IGH somatic hypermutation analysis

Dr. Sci. Sabine Franke
Sabine.Franke@chuliege.be
5/2/2026

Molecular Biology and Cytometry Course 2026

IGH somatic hypermutation analysis

Determination of hypermutation
→ Why to do?
→ What is it?

Different techniques
→ Advantage – Disadvantage

Interpretation
→ Standard cases and special cases

Examples

Reports

Why to do

CLL has a highly heterogeneous clinical course.

Somatic hypermutation (SHM) status of the immunoglobulin heavy variable (IGHV) gene plays a crucial role in determining the prognosis and treatment of patients with chronic lymphocytic leukemia (CLL).

→ IGHV determination important.

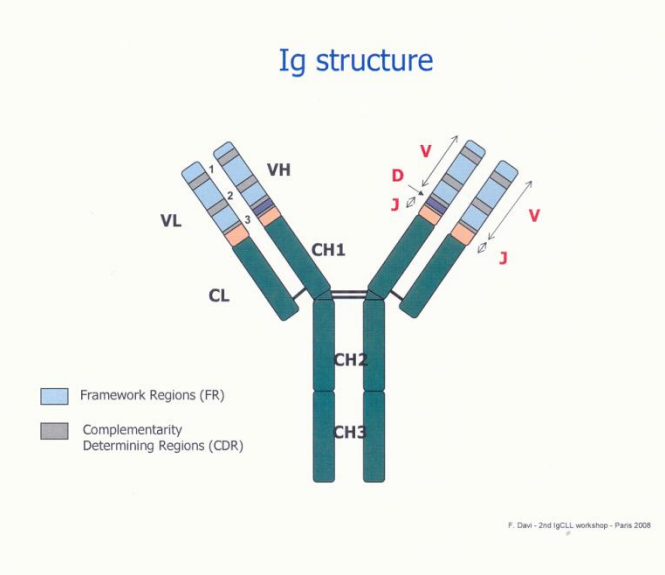
Why to do

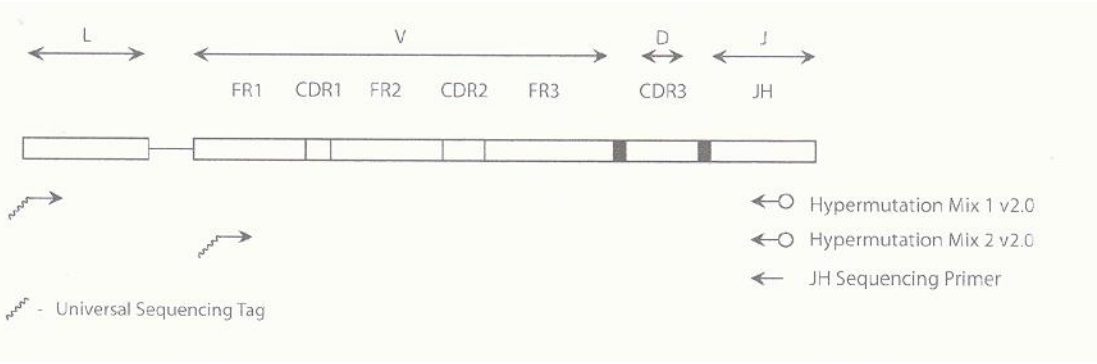
Chance:
Mutational status IGHV only reimbursed
for patients younger than 65y changed to →
reimbursed in all patients with active or advanced disease without del(17p)/*TP53* mutation and in need and fit for therapy (ComPerMed).

The BHS guidelines recommend chemoimmunotherapy in patients without del(17p)/ *TP53* mutation with mutated IGVH.

This biomarker remains stable over time.

What is it





Invivoscribe

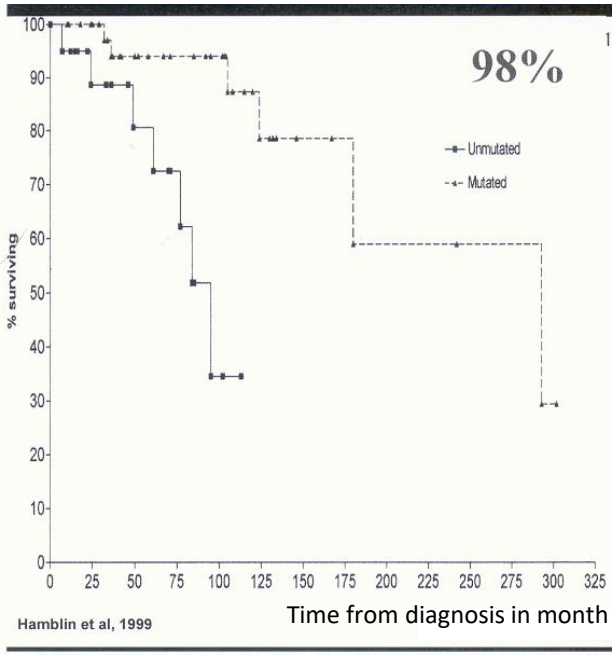
Somatic hypermutation takes place in the V region of both H and L chain genes, introducing a million times point mutations.

This process followed by selection leads to generation of high-affinity antibodies.

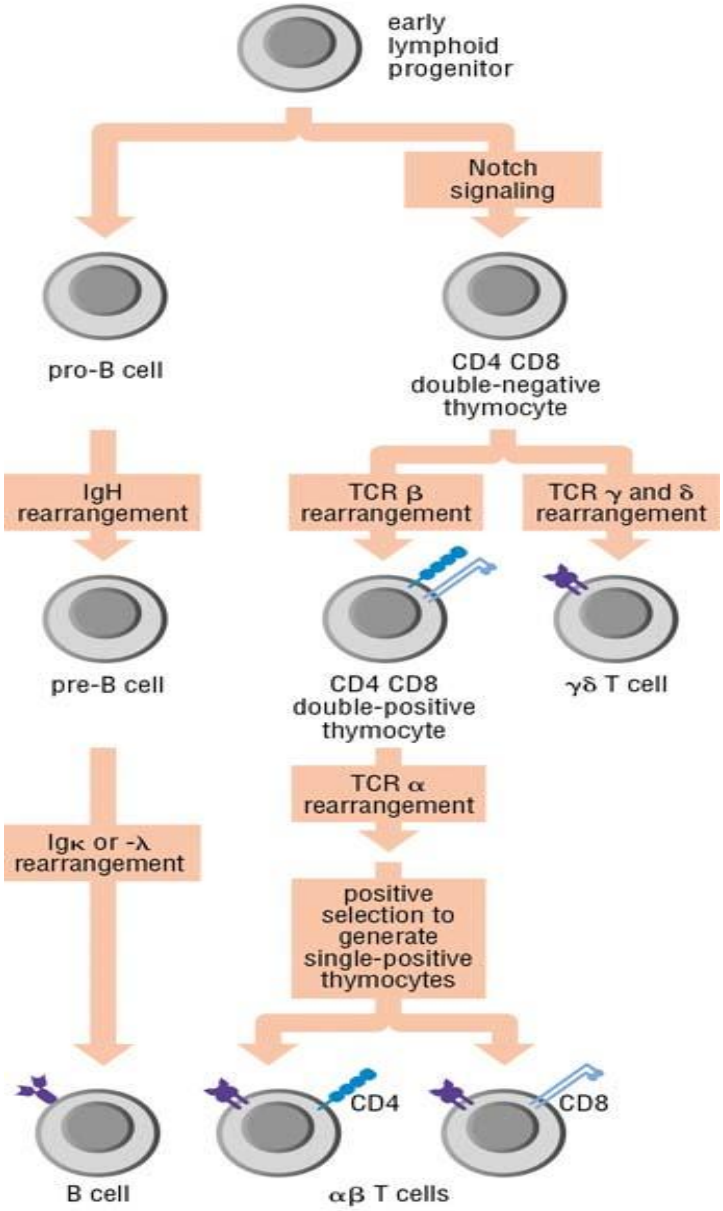
What is it

Sequence of this IGH region allows the determination of homology with the germline sequence.

Homology ≥ 98% = IGHV **unmutated** → worse prognosis
Homology <98% = IGHV **mutated** → good prognosis
(exception when overruled by the stereotype)



From **Immunity: The Immune Response in Infectious and Inflammatory Disease**
by DeFranco, Locksley and Robertson



Gene rearrangements of the antigen receptor genes occur during the lymphoid proliferation

When CLL arises from relatively less differentiated B cells with unmutated heavy chain genes
→ **poor prognosis.**
Patients with unmutated IGVH genes have not generated IgV gene mutations.

When CLL evolves from more differentiated B lymphocytes with somatically mutated heavy chain genes
→ **good prognosis**

Techniques of IG hypermutation determination

Sanger sequencing (standard)

- ↓ Problem if more than one rearrangement (may not differenciate different clonal populations in one sample)
- ↓ Problem of background
- ↓ Time consuming
- ↓ Failure rate 9-18%

Techniques of IG hypermutation determination

NGS : amplification of IGH locus by multiplex PCR
→ **IVS** (LymphoTrack) amplicon based + IVS analysis of FASTQ files
→ **SOPHiA GENETICS** capture panel based + IgCaller analysis of FASTQ files
→ ThermoFisher: Oncomine IGHV Leader-J Panel
→ Euroclonality: NGS IG assays

↑ Sensitive (low infiltration)
↑ NGS-based analysis can reveal the existence in the same patient of minor related clonotypes (corresponding to subclones diversification) or unrelated clonotypes (corresponding to distinct clones)
→ IVS : Can be combined with clonality analysis
→ SOPHiA: Combined with mutation analysis

