What is the cellular pathologist’s role in molecular diagnostics for lymphoma?

Professor Kikkeri Naresh
London
Molecular diagnostics

• Diagnostic tests – Benign or malignant

• Diagnostic tests – to assign a specific diagnosis within the current WHO classification system

• Biomarkers – predict disease behaviour, identify therapeutic targets, disease stratification, personalised medicine

• Rarely molecular monitoring of disease – response and early recurrence
Cellular pathologist’s role in molecular diagnostics for lymphoma
- Diagnostic markers

• Sample quality

• Choice and request of a molecular test

• Interaction with clinical/biomedical scientists

• Result interpretation, integration and clinical context
Sample quality

• Though fresh tissue is preferred, paraffin embedded tissue is more practical

• Optimal fixation across the entire specimen

• Fixation in buffered formalin

• Avoid over-fixation

• Adequate representation of the abnormal population in the sample
Request of a ‘diagnostic’ molecular test

• Should not be part of a general panel of investigations

• Should be requested by an expert haematopathologist following morphological and immunohistochemical / immunophenotypic work-up

• Under the current scenario <20% of the lymphoid lesions require a molecular test
Request of a ‘diagnostic’ molecular test

• A molecular test should only be requested when the result clearly impacts on final diagnosis

• Reactive lymphoid lesions: <10% show monoclonal rearrangements of IG/TCR genes, and ~15% show oligoclonal rearrangements of IG/TCR genes without an apparent explanation.

• Good quality light chain immunostains and application of flow cytometry reduces the requirement of IG gene rearrangement studies.
Kappa

Polytypic

Lambda
Kappa  Polytypic  Lambda
Kappa | Monotypic | Lambda
Kappa  Monotypic  Lambda
Choice of ‘diagnostic’ molecular tests

- FISH based tests investigating translocations (also provide information on copy number changes)

- Clonality tests based on clonal rearrangements of antigen receptor genes

- Mutation analysis

In lymphomas associated with specific chromosomal translocations, interphase-FISH is preferable over antigen receptor gene rearrangement analysis.
Gene targets for clonality analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>IGH</td>
<td>+++</td>
</tr>
<tr>
<td>IGK</td>
<td>+++</td>
</tr>
<tr>
<td>IGK del</td>
<td>+++</td>
</tr>
<tr>
<td>IGL</td>
<td>+</td>
</tr>
<tr>
<td>TCRG</td>
<td>+++</td>
</tr>
<tr>
<td>TCRB</td>
<td>++</td>
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</table>
## Antigen receptor gene rearrangement studies

<table>
<thead>
<tr>
<th>Histological pattern</th>
<th>Diagnostic suspicion</th>
<th>Test</th>
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<tbody>
<tr>
<td>Expansion of interfollicular T-cell areas</td>
<td>Early phase of angioimmunoblastic T-cell lymphoma</td>
<td>T-cell and B-cell clonality</td>
</tr>
<tr>
<td>Angioimmunoblastic T cell lymphoma with large B cells without</td>
<td>Clonal large B cell expansion or evolving DLBCL in the context of angioimmunoblastic</td>
<td>B-cell clonality</td>
</tr>
<tr>
<td>demonstrable light chain restriction</td>
<td>T-cell and B-cell lymphoma</td>
<td></td>
</tr>
<tr>
<td>Medium and large T-cell expansion inside B-cell follicles</td>
<td>Peripheral T-cell lymphoma NOS, follicular variant</td>
<td>T-cell clonality</td>
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<td>Paracortical expansion in a lymph node with mycosis fungoides</td>
<td>LN involvement by mycosis fungoides</td>
<td>T-cell clonality</td>
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<td>T cell infiltrates in skin suspicious but not diagnostic of</td>
<td>Mycosis fungoides and other cutaneous T cell lymphomas</td>
<td>T-cell clonality</td>
</tr>
<tr>
<td>lymphoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-density lymphoid infiltrates in HTLV1 positive patients</td>
<td>Adult T cell leukaemia/lymphoma</td>
<td>T-cell clonality</td>
</tr>
<tr>
<td>Coeliac disease with downregulation of CD8 and clinical</td>
<td>Refractory coeliac disease and Enteropathy associated T cell lymphoma in-situ</td>
<td>T-cell clonality</td>
</tr>
<tr>
<td>refractoriness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRS cells with background atypical T cells</td>
<td>Classical Hodgkin lymphoma vs. T cell lymphoma</td>
<td>T-cell clonality</td>
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</table>
60Y Male
Skin lesions, Lymphadenopathy & renal failure

Diagnosis
Peripheral T cell lymphoma, NOS; lymphoepithelioid var. (Lennert’s lymphoma)

Immunophenotype:
Positive: CD2, CD3, CD5, CD7, CD8
Negative: CD4, PD1, CD30 & B cell markers

TCRG rearrangements studies:
Identical clonal products from skin, LN and renal biopsies
60Y Male  
Lymphadenopathy & Splenomegaly  

Diagnosis  
Classical Hodgkin lymphoma  

Smaller lymphoid cells:  
- CD3+  
- CD2+  
- CD4+  
- PD1+  
- CD10 – occ.  

