IgH hypermutation analysis

Dr. Sci. Sabine Franke

9/2/2024

Molecular Biology and Cytometry Course

Why investigate the Ig gene hypermutation status?

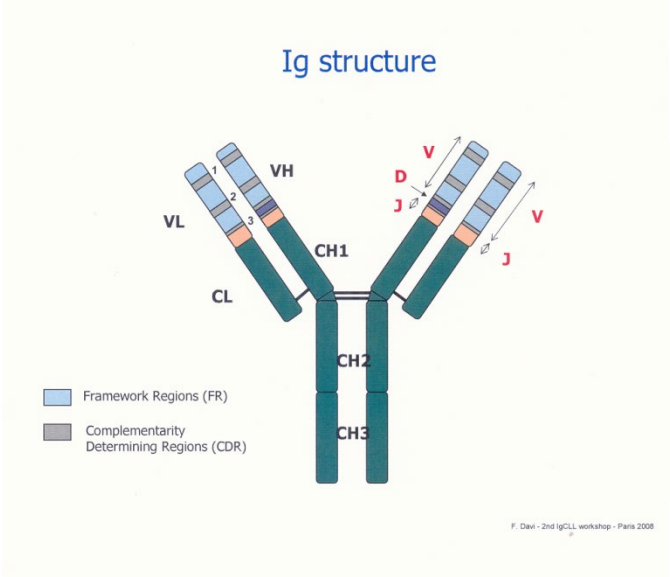
The somatic hypermutation status of the immunoglobulin heavy variable (IGHV) gene is a biomarker for assessing the prognosis of patients with chronic lymphocytic leukemia (CLL) and predictor of responses to therapy.

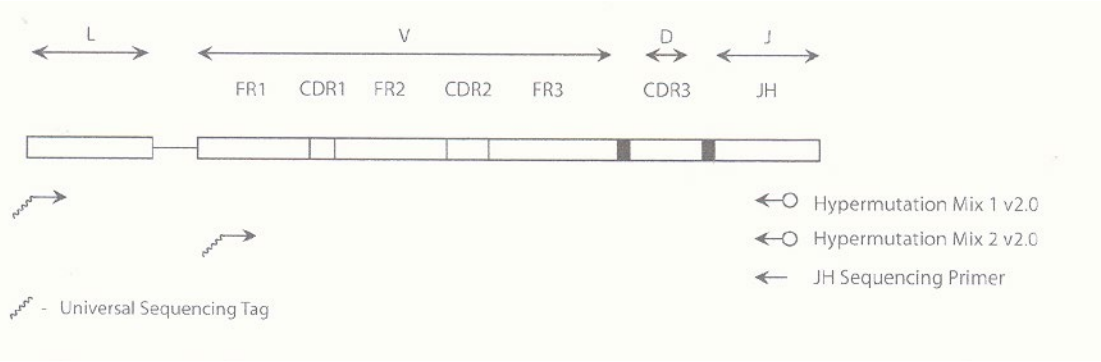
- This biomarker remains stable over time
- This biomarker should be assessed prior to treatment in all patients with CLL

The ComPerMed recommends (1/2024) to test for I GHV status in all patients with active or advanced disease without del(17p)/ TP53 mutation and in need for therapy.