↓ Expensive
↓ Equipment

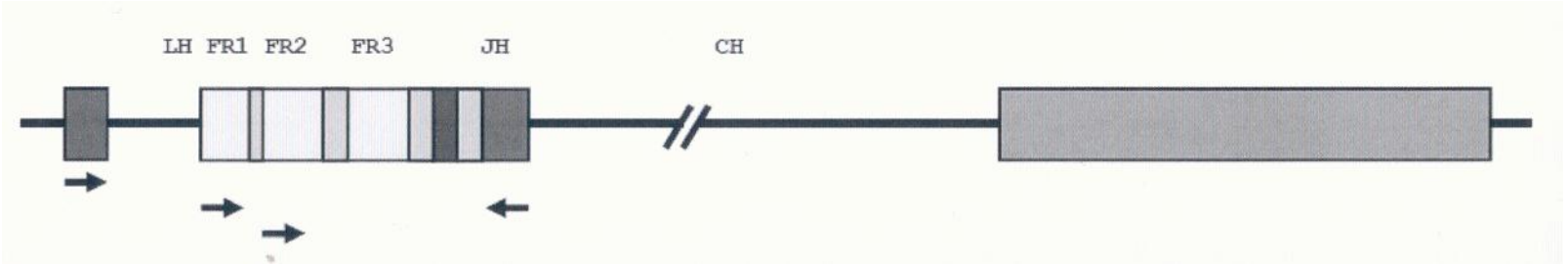
ERIC recommendations

ERIC: European Research Initiative on CLL

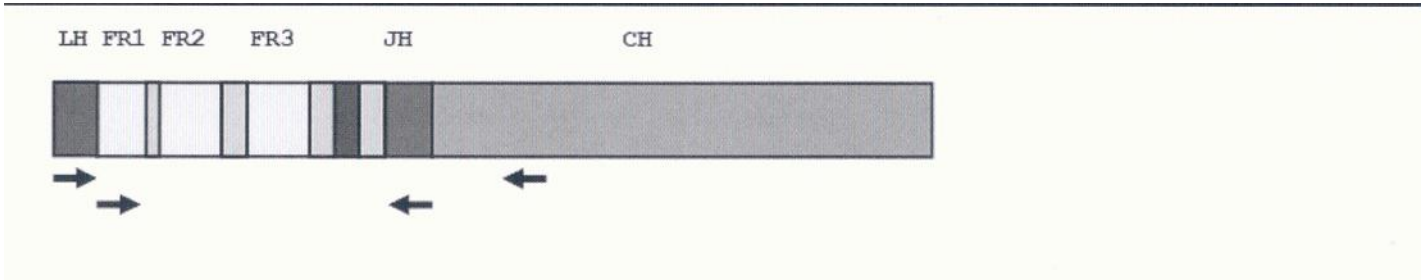
→ Type of nucleid acid
→ PCR primers
→ Sanger Sequencing both strands
→ Interpretation
→ Report

Type of nucleid acid

gDNA:
better for transport
use archival material
but: non-productive rearrangement can also be amplified



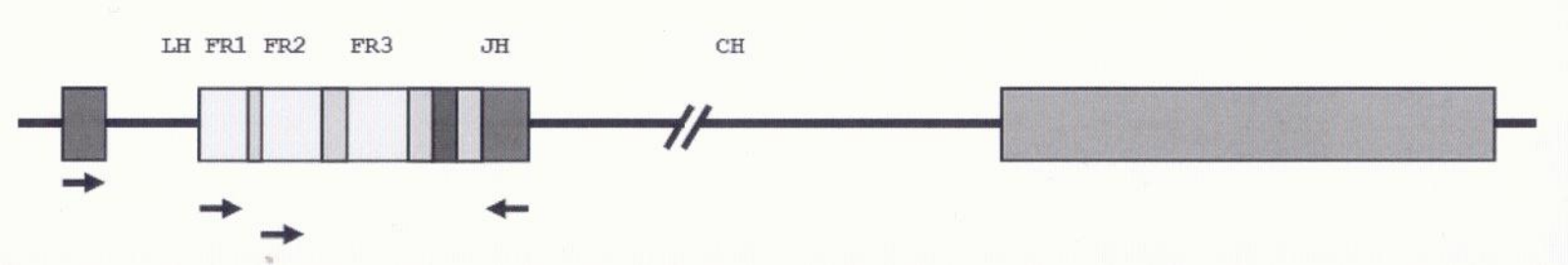
cDNA:
identifies mostly only functional productive rearrangement
but reverse transcription step necessary



PCR primers

IGHV leader:
accurate SHM status
based on whole IGHV gene
less samples in one run

IGHV FR1:
widely used in clonality testing
but lack of 5'V (estimation of SHM level)



Primer sets: IGHV leader primers
IGHV FR1 primers
IGHV FR2 primers: when leader or FR1 are negativ ->NO
IGHV FR3 primers: short IGHV sequences -> NO

NGS Lymphotrack workflow (IVS)

DNA

Amplification

Purification PCR products

Library quantification

Run prep

FASTQ files

Analysis

+ clonality analysis in same run possible

NGS SOPHiA GENETICS workflow

DNA

Amplification

Purification PCR products

Library quantification

Run prep

FASTQ files

Analysis

+ mutation analysis of 23 genes

Information of the IVS software

Length	Raw count	V-gene	J-gene	% total reads	Cumulative %	Mutation rate to V-gene (%)	In-frame (Y/N)	No Stop codon (Y/N)	V-coverage
465	303519	IGHV4-4_02	IGHJ4_02	27,00	27,00	3,04	N	N	99,32
473	50283	IGHV3-53_01	IGHJ4_02	4,47	31,47	1,37	Y	Y	99,66
465	12924	IGHV4-4_02	IGHJ4_02	1,15	32,62	3,38	N	N	99,32
465	12764	IGHV4-4_02	IGHJ4_02	1,14	33,76	3,38	N	N	99,32
465	9377	IGHV4-4_02	none	0,83	34,59	3,04	n/a	N	99,32
465	6971	IGHV4-4_02	IGHJ4_02	0,62	35,21	3,38	N	N	99,32
465	6895	IGHV4-4_02	IGHJ4_02	0,61	35,82	3,38	N	N	99,32
465	6546	IGHV4-4_02	IGHJ4_02	0,58	36,41	3,38	N	N	99,32
465	5610	IGHV4-4_02	IGHJ4_02	0,50	36,91	3,38	N	N	99,32
465	5402	IGHV4-4_02	IGHJ4_02	0,48	37,39	3,38	N	N	99,32
465	4320	IGHV4-4_02	IGHJ4_02	0,38	37,77	3,04	N	N	99,32
465	3693	IGHV4-4_02	IGHJ4_02	0,33	38,10	3,38	N	N	99,32
465	3496	IGHV4-4_02	IGHJ4_02	0,31	38,41	3,04	N	N	99,32
473	3392	IGHV3-53_01	IGHJ4_02	0,30	38,71	1,71	Y	Y	99,66
465	3270	IGHV4-4_02	IGHJ4_02	0,29	39,00	3,04	N	N	99,32
465	3267	IGHV4-4_02	IGHJ4_02	0,29	39,29	3,04	N	N	99,32
465	3105	IGHV4-4_02	IGHJ4_02	0,28	39,57	3,38	N	N	99,32
465	3075	IGHV4-4_02	IGHJ4_02	0,27	39,84	3,04	N	N	99,32
465	2965	IGHV4-4_02	IGHJ4_02	0,26	40,11	3,04	N	N	99,32
465	2911	IGHV4-4_02	IGHJ4_02	0,26	40,37	3,04	N	N	99,32
473	2271	IGHV3-53_01	IGHJ4_02	0,20	40,57	1,71	Y	Y	99,66
465	1913	IGHV4-4_02	IGHJ4_02	0,17	40,74	3,38	N	N	99,32
465	1711	IGHV4-4_02	IGHJ4_02	0,15	40,89	3,72	N	N	99,32
473	1695	IGHV3-53_01	IGHJ4_02	0,15	41,04	1,37	Y	Y	99,66
465	1678	IGHV4-4_02	IGHJ4_02	0,15	41,19	3,38	N	N	99,32

Our table

N° Echantillon	reads total	Rank	Sequence	Length	Raw count	V-gene	J-gene	% total reads	Cumulative %	Mut rate partial V-gene (%)	In-frame	No Stop codon		
HMIGH POS	323286	1	GGTCTTCTGCTTGCTGGCTGTAGCTCCAGGTAAAGGGCCAACTGGTTCC	490	8306	IGHV1-46_03	IGHJ4_02	2,57	2,57	0,00	Y	Y	OK	100,00
		2	GGGATTTTTTCCAGTTTAGAGGACTGTCATTCTCTACTGTGTCCTCTCC	429	1031	IGHV1-46_03	IGHJ4_02	0,32	2,89	0,00	Y	Y		
		3	GGTTTTCCTTGTTACTATATTAGAAGGTGATTCATGGAGAACTAGAGAT	311	271	IGHV3-13_04	IGHJ4_02	0,08	2,97	2,73	n/a	N		
		4	GGTCTTCTGCTTGCTGGCTGTAGCTCCAGGTAAAGGGCCAACTGGTTCC	490	265	IGHV1-46_03	IGHJ4_02	0,08	3,05	0,34	Y	Y		
14-251201-0096	373718	1	GCTTTTTCTTGTGGCTATTTTAAAAGGTTATTCATGGAGAAATAGAAGA	481	161661	IGHV3-23_01	IGHJ4_02	43,26	43,26	2,36	Y	Y		
		2	GCTTTTTCTTGTGGCTATTTTAAAAGGTTATTCATGGAGAAATAGAAGA	481	1915	IGHV3-23_01	IGHJ4_02	0,51	43,77	2,70	Y	N		
		3	GCTTTTTCTTGTGGCTATTTTAAAAGGTTATTCATGGAGAAATAGAAGA	481	1447	IGHV3-23_01	IGHJ4_02	0,39	44,16	2,70	Y	Y		
		4	GCTTTTTCTTGTGGCTATTTTAAAAGGTTATTCATGGAGAAATAGAAGA	481	1059	IGHV3-23_01	IGHJ4_02	0,28	44,44	2,70	Y	Y		

Sequence interpretation

https://www.imgt.org/IMGT_vquest/input

Mostly point mutations

Functionality: Productive or unproductive

Identity: Gene region identified (V, D, J)

Check In frame/ no stop codon

→ IMGT: use option “search for insertions/deletions” when low % identity.

