Molecular Pathology of Lymphoma: Focus on B-cell lymphomas

XXIV International Academy of Pathology – Arab Division (IAPAD)
Update on Molecular Pathology
Khartoum, Sudan – December 7, 2012

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Application of molecular studies to diagnosis of hematopoietic neoplasms
Application of molecular studies to diagnosis of hematopoietic neoplasms
what
Molecular targets
Molecular targets

- Rearrangements
  - physiologic
  - pathologic
Molecular targets

- **Rearrangements**
  - physiologic
  - pathologic

- **Somatic recombination (V-D-J joining)**, addition of N and P nucleotides, transcription and RNA processing in three B cell clones

- **creation of a novel chimeric gene**
  - upregulated/overexpression of a protooncogene

- **t(9;22) ⇒ bcr-abl**
- **t(8;14) ⇒ IgH + c-myc**

**what**

**Rearrangements**
- **physiologic**
- **pathologic**

**Molecular targets**

**what**

**Rearrangements**
- **physiologic**
- **pathologic**

**Molecular targets**

**what**

**Rearrangements**
- **physiologic**
- **pathologic**

**Molecular targets**

**what**

**Rearrangements**
- **physiologic**
- **pathologic**

**Molecular targets**
Molecular targets

- Rearrangements
  - physiologic
  - pathologic
A creation of a novel chimeric gene upregulated/overexpression of a protooncogene

t(9;22) → bcr-abl
t(8;14) → IgH + c-myc

Molecular targets

• Rearrangements
  - physiologic
  - pathologic

• Mutations

Rearrangements - physiologic
- pathologic

Mutations

creation of a novel chimeric gene

upregulated/overexpression of a protooncogene

V1 V2 Vn D1-n J1-n C

Somatic recombination (V-D-J joining), addition of N and P nucleotides, transcription and RNA processing in three B cell clones

V1 D1 J1 C V2 D3 J5 C Vn D2 J2 C

N/P nucleotides N/P nucleotides N/P nucleotides

homogeneity vs heterogeneity

present vs absent

qualitative
quantitative

t(9;22) ⇒ bcr-abl
t(8;14) ⇒ IgH + c-myc
**Molecular targets**

- **Rearrangements**
  - physiologic
  - pathologic

- **Mutations**

- **Additions**

- **Losses**
  - deletions
  - silencing

**what**

- **Rearrangements**
  - physiologic
  - pathologic

- **Mutations**

- **Additions**

- **Losses**
  - deletions
  - silencing

**Creation of a novel chimeric gene**

**Upregulated/overexpression of a protooncogene**

**t(9;22) → bcr-abl**

**t(8;14) → IgH + c-myc**
Major methodologies

Cytogenetics

Molecular genetics

FISH  CGH  SKY
Major methodologies

Cytogenetics

- FISH
- CGH
- SKY

Molecular genetics

SB
Major methodologies

Cytogenetics

- FISH
- CGH
- SKY

Molecular genetics

- SB
- PCR
Major methodologies

Cytogenetics

FISH, CGH, SKY

Molecular genetics

SB, PCR, ?CHIP
Major methodologies

Cytogenetics

FISH  CGH  SKY

Molecular genetics

SB  PCR  ?CHIP  NGS!
## Advantages of molecular methods

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<thead>
<tr>
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<th>Karyotypic</th>
<th>Molecular</th>
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<tr>
<td>Fresh material</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Viable cells</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Dividing cells</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Average TAT</td>
<td>~1 week</td>
<td>~2 days</td>
</tr>
<tr>
<td>Submicroscopic</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>5-10%</td>
<td>0.001-1%</td>
</tr>
<tr>
<td>Numeric</td>
<td>Yes</td>
<td>No*</td>
</tr>
</tbody>
</table>

* CGH → yes
why
why
Major indications for molecular testing
Major indications for molecular testing

why
Antigen receptors

B-cell

T-cell

Immunoglobulin

T-cell receptor

Antigen
IGH@ gene rearrangement and PCR

V<sub>H</sub> segments

D<sub>H</sub> segments

J<sub>H</sub> segments

C<sub>H</sub> segments
1. DJ rearrangement

**IGH@ gene rearrangement and PCR**
IGH@ gene rearrangement and PCR

1. DJ rearrangement
**IGH@ gene rearrangement and PCR**

1. **DJ rearrangement**

   - **V<sub>H</sub> segments:** 1 2 3 4 5 45
   - **D<sub>H</sub> segments:** 1 2 3
   - **J<sub>H</sub> segments:** 5 6
   - **C<sub>H</sub> segments:** μδγαε
**IGH@ gene rearrangement and PCR**

