
- Mutation leads to increased proliferation

TP53

- On chromosome 17p
- Tumor suppressor that prevents uncontrolled cell growth
- Mutation of 17p found in many cancers
  - CLL, DLCL, solid cancers

CIBMTR Disease Forms

Info on molecular testing now being collected

- AML: CEBP-α, FLT3-D835 point mutation, FLT3-ITD mutation, IDH1, IDH2, NPM1, MLL
- ALL: BCR/ABL, TEL-AML/AML1
- MDS: ASXL1, JAK2, ETV6, EZH2, P53, RUNX1
- Lymphoma: BCL-1 (CCND1), BCL-2, BCL-6, IgH, TCR

BMT CTN 1202

- Biomarker protocol
- Obtain samples to correlate molecular signatures with clinical outcomes of transplant
  - DNA, RNA, and Protein
- Data collection for post-transplant complications
  - Acute GVHD, chronic GVHD, lung injury, TMA, VOD, serious infections, relapse, death

Summary

- Molecular testing is a powerful tool
  - Guide treatment decisions
  - Can monitor for low levels of disease
- Constantly evolving field with new discoveries
- Impact of various markers requires large populations of patients to determine true importance