Homology: % (>2% HMS mutated; ≤2% unmutated)

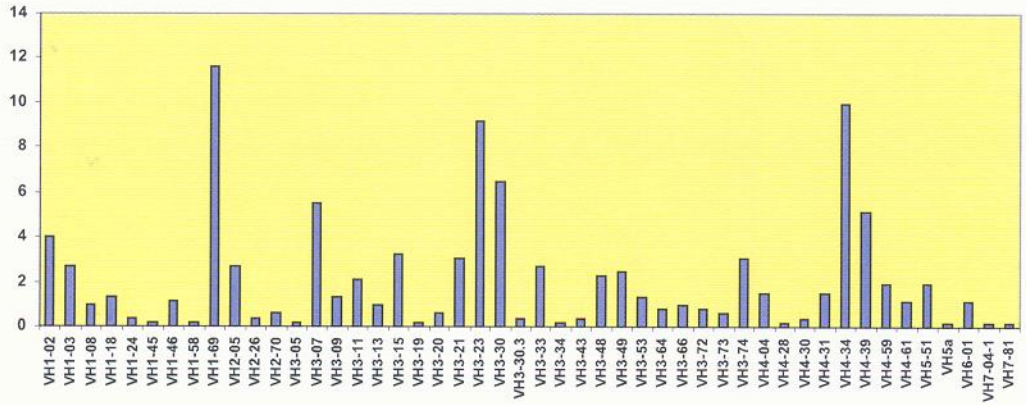
→ Assignment to stereotyped subsets #2 and #8

IMGT/V-QUEST or

ARResT/AssignSubsets (http://bat.infspire.org/arrest/assignsubsets/)

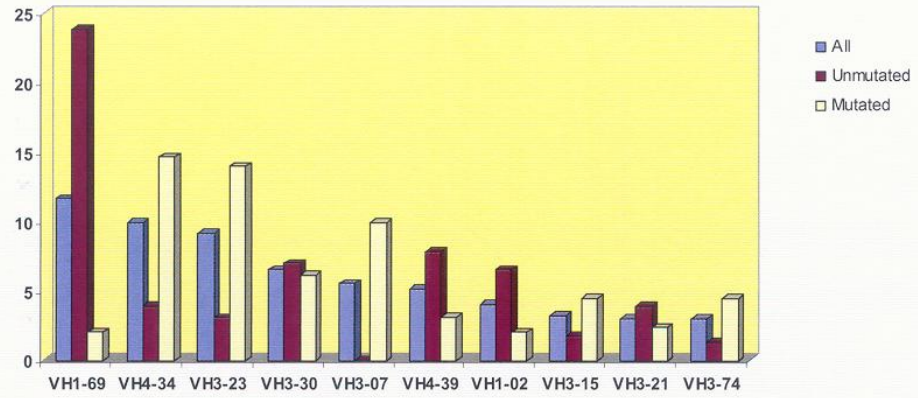
IGHV repertoire in CLL

(Ghia, *Blood 2004*)



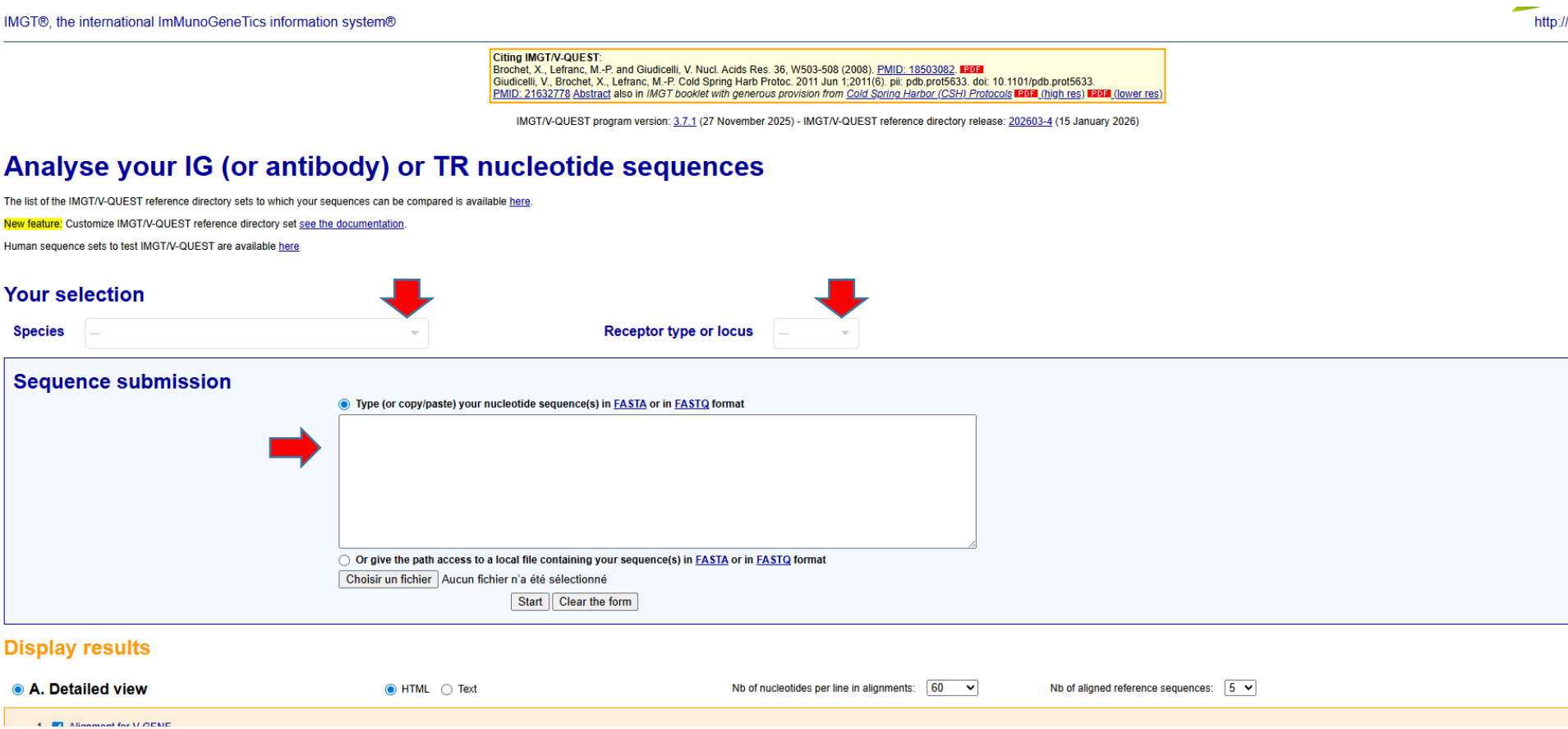
IGHV repertoire in CLL

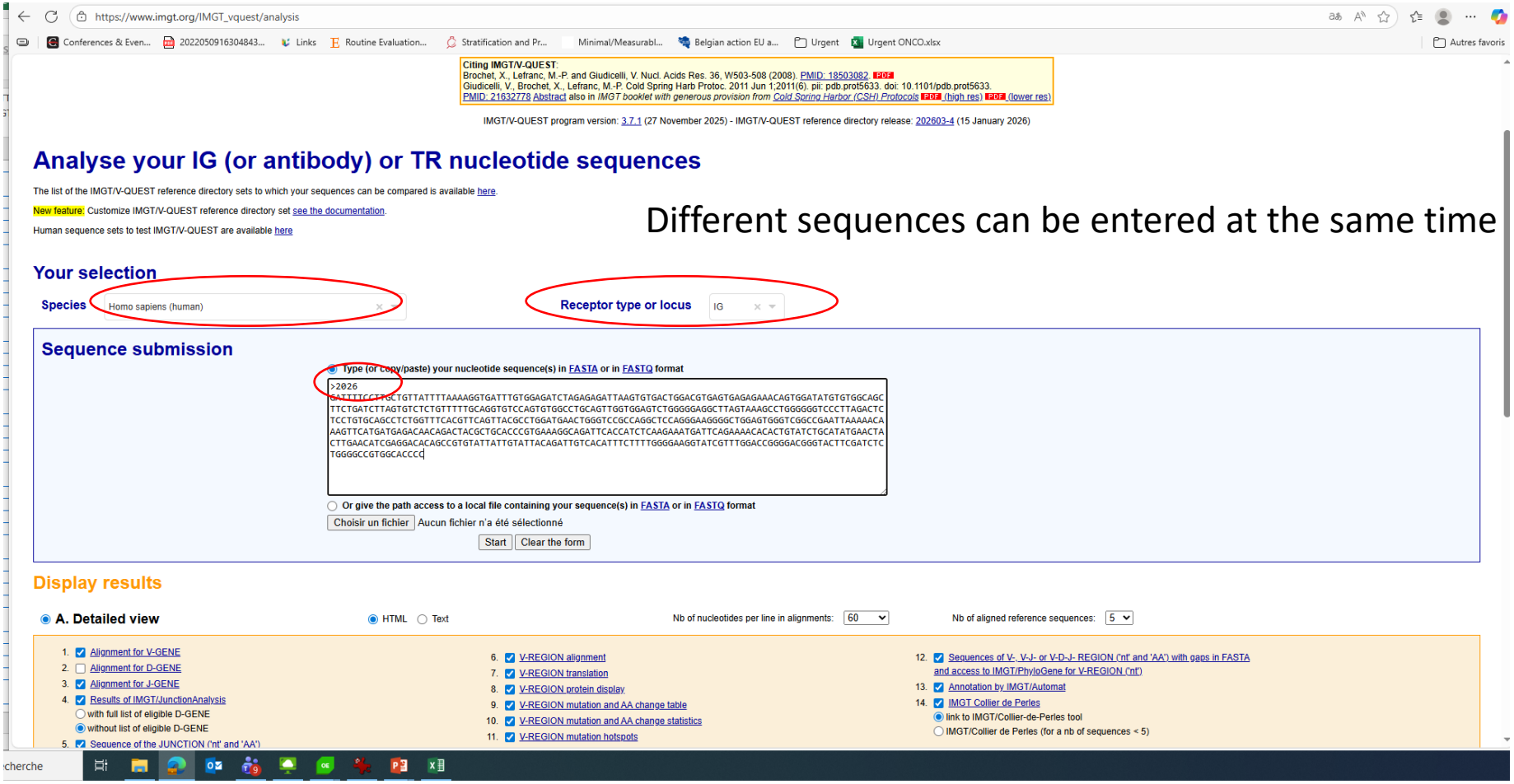
(Ghia, *Blood 2004*)



