The development of clonality testing for lymphomas in the Bristol Genetics Laboratory

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Introduction

- The majority of lymphoid malignancies belong to the B cell lineage (90-95%) with only 5-7% being T cells.

- According to van Dongen et al., (2003) the vast majority of lymphoid malignancies (>98%) contain identically (clonal) rearranged immunoglobulin (Ig) and/or T cell receptor (TCR) genes.

- Ig and TCR gene loci contain many different variable (V), diversity (D) and joining (J) regions. In Ig heavy chains (IGH), TCRB and TCRD there is an initial D-J rearrangement followed by a V to D-J rearrangement whereas there is only direct V-J rearrangements in IGK, IGL, TCRA and TCRG genes.
Introduction cont....

- During the rearrangements of V, D and J gene segments random deletions and insertions occur at the junction sites.

- This results in highly diverse junctional regions, which in turn, leads to an Ig and TCR repertoire of $\sim 10^{12}$. 
IGH gene rearrangement

Germline IgH locus

Partial DH-JH rearrangement

Full VH-DH-JH rearrangement

V genes (124)
D genes (27)
J genes (6)
Constant region

Random insertion and deletion of nucleotides

Germline IgH locus: VH1, VH2, VH3, VH4, VH5, VHn
D genes: DH1, DH2, DH3, DHn
J genes: JH1 - 6
Constant region: Cm, Cd, Cg, Ca

V, N, D, N, J

CDR3
In mature B cells, somatic hypermutation of the V(D)J exon of IGH and Ig light chain genes occurs. This causes single nucleotide mutations or insertions/deletions to occur. As such, mature B cell malignancies can show a mutated or unmutated gene profile.
Introduction – Origin of B lymphoid malignancies

Ig isotype switch with Ig mutations

Mantle cell lymphoma
Follicular Lymphoma
Plasma cell
Myeloma
Marginal Zone lymphoma
Lymphoplasmacytoid lymphoma
Diffuse Large cell lymphoma
B cell chronic lymphocytic leukaemia
Naive B cells
Bone Marrow

Germinal Follicle
Mantle Zone
Marginal Zone

Adapted from Hoffbrand et al., (2008)
Important points to remember when testing for clonality

- **Clonality does not always imply malignancy** – all results must be interpreted in the context of all of the other available diagnostic criteria.

- **Ig and TCR gene rearrangements are not markers for lineage** – Ig and TCR gene rearrangements are not necessarily restricted to B and T cell lineages, respectively. Cross lineage can occur ie a B cell malignancy can be positive for a TCR gene rearrangement and vice versa.

- **Limited sensitivity compared to polyclonal background** – clonality can only be reported if the clonal peak is 3x higher than the 3rd highest peak in the polyclonal background.
BGL and Clonality Testing

- In November 2009, BGL introduced clonality testing for lymphomas using the IdentiClone gene clonality assay kits from InVivoScribe Technologies, adopting the testing strategy laid out by Liu et al., (2007)

- The aim of the Liu et al., (2007) strategy was to “introduce an efficient stepwise process for the routine application of BIOMED-2 PCR assays to deliver a sensitive method for the detection of clonality using the fewest reactions possible for initial testing”.
BGL and Clonality Testing Cont…

DNA sample

DNA size ladder PCR if paraffin-embedded tissue

(If DNA > 300 bp)

B-cell proliferation

\[ IGH_B + IgkA+B \]
91% (58%)

\[ IGH_A+C+D \]
99% (79%)

\[ IGL + IGHE \]
100% (80%)

If not clonal

T-cell proliferation

\[ TCR_GA+B \]
94% (30%)

\[ TCR\alpha\beta^+ \]

\[ TCR\gamma\delta^+ \]

\[ TCR\gamma\delta^+ \]

\[ TCR\alpha\beta^+ \]

\[ TCR\alpha\beta^+ \]

\[ TCR\alpha\beta+C \]

\[ TCR\beta^+ \]

\[ TCR\beta^+ \]

\[ TCR\beta^+ \]

No evidence of presence of clonal B/T-cell population in the sample by PCR
Patient A

Clinical

- Sample type: Paraffin embedded tissue
- 60 year old female
- Referred with extensive mediastinal and abdominal lymphadenopathy. Diagnosis hypercalcaemia ?lymphoma
- Immunology: A small population of large B cells with the phenotype CD19, CD20 and CD79+, strong Kappa+, CD10, 5, 103-
- Cell Pathology Summary: Histology suggestive of diffuse large B cell lymphoma although check immunophenotype and genetic analysis by PCR requested for confirmation.
- Immunohistochemistry: Tumour cells +ve with CD20 and BCL2. +ve nuclear staining with BCL6, MUM1, PAX5 and p53
Patient A Clonality Testing