Larger lymphoid cells:  
- CD30+  
- CD15+  
- Pax5w  
- EBER-  
- CD20-
## Antigen receptor gene rearrangement studies

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<td>Marginal zone expansion in a lymph node, spleen, or an extranodal sample without demonstration of light chain restriction</td>
<td>Marginal zone lymphoma</td>
<td>B-cell clonality</td>
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<tr>
<td>Suspicion of mantle cell lymphoma but overfixed with negative cyclin D1 staining of internal positive control, and failed FISH</td>
<td>Mantle cell lymphoma</td>
<td>B-cell clonality</td>
</tr>
<tr>
<td>BCL2 negative follicles in a sample suspicious of follicular lymphoma, and with negative FISH results</td>
<td>Follicular lymphoma</td>
<td>B-cell clonality</td>
</tr>
<tr>
<td>Multicentric Castleman’s disease with a high density of HHV8+ cells in the mantle zone</td>
<td>‘Micro-lymphoma’</td>
<td>B-cell clonality</td>
</tr>
</tbody>
</table>

20Y Male  
Right groin LN  

**Diagnosis**  
Follicular lymphoma, gr. 1  

**IGH & IGK rearrangements studies:**  
Identical clonal products from needle core and excision biopsies
# Interphase FISH studies as ‘diagnostic’ tests

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| Marginal zone expansion in an extranodal sample without demonstration of light chain restriction | Marginal zone lymphoma                                                                | **MLT1**  
|                                                                                      |                                                                                       | **BCL10**                 |
| BCL2 negative follicles in a sample suspicious of follicular lymphoma                  | Follicular lymphoma                                                                    | **BCL2**  
|                                                                                      |                                                                                       | **BCL6**                  |
| Extensive follicular colonisation                                                      | Distinction of follicular lymphoma and marginal zone lymphoma with follicular colonisation | **BCL2**  
|                                                                                      |                                                                                       | **BCL6**                  |
| Suspicion of mantle cell lymphoma but overfixed with negative cyclin D1 staining of internal positive control | Mantle cell lymphoma                                                                  | **CCND1**                |
| Diagnosis of Burkitt lymphoma unresolved with morphology and immunohistochemistry     | Burkitt lymphoma or a ‘grey’ zone lymphoma / double-hit lymphoma                       | **MYC**  
|                                                                                      |                                                                                       | **BCL2**  
|                                                                                      |                                                                                       | **BCL6**                  |
|                                                                                      |                                                                                       | **IG**                   |
| Diffuse large B cell lymphoma with cyclin D1 expression                                | Distinction of DLBCL from Blastoid MCL                                                 | **CCND1**                |
| CD5+ small B cell lymphomas with features not characteristic of CLL, MCL or MZL       | CD5+ lymphoproliferative disorder associated with t(14;19) **BCL3-IGH**                 | **BCL3**                  |
60Y Male  
Rapid growth of left tonsil

Diagnosis  
Follicular lymphoma gr. 2-3a with marginal zone diff.

**Immunophenotype:**

Positive: CD20, CD79a, BCL6, BCL2, MUM1, IgM, IgD, CD38 & CD44

Negative: CD5, CD10, CD23  
Cyclin D1 ……

**FISH:**

Additional copies of BCL2 and BCL6; no rearrangement

No rearrangement of IGH
70Y Male
Splenomegaly & multiple left large axillary LNs

Diagnosis: DLBCL

Immunophenotype:

Positive: CD20, CD10, BCL6, BCL2, MUM1

Ki-67>90%

Negative: CD5, Cyclin D1, EBER, TdT

FISH:

Two copies of rearranged BCL6; No normal BCL6

No rearrangement of BCL2 or MYC
B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma

t(8;14)(q24;32); der(2)t(2;7)(p1?3;q?11.2); add(13)(q34)
Interphase FISH studies as ‘diagnostic’ tests

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<th>Morphology / immunophenotype</th>
<th>Diagnostic suspicion</th>
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<tr>
<td>Differential diagnosis of splenic marginal zone lymphoma, hairy cell leukaemia and other B cell lymphomas</td>
<td>Splenic marginal zone lymphoma</td>
<td>Del 7q31-32</td>
</tr>
<tr>
<td>CD4+ T cell lymphocytosis with cells having features of prolymphocytes</td>
<td>T-cell prolymphocytic leukaemia</td>
<td>t(14;14)(q11; q32)</td>
</tr>
<tr>
<td>Features of hepatosplenic T cell lymphoma</td>
<td>Hepatosplenic T cell lymphoma</td>
<td>iso7q</td>
</tr>
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Mutation analysis as ‘diagnostic’ tests

- *MYD88* mutation in lymphoplasmacytic lymphoma

- *BRAF* mutation in hairy cell leukaemia
Molecular tests – prognostic markers in current clinical practice

- *IGVH* mutation in CLL and other small B cell lymphomas
- *TP53* mutation
FISH tests – prognostic markers in current clinical practice

- TP53 deletion

- API2-MLT1 translocation in gastric MALT lymphoma

- CLL: 13q- (good prognosis)
  +12, 11q-, 17p- (poor prognosis)
Interaction with clinical/biomedical scientists – pre-analytical

- Mark the most involved area on the section for FISH analysis – saves reagents and time!