More unmutated then mutated cases

www.imgt.org/IMGT_vquest/input




https://www.imgt.org/IMGT_vquest/analysis
Citing IMGT/V-QUEST:
Brochet, X., Lefranc, M.-P. and Giudicelli, V. Nucl. Acids Res. 36, W503-508 (2008). PMID: 18503082
Giudicelli, V., Brochet, X., Lefranc, M.-P. Cold Spring Harb Protoc. 2011 Jun 1;2011(6). pii: pdb.prot5633. doi: 10.1101/pdb.prot5633.
PMID: 21632778 Abstract also in IMGT booklet with generous provision from Cold Spring Harbor (CSH) Protocols (high res) (lower res)
IMGT/V-QUEST program version: 3.7.1 (27 November 2025) - IMGT/V-QUEST reference directory release: 202603-4 (15 January 2026)
Analyse your IG (or antibody) or TR nucleotide sequences
The list of the IMGT/V-QUEST reference directory sets to which your sequences can be compared is available here.
New feature: Customize IMGT/V-QUEST reference directory set see the documentation.
Human sequence sets to test IMGT/V-QUEST are available here
Different sequences can be entered at the same time
Your selection
Species
Homo sapiens (human)
Receptor type or locus
IG
Sequence submission
Type (or copy/paste) your nucleotide sequence(s) in FASTA or in FASTQ format
>2026
GATTTTCCTTGCTGTTATTTTAAAAGGTGATTTGTGGAGATCTAGAGAGATTAAGTGTGACTGGACGTGAGTGAGAGAAACAGTGGATATGTGTGGCAGC
TTCTGATCTTAGTGTCTCTGTTTTTGCAGGTGTCCAGTGTGGCCTGCAGTTGGTGGAGTCTGGGGGAGGCTTAGTAAAGCCTGGGGGGTCCCTTAGACTC
TCCTGTGCAGCCTCTGGTTTCACGTTCAGTTACGCCTGGATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGCCGAATTAAAAACA
AAGTTCATGATGAGACAACAGACTACGCTGCACCCGTGAAAGGCAGATTCACCATCTCAAGAAATGATTCAGAAAACACACTGTATCTGCATATGAACTA
CTTGAACATCGAGGACACAGCCGTGTATTATTGTATTACAGATTGTCACATTTCTTTTGGGGAAGGTATCGTTTGGACCGGGGACGGGTACTTCGATCTC
TGGGGCCGTGGCACCCC
Or give the path access to a local file containing your sequence(s) in FASTA or in FASTQ format
Choisir un fichier
Aucun fichier n'a été sélectionné
Start
Clear the form
Display results
A. Detailed view
HTML
Text
Nb of nucleotides per line in alignments: 60
Nb of aligned reference sequences: 5
1. Alignment for V-GENE
2. Alignment for D-GENE
3. Alignment for J-GENE
4. Results of IMGT/JunctionAnalysis
with full list of eligible D-GENE
without list of eligible D-GENE
5. Sequence of the JUNCTION ('nt' and 'AA')
6. V-REGION alignment
7. V-REGION translation
8. V-REGION protein display
9. V-REGION mutation and AA change table
10. V-REGION mutation and AA change statistics
11. V-REGION mutation hotspots
12. Sequences of V-, V-J- or V-D-J- REGION ('nt' and 'AA') with gaps in FASTA and access to IMGT/PhyloGene for V-REGION ('nt')
13. Annotation by IMGT/Automat
14. IMGT Collier de Perles
link to IMGT/Collier-de-Perles tool
IMGT/Collier de Perles (for a nb of sequences < 5)

myCHU | Zimbra: Envoyé (15) | IMGT/V-QUEST

https://www.imgt.org/IMGT_vquest/analysis;jsessionid=C30D06C8677E252FE3C8ACC1AD3AED4C

Conferences & Eve...

A. Detailed results for the IMGT/V-QUEST analysed sequences

Number of analysed sequences: 1

1. 2022

This release of IMGT/V-QUEST uses IMGT/JunctionAnalysis for the analysis of the JUNCTION

Hyphens (-) show nucleotide identity, dots (.) represent gaps

Sequence: 1 2022

Search for insertions and deletions in V-REGION

YES (low V-region identity)

Analysed sequence length: 467.
Sequence analysis category: 2 (indel search & correction).
Sequence compared with the *Homo sapiens* (human) IG set from the IMGT reference directory (set: F+ORF+ in-frame P)

```
>2022
ggttttccttgttgctattttagaaggtgaatcatggaaaagtagagagatttagtgtgt
gtggatatgagtcagagaaacggtggatgtgtgtgacagtttctgaccaatgtctctctg
tttgcaggtgtccagtgtgaggtgcacctggtggagtctgggggaggcctggtcaagcct
gggggtccctgagactctttctgtgcagcctctggattcactttcagtagttgtaccatg
aactgggtccgccaggctccagggaaggggctggaatgggtctcatccattagtagtagt
agttacatatactacgcagactcagtgaagggccgattcaccatctccagagacaacgcc
aagaactcactgtatctgcaaatgaacagcctgagagccgaggacacggctgtgtattac
tgtgcgagagattctaacggtatggacgtctggggccaagggaccac
```

Result summary: 2022	Nucleotide deletions have been detected (shown by dots in the alignments):				
	localization	nb of deleted nt	causing frameshift	from V-REGION codon	from nt position in user submitted sequence
	CDR2-IMGT	3	no	57	292

IMGT/V-QUEST results after filling the deletion(s) gap(s):
Potentially productive IGH rearranged sequence (no stop codon and in-frame junction)
(Check also your sequence with BLAST against IMGT/GENE-DB reference sequences to eventually identify out-of-frame pseudogenes)

V-GENE and allele	Homsap IGHV3-21*01 F, or Homsap IGHV3-21*02 F	score = 1371	identity = 97.89% (279/285 nt) [97.54% (278/285 nt)]
J-GENE and allele	Homsap IGHJ6*02 F	score = 144	identity = 88.89% (32/36 nt)
D-GENE and allele by IMGT/JunctionAnalysis	Homsap IGHD3-10*01 F	D-REGION is in reading frame 2	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[25.17.38.5]	[8.7.9]	CARDSNGMDVW
JUNCTION length (in nt) and decryption	33 nt = (11)0{0}-24(5)-2{0}-15(17)	(3'V)3'{N1}5'(D)3'{N2}5'(5'J)	

J-REGION partial 3' missing nt nb: **15**

1. Alignment for V-GENE and allele identification

Closest V-REGIONs (evaluated from the V-REGION first nucleotide to the 2nd-CYS codon)

Tapez ici pour effectuer une recherche

4. ☑ V-REGION protein display

8. ☑ Results of IMGT/JunctionAnalysis

Check all |

○ **C. Excel file** ◉ Open in a spreadsheet ○ Download in a zip archive ○ Display 1 CSV file in your brows

1. ☑ Summary
2. ☑ IMGT-gapped-nt-sequences
3. ☑ nt-sequences
4. ☑ IMGT-gapped-AA-sequences
5. ☑ AA-sequences
6. ☑ Junction
7. ☑ V-REGION-mutation-and-AA-change-table
8. ☑ V-REGION-nt-mutation-statistics
9. ☑ V-REGION-AA-change-statistics
10. ☑ V-REGION-mutation-hotspots
11. ☑ Parameters
12. ☐ scFv (only for option "Analysis of single chain Fragment v

Check all |

Advanced parameters

Selection of IMGT reference directory set	F+ORF+ in-frame P
Customize the reference directory set	○ Yes
Search for insertions and deletions in V-REGION	● Yes
Parameters for IMGT/JunctionAnalysis	Nb of accepted D-GENE in IGH (default is 1), TRB (default is 1) or TRD (default is 3) JUNCTION def
Parameters for "Detailed view"	Nb of nucleotides to exclude in 5' of the V-REGION for the evaluation of the nb of mutations (in results 9 and 10) 0

Advanced functionalities

Analysis of single chain Fragment variable (scFv)	○ Yes ◉ No
Clinical application: search for CLL subsets #2 and #8	○ Yes ◉ No

Sequence compared with the IG set from the IMGT reference directory

```
>2026
gattttccttgctgttattttaaaaggtgatttgtggagatctagagagattaagtgtga
ctggacgtgagtgagagaaacagtggatatgtgtggcagcttctgatcttagtgtctctg
tttttgcaggtgtccagtgtggcctgcagttggtggagtctgggggaggcttagtaaagc
ctgggggtcccttagactctcctgtgcagcctctggtttcacgttcagttacgcctgga
tgaactgggtccgccaggctccagggaaggggctggagtgggtcggccgaattaaaaaca
aagttcatgatgagacaacagactacgctgcacccgtgaaaggcagattcaccatctcaa
gaaatgattcagaaaacacactgtatctgcatatgaactacttgaacatcgaggacacag
ccgtgtattattgtattacagattgtcacatttcttttggggaaggtatcgtttggaccg
gggacgggtacttcgatctctggggccgtggcaccccc
```

Standard case: IGHM mutated

Result summary: 2026	**Productive IGH rearranged sequence** (no stop codon and in-frame junction)		
V-GENE and allele	Homsap IGHV3-15*07 F	score = 1254	identity = **91.84%** (270/294 nt)
J-GENE and allele	Homsap IGHJ2*01 F	score = 144	identity = 88.89% (32/36 nt) - (J-REGION partial in 3' missing nt r
D-GENE and allele by IMGT/JunctionAnalysis	Homsap IGHD3-16*02 F	D-REGION is in reading frame 3	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[25.17.38.5]	[8.10.22]	CITDCHISFGEGIVWTGDGYFDLW
JUNCTION length (in nt) and decryption	72 nt = (11)+1{6}-8(24)-5{10}-2(20)	(3'V)3' {N1}5'(D)3'{N2} 5'(5'J)	

1. Alignment for V-GENE and allele identification

Closest V-REGIONs (evaluated from the V-REGION first nucleotide to the 2nd-CYS codon)

	Score	Identity
Homsap IGHV3-15*07 F	1254	91.84% (270/294 nt)
Homsap IGHV3-15*01 F	1227	90.82% (267/294 nt)
Homsap IGHV3-15*06 F	1227	90.82% (267/294 nt)
Homsap IGHV3-15*02 F	1218	90.48% (266/294 nt)
Homsap IGHV3-15*04 F	1218	90.48% (266/294 nt)

Alignment with FR-IMGT and CDR-IMGT delimitations

```
                              <---------------------------------- FR1-IMGT ---------------
2026                          ggcctgcagttggtggagtctgggggga...ggcttagtaaagcctgggggtcccttaga
Homsap IGHV3-15*07 F          -agg-----c-----------------...-----g------------------------
Homsap IGHV3-15*01 F          -agg-----c-----------------...-----g------------------------
Homsap IGHV3-15*06 F          -agg-----c-----------------...-----g------------------------
Homsap IGHV3-15*02 F          -agg-----c-----------------...-c---g------------------------
Homsap IGHV3-15*04 F          -agg-----c-----------------...-----g------------------------

                              ----------------->_____________ CDR1-IMGT ___________<-----
2026                          ctctcctgtgcagcctctggtttcacgttc............agttacgcctggatgaac
```

1. 2023C

This release of IMGT/V-QUEST uses IMGT/JunctionAnalysis for the analysis of the JUNCTION