1. **DJ rearrangement**

2. **V-DJ rearrangement**
**IGH@ gene rearrangement and PCR**

1. **DJ rearrangement**

   - **V<sub>H</sub> segments**
     - 1 2 3 4 5 45
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   - **C<sub>H</sub> segments**
     - μδγαε

2. **V-DJ rearrangement**

   - 1 2
   - 3 5 6
   - μδγαε
**IGH@ gene rearrangement and PCR**

1. DJ rearrangement

2. V-DJ rearrangement
IGH@ gene rearrangement and PCR

1. DJ rearrangement
2. V-DJ rearrangement
3. High power view
IGH@ gene rearrangement and PCR

1. DJ rearrangement

2. V-DJ rearrangement

3. High power view

VH segments

1 2 3 4 5 45

DH segments

1 2 3 5 6

JH segments

5 6

CH segments

μδγαε

VHsegments

1 2 3 4 5

DHSegments

1 2 3

JHsegments

5 6

CHsegments

μδγαε

IGH gene rearrangement and PCR

CDRs, FRs

L FR I CDR I FR II CDR II FR III CDR III FR IV
**IGH@ gene rearrangement and PCR**

1. **DJ rearrangement**
   - V\(_H\) segments
     - 1 2 3 4 5 45
   - D\(_H\) segments
     - 1 2 3
   - J\(_H\) segments
     - 5 6
   - C\(_H\) segments
     - μ\(\delta\)αε

2. **V-DJ rearrangement**
   - μ\(\delta\)αε

3. **High power view**
   - CDRs, FRs and primers

---

**V\(_H\)2**

- L FR I CDR I FR II CDR II FR III CDR III FR IV
IGH@ gene rearrangement and PCR

1. DJ rearrangement

2. V-DJ rearrangement

3. High power view

VH segments | DH segments | JH segments | CH segments
---|---|---|---
1 2 3 4 5 45 | 1 2 3 | 5 6 | μδγαε

1. DJ rearrangement

2. V-DJ rearrangement

3. High power view

CDRs, FRs and primers
IGH@ gene rearrangement and PCR

gel-based PCR product detection

<table>
<thead>
<tr>
<th>Size</th>
<th>Poly</th>
<th>Mono</th>
<th>Mono</th>
<th>Mono</th>
<th>Poly</th>
<th>Neg</th>
</tr>
</thead>
</table>

[Image of a gel with bands indicating PCR products]
IGH@ gene rearrangement and PCR

capillary electrophoresis-based PCR product detection

reactive

neoplastic
Antigen receptor gene rearrangements

Useful in the following situations:

• atypical lymphoproliferations
• limited tissue
• equivocal immunophenotype (??)
• T-cell lymphoproliferations
• baseline for MRD

BM:
• precursor B-cells

But not that helpful in:
• diagnosing specific entities
• unraveling the heterogeneity
Specific lymphoma categories ...
Follicular lymphoma: t(14;18) and BCL-2
Follicular lymphoma: t(14;18) and BCL-2

- only $\sim 85\%$ t(14;18)+
Follicular lymphoma: t(14;18) and BCL-2

- only \(~85\%\) t(14;18)+
- not all t(14;18)+ cases = FL
Follicular lymphoma: t(14;18) and BCL-2

- only ~85% t(14;18)+
- not all t(14;18)+ cases = FL

- FL vs RFH (IHC ✓)
- FL vs other SBCL (IHC ×)
Follicular lymphoma: t(14;18) and BCL-2

- only ~85% t(14;18)+
- not all t(14;18)+ cases = FL

- FL vs RFH (IHC ✔)
- FL vs other SBCL (IHC ✗)

- rare cases:
  - t(14;18)+; IHC-
  - point mutation
Follicular lymphoma: t(14;18) and BCL-2

- only ~85% t(14;18)+
- not all t(14;18)+ cases = FL
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- t(14;18) PCR preferable to IGH PCR
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• rare cases:
  - t(14;18)+; IHC-
  - point mutation

• t(14;18) PCR preferable to IGH PCR

• of the ~15% t(14;18)-negative cases:
  
  ↑ copies chromosome 18/BCL-2 → BCL-2+
  
  t(BCL-6) → BCL-2- (MUM1+, grade 3)
Follicular lymphoma: t(14;18) and BCL-2

- only ~85% t(14;18)+
- not all t(14;18)+ cases = FL
- FL vs RFH (IHC ✔)
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  - copies chromosome 18/BCL-2 → BCL-2+
  - t(BCL-6) → BCL-2- (MUM1+, grade 3)

t(14;18)-negative FL:
- skin, testicle, pediatric, grade 3B,
- diffuse inguinal (del1p), blastoid
BCL2 gene rearrangements

