Technical & Report Guidelines file

1 - Technical considerations for the determination of IGHV somatic hypermutation status in clonotypic IGHV-IGHD-IGHJ gene rearrangements in CLL.

Abbreviations: EDTA: ethylenediaminetetraacetic acid; CPT: citrate/pyridoxal 5'-phosphate/Tris; gDNA: genomic DNA; cDNA: complementary DNA; PAGE: polyacrylamide gel electrophoresis.

**Agathangelidis et al. Blood 2012 (ref. 7)*

Item	Recommendations	Remarks
Material		
Anticoagulants	EDTA (or CPT)	
Cells / Tissue	Blood, bone marrow, tissue biopsy	purification of B cells usually not necessary unless low fraction of leukemic cells
Nucleic acid	gDNA or cDNA	cDNA useful when mutations on the IGHJ gene impair amplification
Production of template for sequencing		
Primers	5': leader 3' : IGHJ or IGHC	VH FR1 might be acceptable if leader fails; VH FR2 and VHFR3 not accepable IGHC primers (on cDNA) useful when mutations on IGHJ gene impair amplification
Amplification	multiplex PCR	parallel simplex (for each 5' primer) PCR may be useful when more than one rearrangement
Detection of IGH rearrangement	GeneScan or PAGE electrophoresis	agarose gel electrophoresis strongly discouraged (lack of resolution)
Cloning	not necessary	except in rare circumstances (more than 1 rearrangement not isolated by simplex PCR)
Sequencing		
Methodology	direct, both strands	both strands mandatory for high quality sequence
Sequence alignment	IMGT/V-QUEST (www.imgt.org)	adjustable parameters : (i) search for insertions/deletions; (ii) number of accepted D genes
IGHV identity (%)	automatic or adjusted	adjusted : use option "search for insertions/deletions" when low % identity
Stereotypic subset identification	ARResT/AssignSubsets (bat.infspire.org/arrest/ ericll.org/pages/services/tool)	applicable for the current 19 major BcR stereotyped subsets in CLL*

2 - Reporting IGHV gene somatic hypermutation status in CLL.

Item	Recommendations
Standard cases	
- Methodology	Report type of: primers, PCR product analysis, sequencing method, bioinformatics tools
- Gene identification	IGHV, IGHD, IGHJ genes and alleles; IGHD might difficult to precisely identify (due to deletions and/or SHM)
- Productive rearrangement	Mutational status determined only on productive rearrangements; if unproductive, mention reasons (out-of-frame junction, stop codon)
- IGHV gene: % of nucleotide identity to germline	Classification: U-CLL ≥98% ; M-CLL <98%; borderline CLL when 97-97.9%
- Subset identification	For subsets with well-established prognostic value (subsets #1, #2, #4, #8)
Difficult cases (frequency[1])	
• Double rearrangements (10.5%)	
- productive + non-productive concordant status (7.8%)	Same as standard cases (mutational status defined by the productive rearrangement)
- productive + non-productive discordant status	
productive U + non-productive M (0.4%)	Mutational status not determined
productive M + non-productive U (0.2%)	Consider as M-CLL

- double productive	
concordant status (1.3%)	Same as standard cases
discordant status (0.7%)	Mutational status not determined
• Multiple (>2) rearrangements (5% *)	
• Single unproductive rearrangement (0.6%)	Mutational status not determined (after failure of alternative PCR attempts)
• Missing anchors (C104 / W118) (0.4%)	Mutational status possible if evidence for IG expression on leukemic cells and/or preserved G-X-G motif in VH FR4

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1 - All frequencies according to Langerak et al. Leukemia 2011 *(ref. 17),* except for * Sanchez et al. Blood 2003 *(ref. 25).*