IGH Tube B VH(1-7) FR2-JH

Clonal peak identified at 271bp

Final Diagnosis: Diffuse Large B Cell Lymphoma
Patient B

Clinical

- Sample Type: Tumour
- Referred with ? Lymphoma. Lymphadenopathy left neck
- Very difficult lymph node to interpret histologically
- Immunohistochemistry: CD10, Cyclin D1 and bcl2 negative
- Working diagnosis: Marginal zone lymphoma with follicular colonisation.
Patient B Clonality Testing

IGH Tube B VH(1-7) FR2-JH

3rd largest peak in polyclonal background ~1000, clonal peak ~4000 therefore clonal peak over 3x the height of the polyclonal background and reported as weakly clonal in a polyclonal background

Final Diagnosis: Nodal marginal zone lymphoma
Patient C

Clinical

- Sample type: Bone Marrow
- No immunology or histology reports available
- Cytogenetics: 46,XY[50]. *In situ* hybridisation studies using Abbot/Vysis TCRA/D dual colour break-apart probe specific for the locus on chromosome 14q11 and an IGH dual colour break-apart probe specific for the locus on chromosome 14q32 showed a normal signal pattern in 150 interphase nuclei
Patient C Clonality Testing

TCRG (tube B) Vγ9Vγ11 – Jγ1.3/2.3 + Jγ1.1/2.1

3\textsuperscript{rd} largest peak in polyclonal background \sim 10000, clonal peak Vs \sim 32000 (in green) 
\sim 8000 polyclonal background peak Vs \sim 33000 polyclonal peak therefore clonal peak over 3x the height of the polyclonal background and reported as weakly clonal in a polyclonal background

Final Diagnosis: T cell lymphoblastic lymphoma with BM involvement
Patient D

Clinical

- Sample: PET
- Rt groin biopsy? Lymphoma – anaemic axillary, inguinal and para-aortic lymphadenopathy – Urgent
- Initial histology: reactive hyperplasia but sent to lymphoma expert for opinion who then sent a PET section to BGL for B and T cell clonality testing
- Concurrently, a peripheral blood sample was sent for JAK2 V617F testing – no mutation present.
- Immunology showed a 1-2% population of Lambda +ve cells
- Initial B and T cell screen on PET = no clonality identified = reactive rather than malignant
- Further testing, an initial B and T cell screen on peripheral blood showed........
Patient D Clonality Testing (PET and Peripheral blood)

TCRG (tube A) VγIf Vγ10 – Jγ1.3/2.3 + Jγ1.1/2.1

Negative for the detection of clonal TCRG chain rearrangements

3rd largest peak in polyclonal background ~25000, clonal peak ~75000 therefore clonal peak over 3x the height of the polyclonal background and reported as weakly clonal in a polyclonal background
What went wrong with the clonality testing?

- Nothing!!
- We reported that in the context of overall diagnostic criteria, clonal cell populations can indicate the presence of haematological malignancy.
- While monoclonality is a key feature of tumour cell populations it does not always imply malignancy because some reactive processes contain large clonal lymphocyte populations. Therefore we need to take into consideration the whole clinical picture rather than just the result of the clonality testing.
- The patients flow results showed a 1-2% population of malignant cells which were Lambda +ve, this is part of the extended panel which also includes other B cell markers (Framework 1 and 3 and the incomplete DH’s)
- However, its absence would not detract from the clonality already identified in the T cells
An extended B cell screen was initiated and was negative for the detection of IGH rearrangements.

A second core biopsy was taken. The histology showed HHV8 (human herpes virus-8) positive Kaposi’s sarcoma and HHV8 associated lymphoproliferative disorder. HIV negative patient, probably common variable immunodeficiency.

HHV8 positive plasma cells are monotypic but polyclonal. (On review first biopsy also HHV8+ but not Castleman’s Disease))

Patient now treated with Rituximab (anti-CD20 monoclonal antibody therapy) and responding well
References

- Van Dongen et al., Design and standardisation of PCR primers and protocols for detection of clonal immunoglobulin and T cell receptor gene recombinations in suspect lymphoproliferations. Leukemia, 2003. 17: 2257-2317


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