Hyphens (-) show nucleotide identity, dots (.) represent gaps

Sequence : 1 2023C

Analyzed sequence length: 485 .
Sequence analysis category: 2 (indel search & correction) .
Sequence compared with the IG set from the IMGT reference directory

```
>2023C
ggttttcctcgttgctcttttaagaggtgattcatggagaaatagagagactgagtgtga
gtgaacatgagtgagaaaaactggatttgtgtggcattttctgataacggtgtccttctg
tttgcaggtgtccagtgtcaggtgcagctggtggagtctgggggaggcgtggtccagcct
gggaggtccctgagactctcctgtgcagcctctggattcaccttcagtagctatgctatg
cactgggtccgccaggctccaggcaaggggctggagtgggtggcagttatatcatatgat
ggaagcaataaatactacgcagactccgtgaagggccgattcaccatctccagagacaat
tccaagaacacgctgtatctgcaaatgaacagcctgagagctgaggacacggctgtgtat
tactgtgcgagagggccaaggggtgactacgccttttctttgactactggggccaggga
accct
```

Result summary: 2023C	Productive IGH rearranged sequence (no stop codon and in-frame junction)		
V-GENE and allele	Homsap IGHV3-30-3*01 F	score = 1440	identity = 100.00% (288/288 nt)
J-GENE and allele	Homsap IGHJ4*02 F	score = 119	identity = 87.10% (27/31 nt) - (J-REGION partial in 3' missing nt nb= 16)
D-GENE and allele by IMGT/Junc[illegible]is	Homsap IGHD4-17*01 F	D-REGION is in reading frame 2	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[25.17.38.5]	[8.8.14]	CARGPRGDYAFSFDYW
JUNCTION length (in nt) and decryption	48 nt = (10)-1{8}-7(9)+1{8}-5(12)	(3'V)3-(N1)5-(D)9-(N2)-5'(J)3'	

1. Alignment for V-GENE and allele identification

Closest V-REGIONs (evaluated from the V-REGION first nucleotide to the 2nd-CYS codon)

	Score	Identity
Homsap IGHV3-30-3*01 F	1440	100.00% (288/288 nt)
Homsap IGHV3-30*04 F	1431	99.65% (287/288 nt)
Homsap IGHV3-30-3*02 F	1431	99.65% (287/288 nt)
Homsap IGHV3-30-3*03 F	1431	99.65% (287/288 nt)
Homsap IGHV3-30*01 F	1422	99.31% (286/288 nt)

Alignment with FR-IMGT and CDR-IMGT delimitations

```
                          <---------------------------------- FR1-IMGT ----------------
2023C                     caggtgcagctggtggagtctgggggga...ggcgtggtccagcctgggaggtccctgaga
Homsap IGHV3-30-3*01 F    ---------------------------...----------------------------
Homsap IGHV3-30*04 F      ---------------------------...----------------------------
Homsap IGHV3-30-3*02 F    ---------------------------...----------------------------
```

Standard case: IGHM not mutated

1. 2026b

☞ This release of IMGT/V-QUEST uses IMGT/JunctionAnalysis for the analysis of the JUNCTION

☞ Hyphens (-) show nucleotide identity, dots (.) represent gaps

Sequence : 1 2026b

Analyzed sequence length: 470 .
Sequence analysis category: 2 (indel search & correction) .
Sequence compared with the IG set from the IMGT reference directory

>2026b
ggttttccttgttgctattttagaaggtgaatcatggaaaagtagagagatttagtgtgt
gtggatatgagtgagagaaacggtggatgtgtgtgacagtttctgaccaatgtctctctg
gttgcaggtgtccagtgtgaggtgcagctggtggagtctgggggaggcctggtcaagcct
gggggtccctgagactctcctgtgcagcctctgggttcaccttcagtaactataacatg
aactgggtccgccaggctccagggaaggggctggagtgggtctcatccattagtagtagt
actacttacatatactgcgcagactcagtgaagggccgattcaccatctccagagacaac
gccaagaattcactgtatctgcaaatgaacagcctgagagccgaggacacggctgtgtat
tactgtgtagtggaccggaacggtatggacgtctggggccaagggaccac

V3-21 case

Result summary: 2026b	Productive IGH rearranged sequence (no stop codon and in-frame junction)		
V-GENE and allele	Homsap IGHV3-21*06 F	score = 1386	identity = 97.92% (282/288 nt)
J-GENE and allele	Homsap IGHJ6*02 F	score = 125	identity = 76.74% (33/43 nt) - (J-REGION partial in 3' missing nt nb
D-GENE and allele by IMGT/JunctionAnalysis	Homsap IGHD2-8*02 F	D-REGION is in reading frame 1	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[25.17.38.5]	[8.8.9]	CVVDRNGMDVW
JUNCTION length (in nt) and decryption	33 nt = (4)-7{0}-4(11)-16{1}-15(17)	(3'V)3' {N1}5'(D)3'{N2} 5'(5'J)	

1. Alignment for V-GENE and allele identification

www.ncbi.nlm.nih.gov/igblast/

Closest V-REGIONs (evaluated from the V-REGION first nucleotide to the 2nd-CYS codon)

Subset

Immunogenetic analysis in CLL has revealed that different patients may express (quasi)identical, stereotyped B cell receptor immunoglobulin (BcR IG) and are classified into subsets based on this common feature.

http://bat.infspire.org/arrest/assignsubsets/

ARResT/AssignSubsets

cite us!

assigning **new** members to **existing** subsets of stereotyped antigen receptor sequences, currently applicable to the 19 major subsets of stereotyped B-cell receptors in chronic lymphocytic leukemia (CLL)

07.01.22 | powered by ARResT/SeqCure ; ARResT/Subsets ; IMGT/V-QUEST ; IMGT/CLL-DB

please consider using Chrome / Firefox / Safari for best viewing and full functionality

your antigen receptor sequences

provide up to 50 FASTA-formatted FULL NUCLEOTIDE IG sequences - *check example below, ~100kb upload limit*

```
>2026b
GGTTTTCCTTGTTGCTATTTTAGAAGGTGAATCATGGAAAAGTAGAGAGATTTAGTGTGTGTGGATATGAGTGAGAGAAACGGTGGATGTGTGTGACAGTTTCTGA
CCAATGTCTCTCTGGTTGCAGGTGTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTC
TGGGTTCACCTTCAGTAACTATAACATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCATCCATTAGTAGTAGTACTACTTACATATACTGC
GCAGACTCAGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAATTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACT
```

Choisir un fichier | Aucun fichier n'a été sélectionné clear browsed file

or click to load example

FASTA

DISCLAIMER - there is no guarantee that ARResT/AssignSubsets will be able to properly assign all your sequences to subsets, please bear this in mind when making decisions, especially important ones on e.g. clinical care, and especially with 'borderline'- or 'low'-confidence assignments. To help us improve ARResT/AssignSubsets, please contact us.

Assign to Subsets or reset

citation and acknowledgements

We're now published in Bioinformatics:

ARResT/AssignSubsets: a novel application for robust subclassification of chronic lymphocytic leukemia based on B cell receptor IG stereotypy

Vojtech Bystry; Andreas Agathangelidis; Vasilis Bikos; Lesley Ann Sutton; Panagiotis Baliakas; Anastasia Hadzidimitriou; Kostas Stamatopoulos; Niko Darzentas

Bioinformatics 2015

ARResT/AssignSubsets

assigning new members to existing subsets of stereotyped antigen receptor sequences

we're running ARResT/AssignSubsets - please follow our progress below...

```
(?) checking IMGT accessibility
(?) running ARResT/SeqCure with your sequences...
(=) ARResT/SeqCure report
(?) model is running...

(=) 1 / 1 / 1 were assigned / 'healthy' / submitted
```

DISCLAIMER - there is no guarantee that ARResT/AssignSubsets will be able to properly assign all your sequences to subsets, please bear this in mind when making decisions, especially important ones on e.g. clinical care, and especially with 'borderline'- or 'low'-confidence assignments. To help us improve ARResT/AssignSubsets, please contact us.

plain-text-formatted results table (best viewable in a spreadsheet), or see below

click to open/close quick help »

assignment frequencies table

CLL#2	CLL#1	CLL#4	CLL#6	CLL#5	CLL#3	CLL#8	CLL#31	CLL#16	CLL#77
2.8%	2.4%	1.0%	0.9%	0.7%	0.6%	0.5%	0.4%	0.3%	0.3%

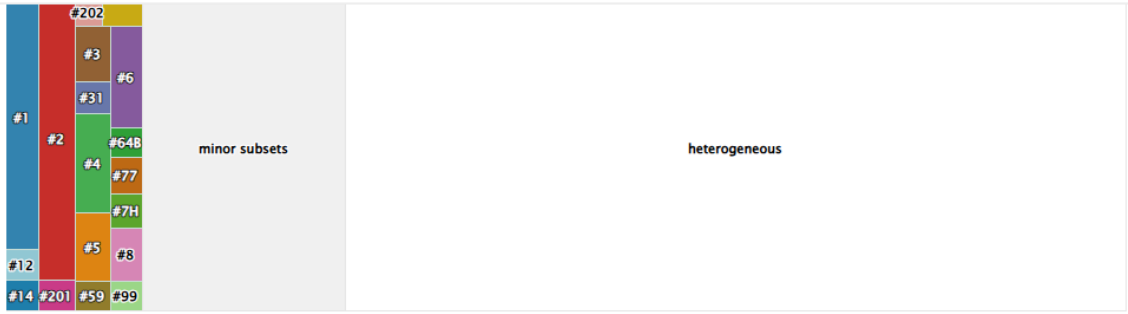
1

CLL#7H	CLL#28A	CLL#201	CLL#12	CLL#59	CLL#14	CLL#64B	CLL#99	CLL#202
0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%

assignment report table

label [+ heat map, if appl.]	SeqCure	subset	confidence	score
2026b	OK	CLL#2	high	74.65