Removed: Mutational status IGHV
Only reimbursed for patients younger than 65y

Ig gene structure





Invivoscribe

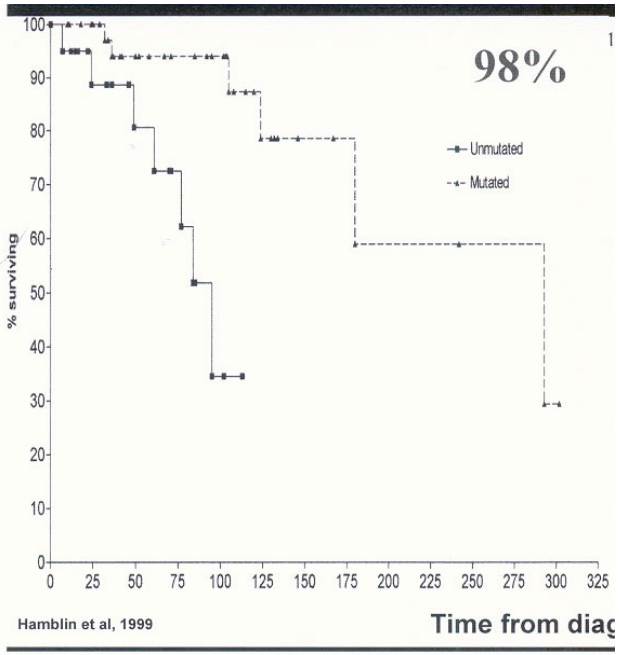
Somatic hypermutation takes place in the V region of both H and L chain genes, introducing a million times point mutations. This process followed by selection leads to generation of high-affinity antibodies.

IGHV mutation status

CLL arises from relatively less differentiated B cells with unmutated heavy chain genes and has a poor prognosis.
Patients with unmutated IgVH genes have not generated IgV gene mutations.

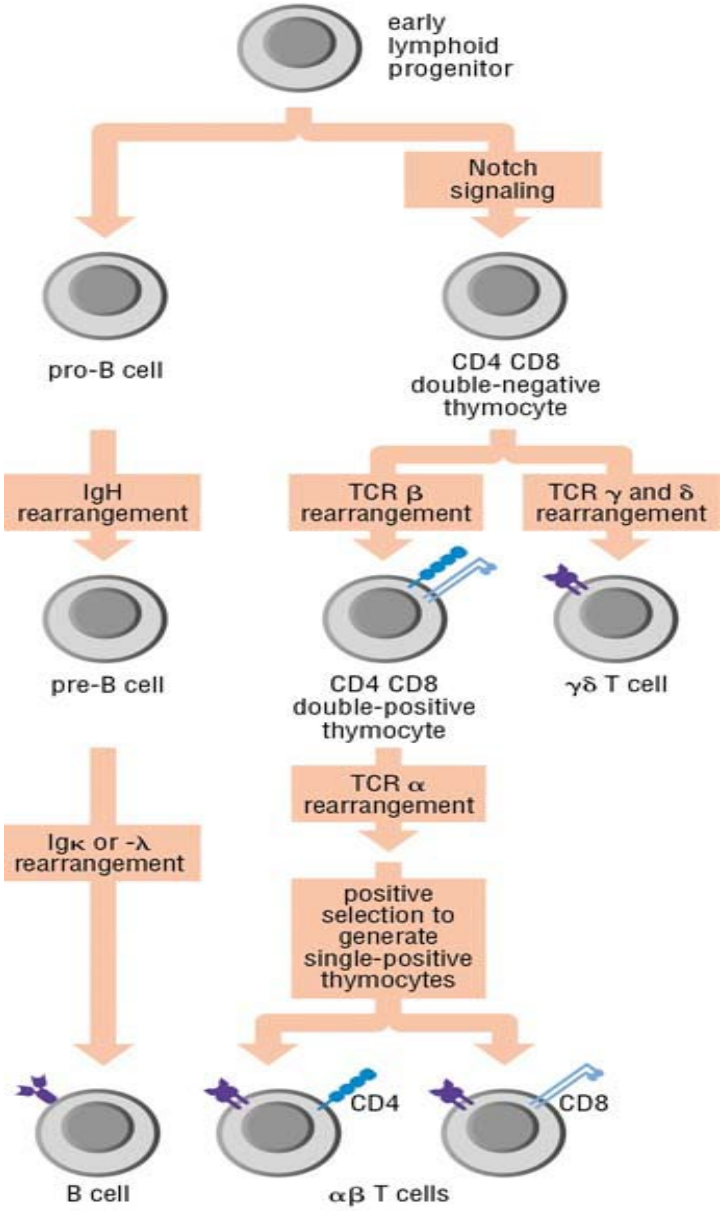
When CLL evolves from more differentiated B lymphocytes with somatically mutated heavy chain genes it has a good prognosis.