BCL2 on 18q21

IGH on 14q32

1

2

3

J

J_H
BCL2 gene rearrangements

BCL2 on 18q21

IGH on 14q32
BCL2 gene rearrangements

BCL2 on 18q21

1

2

3

IGH on 14q32

J_H

MBR

MCR

PCR [~75%]
BCL2 gene rearrangements

BCL2 on 18q21

IGH on 14q32

VCR

MBR

ICR

MCR

PCR (~75%)
BCL2 gene rearrangements

BCL2 on 18q21

1

VCR

2

MBR

3

ICR

MCR

IGH on 14q32

J_H

PCR [~75%]

Southern blot
BCL2 gene rearrangements

BCL2 on 18q21

IGH on 14q32

VCR

MBR

ICR

MCR

PCR [~75%]

Southern blot

FISH
BCL2 gene rearrangements

BCL2 on 18q21

1

2

3

VCR

MBR

ICR

MCR

IGH on 14q32

J_H

PCR [~75%]

Southern blot

FISH

Classical cytogenetics
Chronic lymphocytic leukemia/SLL
CLL: Unraveling the heterogeneity

Conventional parameters

Novel parameters
CLL: Unraveling the heterogeneity

Novel parameters

- Cytogenetics
- Cell of origin
Cytogenetics
Cytogenetics

del(13q14) ~55%
del(11q22) ~18%
+12 ~16%
del(17p13) ~7%
Cytogenetics: molecular

- del(13q14)
- del(11q22)
- +12
- del(17p13)

Approximate frequencies:
- del(11q22): ~18%
- +12: ~16%
- del(17p13): ~7%
Cytogenetics: molecular

- del(13q14)
- del(11q22)
- +12
- del(17p13)
- ATM
  - ~16%
- ?miRNA
  - ~7%
Cytogenetics: molecular

del(13q14)  
?miRNA

del(11q22)  
ATM

+12  
? CDK4

del(17p13)  
~7%
Cytogenetics: molecular

- del(13q14)
- del(11q22)
- +12
- del(17p13)
- ATM
- p53
- ?CDK4
- ?miRNA
Prognostication by cytogenetics

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Prognostication by cytogenetics

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Prognostication by cytogenetics


del(11q)  SF3B1  trisomy 12  NOTCH1
Cell of origin

The dogma

CLL is a neoplasm of naïve B-cells ...
Cell of origin

The dogma

CLL is a neoplasm of naïve B-cells ...

... but a KARma has run over this DOGma
Chronic lymphocytic leukemia

* pre-GC
  - non-mutated IgH
  - “naïve”
  - poorer prognosis

♦ post-GC
  - mutated IgH
  - memory
  - better prognosis
Impact of SHM on prognosis

Chronic lymphocytic leukemia

* pre-GC
  - non-mutated IgH
  - “naïve”
  - poorer prognosis
  - (CD38+)

* post-GC
  - mutated IgH
  - memory
  - better prognosis
  - (CD38-)
Chronic lymphocytic leukemia

*pre-GC*
- non-mutated IgH
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↑ZAP70
Chronic lymphocytic leukemia

- **pre-GC**
  - non-mutated IgH
  - “naïve”
  - poorer prognosis

- **post-GC**
  - mutated IgH
  - memory
  - better prognosis

↑ ZAP70

?↓ miR15/16
ZAP70 by IHC on PB

H&E  |  CD79a  |  CD3
ZAP70 by IHC on PB
<table>
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<th>H&amp;E</th>
<th>CD79a</th>
<th>CD3</th>
<th>ZAP70</th>
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ZAP70 by IHC on PB

SHM-  

SHM+
Diffuse Large B-cell Lymphoma (DLBCL)

- **Morphologic:**
  - centroblastic
  - immunoblastic
  - T-cell/histiocyte-rich
  - anaplastic
  - plasmablastic
  - lymphomatoid granulomatosis type

- **Clinicopathologic:**
  - primary mediastinal (thymic) large B-cell lymphoma
  - primary CNS lymphoma
  - primary effusion lymphoma
  - primary cutaneous large B-cell lymphoma (of the leg!)
  - intravascular large cell lymphoma
Separation of DLBLs into two broad groups:
- germinal center
- activated B-cell
That’s all very nice and impressive, but ...
That’s all very nice and impressive, but ...

- Highly complex (10,000’s of genes)
- Expensive
- Need fresh/frozen tissue
That’s all very nice and impressive, but ...

- Highly complex (10,000’s of genes)
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- Need fresh/frozen tissue

So, what’s a humble, information-overloaded pathologist to do?
That’s all very nice and impressive, but ...

- Highly complex (10,000’s of genes)
- Expensive
- Need fresh/frozen tissue

So, what’s a humble, information-overloaded pathologist to do?