ARREST result

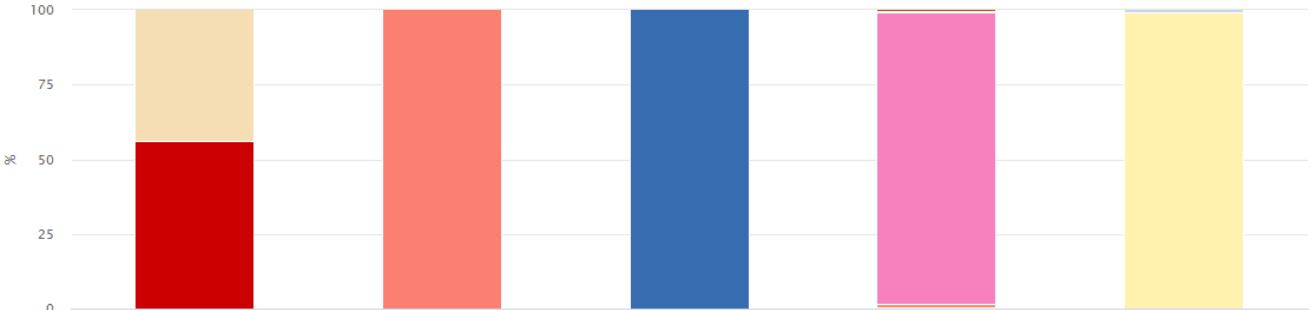


nothing

DESCRIPTION: major and minor subsets, and the heterogeneous cohort, organised as an interactive map
NOTE: by default, the placement of subsets is random, and therefore any conclusions apart from relative sizes is not relevan
- but, select e.g. 'mutational status' from the widget and subsets are organised per such category,
in this case 'unmutated' and 'mutated'
- then double-clicking on subsets zooms in, and hovering-over will show you such categories, then you can zoom in again...

cases	mutational status	V clan	CDR3 len	V gene	J gene	sequence logo	clinical	cytogenomic
#2	212	mixed	clanIII	9	IGHV3-21	IGHJ6	5	♦ – ♦ aggressive ♦ 1.9 (0–7.9) ♦ na [ref]	♦ SF3B1 mut: 36–45% ♦ ATM mut: 26% (21/81 ca ♦ TP53 aberrations: TP53 [ref][ref][ref][ref][ref][ref][ref]

barplot overview



Subset #2 and #8

Subsets #2 and #8 → associated with aggressive disease

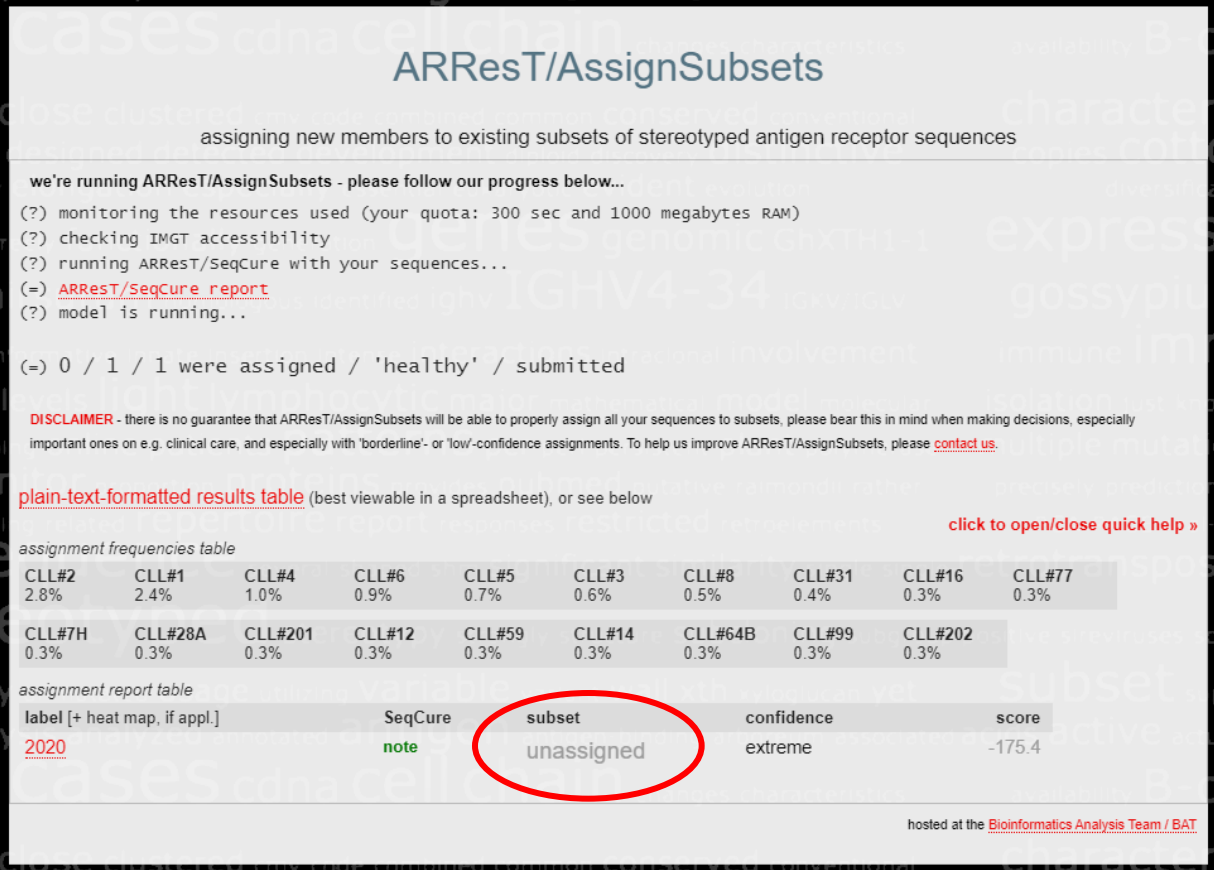
Subset #2 patients experience a particularly aggressive disease course, irrespective of their IGHV gene SHM status !

Membership of subset #2 was found to be an independent prognostic marker for shorter time-to-next-treatment (TTNT) and progression free survival (PFS), irrespective of the SHM status (Baliakas et al. Blood 2015).

Subset #8 has been associated with the highest risk for Richter's transformation among all CLL.

When another or no subset is identified the prognostic is dependant of the homology status of IGHM.

No subset


ARResT/AssignSubsets
assigning new members to existing subsets of stereotyped antigen receptor sequences
we're running ARResT/AssignSubsets - please follow our progress below...
(?) monitoring the resources used (your quota: 300 sec and 1000 megabytes RAM)
(?) checking IMGT accessibility
(?) running ARResT/SeqCure with your sequences...
(=) ARResT/SeqCure report
(?) model is running...
(=) 0 / 1 / 1 were assigned / 'healthy' / submitted
DISCLAIMER - there is no guarantee that ARResT/AssignSubsets will be able to properly assign all your sequences to subsets, please bear this in mind when making decisions, especially important ones on e.g. clinical care, and especially with 'borderline'- or 'low'-confidence assignments. To help us improve ARResT/AssignSubsets, please contact us.
plain-text-formatted results table (best viewable in a spreadsheet), or see below
click to open/close quick help »
assignment frequencies table
CLL#2 2.8%
CLL#1 2.4%
CLL#4 1.0%
CLL#6 0.9%
CLL#5 0.7%
CLL#3 0.6%
CLL#8 0.5%
CLL#31 0.4%
CLL#16 0.3%
CLL#77 0.3%
CLL#7H 0.3%
CLL#28A 0.3%
CLL#201 0.3%
CLL#12 0.3%
CLL#59 0.3%
CLL#14 0.3%
CLL#64B 0.3%
CLL#99 0.3%
CLL#202 0.3%
assignment report table
label [+ heat map, if appl.]
SeqCure
subset
confidence
score
2020
note
unassigned
extreme
-175.4
hosted at the Bioinformatics Analysis Team / BAT

Special case

278	2083	IGHV3-30_18	IGHJ6_02	10,90	10,90	0,00	Y	Y	100,00	Clonal	100,00
185	807	IGHV3/OR16-9_01	none	4,22	15,12	8,00	n/a	N	40,00	Clonal	92,00
291	282	IGHV4-4_07	IGHJ4_02	1,48	16,60	10,09	Y	Y	100,00	IGMT	IGHD3-16*01 F
296	230	IGHV3-7_03	IGHJ2_01	1,20	17,80	8,37	Y	Y	99,12	IGMT	

•(a) Low V-REGION identity (66.49%): this may indicate potential nucleotide insertion(s) and/or deletion(s) which are not dealt in this release.

Result summary: v	**Nucleotide deletions have been detected** (shown by dots in the alignments):			
localization	**nb of deleted nt**	**causing frameshift**	**from V-REGION codon**	**from nt position in user submitted sequence**
FR3-IMGT	**33**	**no**	**67**	**109**
IMGT/V-QUEST results after filling the deletion(s) gap(s): **No rearrangement found** (stop codons) (a) (Check also your sequence with BLAST against IMGT/GENE-DB reference sequences to eventually identify out-of-frame pseudogenes)				
V-GENE and allele	Homsap IGHV3-11*01 F	score = 367	identity = **66.49%** (123/185 nt) [**65.95%** (122/185 nt)] - (V-REGION partial in 5', missing nt nb= **69**)	
FR-IMGT lengths, CDR-IMGT lengths	[8.8.X]			

Single unproductive rearrangement

<0.1% of all CLL

Use an alternative set of primers
Use cDNA when gDNA was used
Repeat with a new sample
Perform NGS analysis

- carry pseudogenes
- out-of-frame rearrangement (deletion/insertion)
- stop codon introduced by somatic hypermutation

leading to frameshifts within the coding part of the sequence.

Unproductive rearrangement – SHM status not determined:
NO clinical association possible

Double rearrangements

one productive/one unproductive (~8% of CLL)

IGHV3-9_01	IGHJ6_02	32,97	32,97	0,00	N	N	99,56	Clonal	100,00	Non Muté	Non Muté	Non fonctionnelle
IGHV1-2_02	IGHJ4_02	14,40	47,38	0,00	Y	Y	100,00	Clonal	100,00	Non Muté		fonctionnelle
IGHV3-7_03	IGHJ3_02	38,05	38,05	6,17	Y	Y	98,24	Clonal	93,83	Muté		fonctionnelle
IGHV3-66_03	IGHJ4_02	21,17	59,22	6,70	n/a	N	93,30	Clonal	93,30	Muté		non fonctionnelle

No evidence supporting any kind of biological and/or clinical relevance for unproductive IG gene rearrangements in CLL.

Same as for standard cases: mutational status defined by the productive rearrangement, irrespective of the SHM status of the unproductive rearrangement.

Double rearrangements

Double productive: concordant SHM status

IGHV3-23_04	IGHJ5_02	34,70	34,70	3,52	Y	Y	97,36	Clonal	96,48	Muté	Muté	fonctionnelle
IGHV3-23_04	IGHJ5_02	13,94	48,63	3,08	Y	Y	97,36	Clonal	96,92	Muté		fonctionnelle

Same as for standard cases i.e., consider as M-CLL or U-CLL, according to the SHM status.

