Antigen Receptor Gene Rearrangement Studies by PCR in Lymphoma Diagnosis

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Biomed-2 Concerted Action

- 1998-2002; major European collaboration to optimise PCR to detect immunoglobulin (heavy and light chains) and T-cell receptor gene rearrangements
- Primer design and protocol optimisation
- Use in FFPE tissue was one (small) workstream
- Provided an international gold standard of immense practical utility
Model of Antigen Receptor Gene Diversity Generation and PCR

V-region genes
D-region genes
N nucleotides
C-region genes
Immunoglobulin genes
Junctional diversity

primer
PCR
IGH Primers

- **FR1 \((V_H1-J_H)\):**
  - products 310-360 nt
- **FR2 \((V_H2-J_H)\)**
  - products 250-295 nt
- **FR3 \((V_H3-J_H)\)**
  - Products 100-170 nt
IGK and L Primers

- IGK (KA) $V_K$-$J_K$: 8 primer combinations
  - 120-160 nt
  - 190-210 nt
  - 260-300 nt

- IGK (KB) $V_K$-$K_{de}$: 8 primer combinations
  - 120-160 nt
  - 190-210 nt
  - 260-300 nt

- IGL $V_L$-$J_L$: 2 primer combinations
  - 140-165 nt
TCR-Beta Primers

• Two $V_B$-$J_B$ multiplexes:
  – BA (15 combinations) gives products 254-286 nt
  – (BB: 11 combinations) gives products 258-293 nt

• $D_B$-$J_B$ multiplex (run in 1 or 2 tubes)
  – BC1 (8 combinations)
    • 304-320 nt
  – BC2 (3 combinations)
    • 182-191 nt
TCR-Gamma Primers

- Two $V_G$-$J_G$ multiplexes:
  - (GA) products
    - 145-175 nt
    - 175-195 nt
    - 195-230 nt **
    - 230-255 nt **
  - (GB) products
    - 80-110 nt
    - 110-140 nt
    - 160-195 nt **
    - 195-220 nt
ELA014_W4730_GA.E08_10100108YY with dye4  Clonal

ELA014_W4730_GB.F08_10100108Z0 with dye4  Polyclonal
Present and Future...

• Biomed-2 consortium members have an ongoing remit to improve quality and reliability

• Hold highly successful annual Euroclonality Workshops for paired molecular scientists and haematopathologists
  – (workshop@euroclonality.org)

• Euroclonality Consortium to develop next-generation sequencing for IG and TCR immunogenetic analysis
Next-generation sequencing for clonality analysis, repertoire, MRD

• Considerations:
  – Choice of platform: Roche 454, MiSeq, Ion Torrent
  – Complete redesign of primers to yield short amplicons (100-150 nt)
  – Artefacts: formalin-induced deamination, PCR errors, detection of normal variation

• [www.euroclonality.org](http://www.euroclonality.org)
SP10-9765

- Lymphoid tissue mass at tongue base
- Morphology and immunohistochemistry equivocal - ?reactive or B-NHL

- PCR for IGH rearrangements
SP07-3417

- Retrospective analysis of FFPE lung specimen
- In 2010, patient was diagnosed with DLBCL
- Lymphoid infiltrate in lung in 2007 reviewed - ?reactive or low grade B-NHL
- PCR for IGH rearrangements
OC10-11907

- FFPE Lymph Node
- Morphology and immunohistochemistry raise suspicion of follicular lymphoma obscured by reactive lymphocytes
- PCR for IGH rearrangements
ELA006_W4626_FR1.A03_100729134N with dye4  Polyclonal (low signal)

ELA006_W4626_FR2.B03_100729134O with dye4  Clonal (fragment size out of expected range 250-295)
ELA006_W4626_FR2.B03_100729134O with dye4  Clonal (fragment size out of expected range 250-295)

ELA006_W4626_FR3.C03_100729134Q with dye4  Polyclonal
SP11-9578

• Sjogren’s syndrome; recent unilateral progression of parotid enlargement
• Histology (and immunophenotype) suggest development of MALT lymphoma

• PCR for IGH rearrangements
Dominant clone in polyclonal background
Dominant clone in polyclonal background

SP11-9578
• FFPE enlarged lymph node in patient with previous adult T-cell lymphoma/leukaemia
• In remission for several years; new 8mm cervical lymph node
• Histology and immunohistochemistry indicate dermatopathic but scattered CD25+ve T-cells; ?reactive/neoplastic
• PCR for TCR rearrangements
SP10-9772

- FFPE, EDTA-decalcified bone marrow trephine core
- Known previous lymphoplasmacytic lymphoma
- Morphology and immunohistochemistry indicate recurrence but also prominent and cytologically atypical T-cell infiltrate
- PCR for TCR rearrangements
4544_BC1.C10_10062110Q5 with dye4  Clonal

4544_BC2.D10_10062110Q6 with dye4  Polyclonal - ?oligoclonal
Clonal – bi-allelic/biclinal

Oligoclonal
• FFPE, EDTA-decalcified bone marrow trephine core
• Thrombocytopenia - ?ITP
• Histology and immunophenotype; T-cell lymphocytosis in marrow otherwise c/w ITP. No cytological atypia

• PCR for TCR rearrangements
SP10-16160

- FFPE lymph node
- Histology and immunohistochemistry suggest angio-immunoblastic T-cell lymphoma
- High content of EBER+ve large B-cells
- PCR for *IGH* and *TCR* rearrangements
Dominant clone in polyclonal background
Dominant clone in polyclonal background

Polyclonal (??DC)
ELA013_W4720_GA.E09_10092408TW with dye4  Clonal

ELA013_W4720_GB.F09_10092408TX with dye4  Polyclonal