- Wait ...
  ... for the dust to settle ...
  ... and it might be ...
  ... and use IHC ... (and only 3 markers at that!)
CD10

GCB

BCL6

non-GCB
CD10

GCB

non-GCB

BCL6

MUM1
CD10

GCB

BCL2

non-GCB

FOXP1

BCL6

MUM1

GCET1
Pitfalls and caveats: IG and TCR PCR

“False” positives
“False” positives
- contamination
Pitfalls and caveats: IG and TCR PCR

“False” positives

- contamination
- pseudoclonality (small biopsies)
Pitfalls and caveats: IG and TCR PCR

“False” positives
- contamination
- pseudoclonality (small biopsies)
- reactive/inflammatory scenarios
  - H. pylori gastritis (but ...)
  - Hepatitis C (but ...)
  - Viral infections (HIV, mumps, EBV, CMV)
  - Sjögren syndrome
  - Rheumatoid arthritis
Pitfalls and caveats: IG and TCR PCR

“False” positives

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  - Sjögren syndrome
  - Rheumatoid arthritis
- canonical (TCR_{\gamma})
“False” positives

- contamination
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  - Hepatitis C (but ...)
  - Viral infections (HIV, mumps, EBV, CMV)
  - Sjögren syndrome
  - Rheumatoid arthritis
- canonical (TCR$\gamma$)
- immune reconstitution post BMT
- immune response to tumor
Pitfalls and caveats: IG and TCR PCR

“False” positives
- contamination
- pseudoclonality (small biopsies)
- reactive/inflammatory scenarios
  - H. pylori gastritis (but ...)
  - Hepatitis C (but ...)
  - Viral infections (HIV, mumps, EBV, CMV)
  - Sjögren syndrome
  - Rheumatoid arthritis
- canonical (TCRγ)
- immune reconstitution post BMT
- immune response to tumor
- “clonal dermatitis”
Pitfalls and caveats: IG and TCR PCR

False negatives
Pitfalls and caveats: IG and TCR PCR

**False negatives**

- Preanalytic variables
- Technical
- Biologic
Pitfalls and caveats: IG and TCR PCR

False negatives

- Preanalytic variables [degradation, fixation, representative sampling]
- Technical
- Biologic

- Somatic hypermutation (primary/ongoing)
  - (follicular lymphoma, myeloma)
- IgH deletions (~1/10 lymphomas)

- Ongoing rearrangements at relapse
- False negatives

- IG and TCR PCR
Pitfalls and caveats: IG and TCR PCR

False negatives

- Preanalytic variables [degradation, fixation, representative sampling]
- Technical
  - consensus primers
    - using CDR3 IGH primers only
- Biologic
False negatives

- Preanalytic variables [degradation, fixation, representative sampling]
- Technical
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  - using CDR3 IGH primers only
- Biologic
  - pre GC/
  - precursor B-cells
  - intra/post GC

Pitfalls and caveats: IG and TCR PCR
False negatives

- Preanalytic variables [degradation, fixation, representative sampling]
- Technical
  - consensus primers
  - using CDR3 IGH primers only
- Biologic
  - pre GC/
  - precursor B-cells
  - intra/post GC
  - partial DJ
  - oligoclonal
    (~1/3 precursor B-ALL)
  - ongoing rearrangements at relapse

Pitfalls and caveats: IG and TCR PCR
False negatives

- Preanalytic variables [degradation, fixation, representative sampling]
- Technical
  
  consensus primers
  using CDR3 IGH primers only
- Biologic
  
  pre GC/
  precursor B-cells
  
  • partial DJ
  • oligoclonal
    (~1/3 precursor B-ALL)
  • ongoing rearrangements at relapse
  
  intra/post GC
  
  • somatic hypermutation
    (primary/ongoing)
    (follicular lymphoma, myeloma)
  • IGH deletions
    (~1/10 lymphomas)
The last slide ...

- Powerful ... but one piece of the puzzle
The last slide ...

- Powerful ... but one piece of the puzzle

- Positive result: not always = neoplastic
The last slide ...

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- Positive result: not always = neoplastic
- Negative result: not always = not neoplastic
The last slide ...

- Powerful ... but one piece of the puzzle
- Positive result: not always = neoplastic
- Negative result: not always = not neoplastic
- Integrate: with morphologic, immunophenotypic, clinical data
The last slide ...

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- Positive result: not always = neoplastic
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- Integrate: with morphologic, immunophenotypic, clinical data
- Decision to perform/ability to interpret: contextual
The last slide ...

- Powerful ... but one piece of the puzzle
- Positive result: not always = neoplastic
- Negative result: not always = not neoplastic
- Integrate: with morphologic, immunophenotypic, clinical data
- Decision to perform/ability to interpret: contextual
- More rational, biologically-based diagnosis: more appropriate, targeted Rx