Double rearrangements

Double productive: disconcordant SHM status (<0.1 %)

IGHV1-69_13	IGHJ6_02	35,10	35,10	0,00	Y	Y	99,56	Clonal	100,00	Non Muté	Non Concluant	fonctionnelle
IGHV3-21_02	IGHJ4_02	24,65	59,75	2,64	Y	Y	100,00	Clonal	97,36	Muté		fonctionnelle
IGHV7-4-1_01	IGHJ5_02	26,22	26,22	5,10	n/a	N	98,98					94,90
IGHV3-21_04	IGHJ6_02	14,77	41,00	6,42	Y	Y	97,97					93,58
IGHV1-69_13	IGHJ6_02	35,10	35,10	0,00	Y	Y	99,56	Clonal		100,00		Non Muté
IGHV3-21_02	IGHJ4_02	24,65	59,75	2,64	Y	Y	100,00	Clonal		97,36		Muté
IGHV1-69_13	IGHJ6_02	5,39	65,14	0,00	Y	Y	99,56	IGHD3-3*01 F		IGMT 100.00%		productif
IGHV1-69_13	IGHJ6_02	0,47	65,62	0,44	Y	Y	99,56	IGHD6-19*01 F		IGMT 97.26%		productif

Check immunophenotype for the presence of 2 clonal populations.
Recommend to the physician that it is safer to consider as U-CLL; close follow-up.

Double rearrangements

The identification of two, unrelated productive IGH rearrangements could be due to the co-existence of two independent B cell clones:
either a CLL clone and a separate, non-CLL B cell clone (i.e., a different malignancy)
or two distinct CLL cell clones.

In terms of prognosis, CLL cases with two B cell clones (a CLL and a non-CLL) have been reported to display earlier need for treatment against cases with monoclonal CLL; this may reflect a stronger clinical relevance of the other B cell malignancy compared to CLL

Multiple rearrangements

Multiple (>2) productive rearrangements
Check immunophenotype for the presence of 2 or more clonal populations.

Perform NGS to assess the relative frequency of each clonotype and consider the predominant clonotype, if it is clearly identified

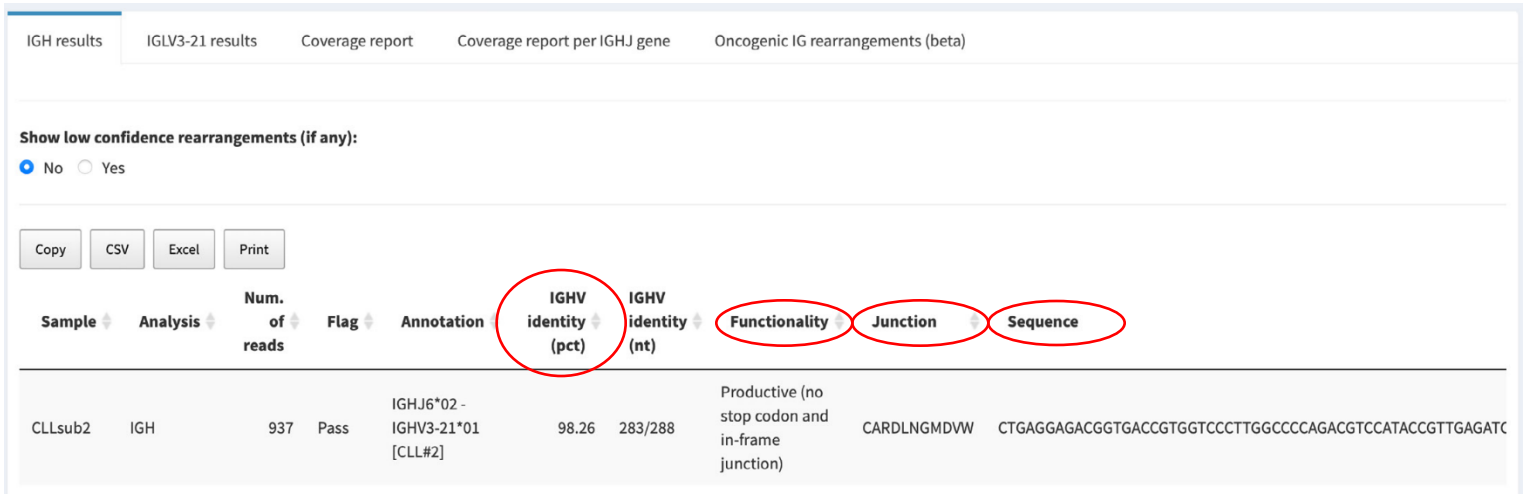
Kit NGS SOPHiA GENETICS

Kit SOPHIA DDM™ Custom Solution (CCLL_A_V3) ; SOPHiA GENETICS = **all in one application**
Combination with SHM to detect prognostically important CLL single nucleid variants, insertions/deletions and copy number variants (*TP53*).

Identification of
23 CLL-specific genes for SNVs, InDels and CNVs including *NOTCH1*, *SF3B1*, *ATM*, *TP53*, IGLV3-21, *BTK*, *PLCG2*, *BCL2*, del13q, del11q, del17p and trisomy 12, all in **one single NGS workflow**.

List of genes:
ATF1, ATM, BCL2, BIRC3, BTK (exon 15), *CDK4, CUL4A* (exon 1-5), *CXCR4* (exon 1-3), *DLEU1, EGR2, FBXW7, KLF5, KRAS, MYD88, NFKBIE, NOTCH1* (exon 34), *PLCG2* (exon 19, 20, 24), *POT1, PROZ, RB1, SF3B1* (exon 14-16, 18), *TP53, XPO1* (exon 15, 16).

IgCaller (SOPHiA GENETICS)



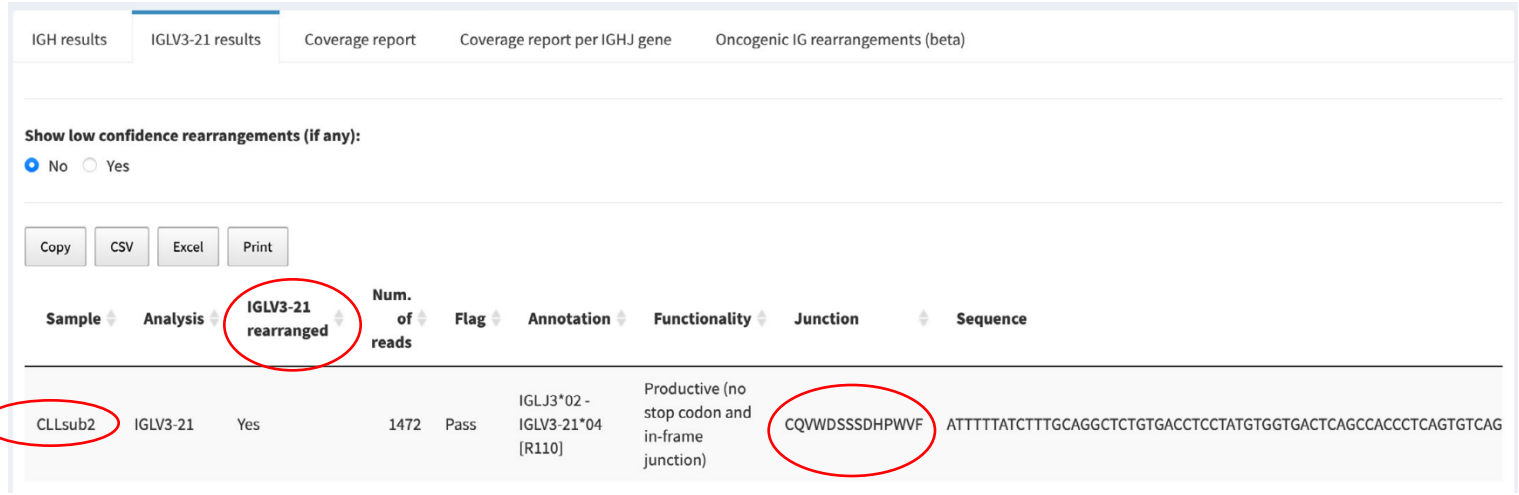
IGH results | IGLV3-21 results | Coverage report | Coverage report per IGHJ gene | Oncogenic IG rearrangements (beta)

Show low confidence rearrangements (if any):

No Yes

Copy CSV Excel Print

Sample	Analysis	Num. of reads	Flag	Annotation	IGHV identity (pct)	IGHV identity (nt)	Functionality	Junction	Sequence
CLLsub2	IGH	937	Pass	IGHJ6*02 - IGHV3-21*01 [CLL#2]	98.26	283/288	Productive (no stop codon and in-frame junction)	CARDLNGMDVW	CTGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAGACGTCCATACCGTTGAGATC



IGH results | IGLV3-21 results | Coverage report | Coverage report per IGHJ gene | Oncogenic IG rearrangements (beta)

Show low confidence rearrangements (if any):

No Yes

Copy CSV Excel Print

Sample	Analysis	IGLV3-21 rearranged	Num. of reads	Flag	Annotation	Functionality	Junction	Sequence
CLLsub2	IGLV3-21	Yes	1472	Pass	IGLJ3*02 - IGLV3-21*04 [R110]	Productive (no stop codon and in-frame junction)	CQVWDSSSDHPWVF	ATTTTTATCTTTGCAGGCTCTGTGACCTCCTATGTGGTGACTCAGCCACCCTCAGTGTCAG

Conclusion for the CLL SOPHiA GENETICS kit

One workflow offers analysis of SNVs, InDels and CNVs and HMIGH (incl. IgL)

Rapid analysis of all what is recommended in CLL
Check *TP53* variants

IgCaller interpretation has also to be done with IMGT !