- Mention the content of B cells, T cells or presumed neoplastic cells for clonality tests – beware of pseudoclonality due to low-levels of specific template

- Mention the provisional histological diagnosis for clonality tests -
  - somatic hypermutation process can hamper primer binding and result in false negative test results
  - florid reactive process may show oligoclonality/monoclonality.

- Ideal for cellular pathologists involved in haematopathology and staff in involved in molecular pathology to be located in the same laboratory or work area
Interaction with clinical/biomedical scientists – post-analytical

• Get involved in fluorescent microscopy in cases posing difficulties in interpretation of FISH results – most cases are straightforward.

• Closer interaction with biomedical/clinical scientists is preferred for reporting of antigen receptor gene rearrangements.

• Involve biomedical/clinical scientists in integrated reporting.
False positive results commonly encountered with antigen receptor gene rearrangement studies

- Contamination
- Pseudoclonality (small biopsies)
- Reactive / inflammatory pathology: H.pylori gastritis; Hepatitis; viral infections; Sjögren's syndrome, Rheumatoid arthritis
- Canonical TCRγ
- Immune reconstitution following BMT
- Immune response to tumour
- Clonal lymphoid infiltrates in skin
False negative results commonly encountered with antigen receptor gene rearrangement studies

- Sample issues: representativeness, fixation issues, degradation of DNA

- Technical: Not using the complete panel of primers

- Precursor B cell expansions:
  - Partial DJ rearrangements
  - Oligoclonal (1/3 of B-ALL)
  - Ongoing rearrangements at relapse

- Germinal centre and post-germinal centre expansions:
  - Somatic hypermutations
  - IgH deletion
Molecular subtyping of DLBCL

Alizadeh AA. Nature. 2000 Feb 3;403(6769):503-11
DLBCL molecular subtypes

Immunohistochemistry based algorithms show concordance with GEP
All the algorithms tested showed significant difference in survival

Hans et al, 2004

Natkunam et al, 2008

LMO2 $\geq 30\% \rightarrow$ GCB
LMO2 $< 30\% \rightarrow$ ABC

Choi et al, 2009

Meyer et al, 2011

GCB
- CD10 (+ or -)
- GCET1 (+ or -)
- Score (0, 1, 2)

ABC
- Mum1 (+ or -)
- FoxP1 (+ or -)
- Score (0, 1, 2)

Score
- GCB $>$ ABC
- ABC $>$ GCB

If GCB Score = ABC Score:
- LMO2 $\geq 30\% \rightarrow$ GCB
- LMO2 $< 30\% \rightarrow$ ABC

DLBCL – Molecular subtypes
Alternate algorithms

DLBCL molecular subtypes
Comparison of impact of immunohistochemistry-based algorithms & GEP-based classification on overall survival
62 patients on immuno-chemotherapy

Misclassification of GEP-defined GCB by immunohistochemistry based algorithms: 30-60%
Impact of Bortezomib on molecular subsets of relapsed DLBCL

Dunleavy K et al. Blood. 2009 Jun 11;113(24):6069-76.
REMoDL-B study
Univ. of Southampton, UK
Hypothesis: Bortezomib improves survival in ABC-DLBCL subset
MYC translocation and protein expression in DLBCL

Horn et al, Blood. 2013;121(12):2253-2263
Genomic alterations in DLBCL

Somatically acquired genetic alterations
- Copy Number Alterations
- Intergenic Mutations
- Regulatory Region Mutations
- Coding Region Mutations

Zhang et al, PNAS 2012
Genomic alterations in DLBCL

Proportion of Cases by Subtype

ABC DLBCL
GCB DLBCL

Gene Ontology
- Apoptosis*
- Cell Adhesion*
- Cell Cycle*
- Cell Differentiation
- Metabolism*
- Chromatin Modification
- DNA Repair*
- Immune Response*
- Membrane Transport
- Protein Modification
- Signal Transduction*
- Ubiquitin Cycle

Zhang et al, PNAS 2012
Genomic alterations in Burkitt lymphoma

Love et al, Nature Genetics, 2012
Mutations in BL vs. DLBCL

Love et al, Nature Genetics, 2012
Lymphoma diagnosis and work-up

• Targeted NGS platforms for mutation based disease classification, prognostication/prediction and identification of drug-able targets.

• Immunohistochemistry based assays as surrogates for mutations?
Acknowledgement

- Dr. Rashpal Flora
- Dr. Raida Ahmad
- Dr. Hazem Ibrahim
- Mr. Pritesh Trivedi
- Ms. Donna Horncastle
- Dr. Elisabet Nadal
- Ms. Rachael Beattie
- Dr. Alistair Reid
- Ms. Philippa May
- Mr. Inigo Ortiz DeMendibil
- Prof. Letizia Foroni
- Dr. Mikel Valganon
- Dr. Natalie Killeen

Thanks: To all colleagues who refer difficult and challenging cases