SPECIAL REPORT | JUNE 21, 2018

iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL

Clinical Trials & Observations

Michael Hallek, Bruce D. Cheson,Daniel Catovsky,Federico Caligaris-Cappio,Guillermo Dighiero,Hartmut Döhner,Peter Hillmen,Michael Keating,Emili Montserrat,Nicholas Chiorazzi,Stephan Stilgenbauer,Kanti R. Rai,John C. Byrd,Barbara Eichhorst,Susan O'Brien,Tadeusz Robak, John F. Seymour, Thomas J. Kipps

Immunoglobulin Gene Sequence Analysis in Chronic Lymphocytic Leukemia: The 2022 Update of the Recommendations by ERIC, the European Research Initiative on CLL (*Agathangelidis*, Leukemia 2022)

https://www.compermed.be/fr/workflows/chronic-lymphocytic-leukemia-cll

LETTER
2024 Feb 16;38(3):679–680. doi: 10.1038/s41375-024-02163-4

Updates of the ERIC recommendations on how to report the results from immunoglobulin heavy variable gene analysis in chronic lymphocytic leukemia

Thomas Chatzikonstantinou [1], Andreas Agathangelidis [1,2], Anastasia Chatzidimitriou [1,3], Cristina Tresoldi [4], Zadie Davis [5], Véronique Giudicelli [6], Sofia Kossida [6], Chrysoula Belessi [7], Richard Rosenquist [3,8], Paolo Ghia [9,✉], Anton W Langerak [10], Frédéric Davi [11], Kostas Stamatopoulos [1,3]; ERIC, the European Research Initiative on CLL
•PMCID: PMC10912022 PMID: 38366088

IGHV unmutated
Patients display shorter progression-free survival when treated with fixed-duration treatment such as venetoclax plus obinutuzumab.

Subset #2
Patients belonging to CLL stereotype subset #2 is a prognostically adverse patient group regardless the SHM status.

Report

- **Basic data**: patient data
  tissue type
  sample arrival date
  reference

- **Technique**: Sanger sequencing, NGS, …
  gDNA or cDNA
  PCR primers/kit
  bioinformatics tools for SHM status assessment

- **Results**: % identity to germline to 2 decimal points
  cut-off U-CLL ≥ 98%; M-CLL < 98%;
  remark when 97- 97.99%.
  IGHV/IGHD/IGHJ gene usage
  functionality: productive/unproductive

- SHM status determined only for **productive** rearrangements

- **Conclusion**: interpretation of data (mutated, unmutatd, borderline)
  clinical association: poor/good prognostic

- No clear interpretation possible -> prognostic implication cannot be determined

- **Subset** identification/BcR IG stereotype for subsets with well-established prognostic value (currently, subsets #2 and #8)

Example typical case I

IGHV: 3-72
IGHD: 2-2
IGHJ: 6
Identity: **95.7%**
Functionality: Productive

IGHV mutated rearrangement
Mutated IGHV genes >98% identity have been associated with a **good clinical outcome**

1. Example of the IG report, IG - mutated

Name of the Hospital/Lab

Determination of IGHV gene SHM status

ERIC example

Date of result:

22/01/2022

Date of sample collection:

09/01/2022

Patient name: ***
Diagnosis: CLL
Tissue type: blood
Molecule type: genomic DNA

Utilized methodology
PCR amplification of IGHV-IGHD-IGHJ gene rearrangements with leader primers.
Genescan analysis
Bidirectional Sanger sequencing
Immunoinformatics analysis: IMGT/V-QUEST

Result: a productive IGHV3-23*01/IGHD4-17*01/IGHJ4*02 gene was detected. The rearranged IGHV gene had 96.2% nucleotide identity with the germline sequence of the IGHV3-23*01 gene.

Interpretation: following the 98% germline identity cut-off value which is used for discriminating CLL cases into the IG-mutated or IG-unmutated category, this case belongs to the IG-mutated category which is generally associated with favorable prognosis.

Example typical case II

IGHV: 1-69
IGHD: 3-3
IGHJ: 4
Identity: **100%**
Functionality: Productive

IGHV unmutated rearrangement
Unmutated IGHV genes ≥98% identity have been associated with a **poor clinical outcome**.

New:

Patients display shorter progression-free survival when treated with fixed-duration treatment such as venetoclax plus obinutuzumab.

Name of the Hospital/Lab

Determination of IGHV gene SHM status

ERIC example

Date of result: 05/09/2016
Date of sample collection: 12/08/2016

Patient name: ***
Diagnosis: CLL
Tissue type: blood
Molecule type: genomic DNA

Utilized methodology
PCR amplification of IGHV-IGHD-IGHJ gene rearrangements with leader primers.
Genescan analysis
Bidirectional Sanger sequencing
Immunoinformatics analysis: IMGT V-Quest

Result: a productive IGHV3-49*01/IGHD3-9*01/IGHJ4*02 gene was detected. The rearranged IGHV gene had 100% nucleotide identity with the germline sequence of the IGHV3-49*01 gene.

Interpretation: following the 98% germline identity cut-off value which is used for discriminating CLL cases into the IG-mutated or IG-unmutated category, this case belongs to the IG-unmutated category which is generally associated with adverse prognosis.

Signatures

Example borderline

IGHV: 3-48
IGHD: 2-21
IGHJ: 3
Identity: **97.8%**
Functionality: Productive

IGHV mutated rearrangement with borderline identity (close to cut-off of 98%)
Caution should be taken with the interpretation of the clinical correlation

Name of the Hospital/Lab

Determination of IGHV gene SHM status

ERIC example

Date of result: 05/09/2016
Date of sample collection: 12/08/2016

Patient name: ***
Diagnosis: CLL
Tissue type: blood
Molecule type: genomic DNA

Utilized methodology
PCR amplification of IGHV-IGHD-IGHJ gene rearrangements with leader primers.
Genescan analysis
Bidirectional Sanger sequencing
Immunoinformatics analysis: IMGT V-Quest

Result: a productive IGHV3-49*01/IGHD3-9*01/IGHJ4*02 gene was detected. The rearranged IGHV gene had 97.3% nucleotide identity with the germline sequence of the IGHV3-49*01 gene.

Interpretation: following the 98% germline identity cut-off value which is used for discriminating CLL cases into the IG-mutated or IG-unmutated category, this case belongs to the IG-mutated category. However, the identity percentage is close to the cut-off and, thus, the case can be considered as borderline-mutated. In such cases, caution is warranted regarding the precise prognostic implications.

Signatures

Example special case I

IGHV: **3-21**
IGHD:-
IGHJ: 6
Identity: 96.7%
Functionality: Productive
Subset #2

IGHV3-21 mutated rearrangement with a stereotyped **subset #2**.
The presence of a mutated IGHV3-21 with stereotypy have been associated with a **poor clinical outcome**.

New:

Patients belonging to CLL stereotype subset #2 is a prognostically adverse patient group regardless the SHM status.

Name of the Hospital/Lab

Determination of IGHV gene SHM status

ERIC example

Date of result: 05/09/2016
Date of sample collection: 12/08/2016

Patient name: ***
Diagnosis: CLL
Tissue type: blood
Molecule type: genomic DNA

Utilized methodology
PCR amplification of IGHV-IGHD-IGHJ gene rearrangements with leader primers.
Genescan analysis
Bidirectional Sanger sequencing
Immunoinformatics analysis: IMGT V-Quest, ARResT/AssignSubsets tool

Result: a productive IGHV3-21*01/IGHD: not determined/IGHJ6*02 gene was detected. The rearranged IGHV gene had 96.8% nucleotide identity with the germline sequence of the IGHV3-21*01 gene.

Interpretation: following the 98% germline identity cut-off value which is used for discriminating CLL cases into the IG-mutated or IG-unmutated category, this case belongs to the IG-mutated category. However, this particular rearrangement belongs to stereotyped subset #2 which is associated with adverse prognosis regardless of the somatic hypermutation status (Baliakas et al. Blood 2015).

Signatures

Example special case II

Rearrangement 1
IGHV: 1-69
IGHD: 3-16
IGHJ: 6
Identity: **99.6%**
Functionality: **Productive**

Rearrangement 2
IGHV: 3-30
IGHD: 3-3
IGHJ: 4
Identity: **100%**
Functionality: **Productive**

2 unmutated IGHV rearragements and would be interpretated as expressing **unmutated IGHV** genes. Unmutated IGHV genes (≥98%) are associated with a poor clinical outcome.

Example special case III

Rearrangement 1
IGHV: 1-69
IGHD: 3-3
IGHJ: 6
Identity: **99.6%**
Functionality: **Productive**

Rearrangement 2
IGHV: 4-34
IGHD: 3-22
IGHJ: 4
Identity: **100%**
Functionality: **Unproductive**

Productive IGHV unmutated rearrangements and an unproductive IGHV unmutated rearrangement. Altogether this has to be interpretated as a case with unmutated IGHV genes. Unmutated IGHV genes (≥98% identity) are associated with a poor clinical outcome.

Example difficult case I

Rearrangement 1
IGHV: 1-69
IGHD: 3-3
IGHJ: 6
Identity: **93.6%**
Functionality: Productive

Rearrangement 2
IGHV: 4-34
IGHD: 3-22
IGHJ: 4
Identity: **100%**
Functionality: Productive

A **productive IGHV mutated rearrangements** and a **productive IGHV unmutated** rearrangement.
Consider as unmutated IGHV; close follow-up.

Example difficult case II

IGHV: 1-3
IGHD: 3-3
IGHJ: 6
Identity: 100**%**
Functionality: **Unproductive**

An **unproductive IGHV unmutated** rearrangement (≥98% identity).
At present the clinical correlation cannot be defined.

Double rearrangements ~10%

Same mutational status
Mutated/mutated or unmutated/unmutated

Productive + productive

Interpretation
Mutated or unmutated

Productive + unproductive

Interpretation

Productive unmutated + unproductive unmutated = unmutated

Productive mutated + unproductive mutated = mutated

Different mutational status

Unprod mutated + prod unmutated = Unmutated

Unprod unmutated + prod mutated = Mutated

Prod mutated + prod unmutated = Consider as unmutated

Advice

Follow:

ERIC guidelines

Difficult cases: ERIC helps www.ERICLL.org

ERIC site helpdesk

www.ericll.org

- Ghia P et al. ERIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukemia. Leukemia 2007;21:1-3.
- Rosenquist R et al. and ERIC. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: updated ERIC recommendations. Leukemia 2017; 31(7): 1477-1481.

- Difficult cases: ERIC helps
- (https://barcelo.eventsair.com/submission-of-ighv-sequences/ighv-sequences/Site/Register)

- QC

- Workshops

Become an ERIC Member
Joining ERIC could not be easier:
It is quick, simple and free!

Advances of analysis by NGS

- Accurate (ERIC 3-4% no interpretation with other technique)
- Easy interpretation
- More detailed insight into subclonal architecture and intraclonal diversity
- Combination with other assays in one NGS workflow
- Very sensitive
- Quantitative
- Monitoring of clonal proliferations possible

- Need NGS equipment, expensive

Take home message

NGS better interpretation rate
than Sanger sequencing

Follow the ERIC guidelines

Interpretation always with IMGT (+ BLAST)

Think of ERIC helpdesk