Compare patient's sequence with consensus germline sequence



Homology ≥ 98% = unmutated
Homology <98% = mutated

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

Patients with unmutated IGVH genes have not generated
IgV gene mutations.

Recommondations

The presence of mutated IGHV genes, with additional prognostic factors (favorable cytogenetics), characterizes a CLL patient subgroup with excellent outcome.

The BHS guidelines recommend chemoimmunotherapy in patients without del(17p)/ TP53 mutation with mutated IGVH.5Techniquenext-generation.
Technique: next generation sequencing (NGS), Sanger sequencing

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

ERIC certificate

IG CERTIFIED CENTRES
BELGIUM
Brugge, **AZ Sint-Jan Brugge (February 2020 / July 2023)**
Brussels, **Erasme Hospital (June 2017)**
Brussels, **Universitair Ziekenhuis Brussel (December 2018)**
Liege, **CHU Liège (September 2016 / January 2021)**
Leuven, **UZ Leuven (June 2017)**

Technics

Heteroduplex analysis:
Advantage:
- unlabeled products direct sequencing
Disadvantage:
- lower detection limit

GeneScan fragment analysis:
Advantage:
- Optimal visualization
- Cost

Disadvantage:
- Monoallelic with background/biallelic: no direct sequencing: demands gel excision + elution
- Limited sensitivity

NGS (next generation sequencing)
Advantage:
- Sensitive (low infiltration)
- Acurate (ERIC other technic 3-4% no interpretation)
- Analysis of monoallelic with background/biallelic (pas gel)
- More detailed insight into the existence of minor related clonotypes or unrelated clonotypes
- Combine with other clonality assays in one NGS workflow

Disadvantage:
- Expensive
- Equipment

Somatic Hypermutation

Invivoscribe: GeneScan fragment analysis
Biomed

Invivoscribe: LymphoTrack Dx Assay Kit A MiSeq
IGHV leader
(FR1, FR2, FR3, kappa, TRG, TRB)
+ LymphoTrack software IVS (based on IgBlast database)

ThermoFisher: Oncomine IGHV Leader-J Panel, …

Euroclonality: NGS IG/TR assays

…….

Think also about IVDR

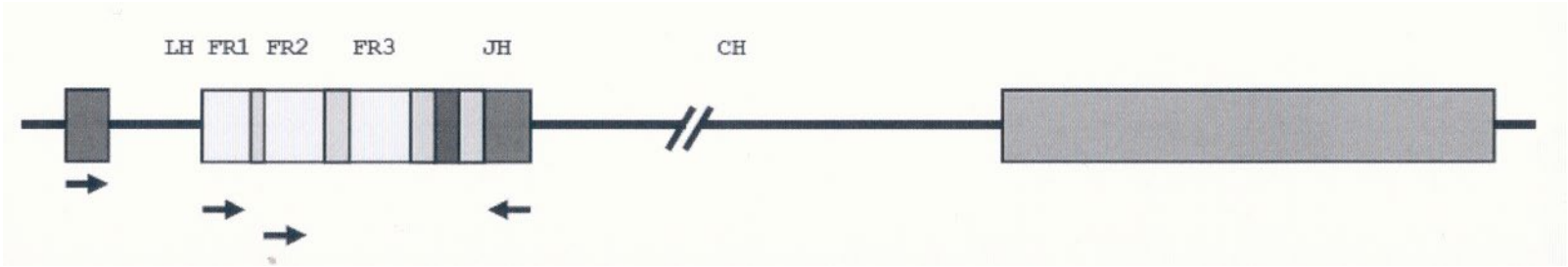
Type of nucleid acid

gDNA:

better for transport

use archival material

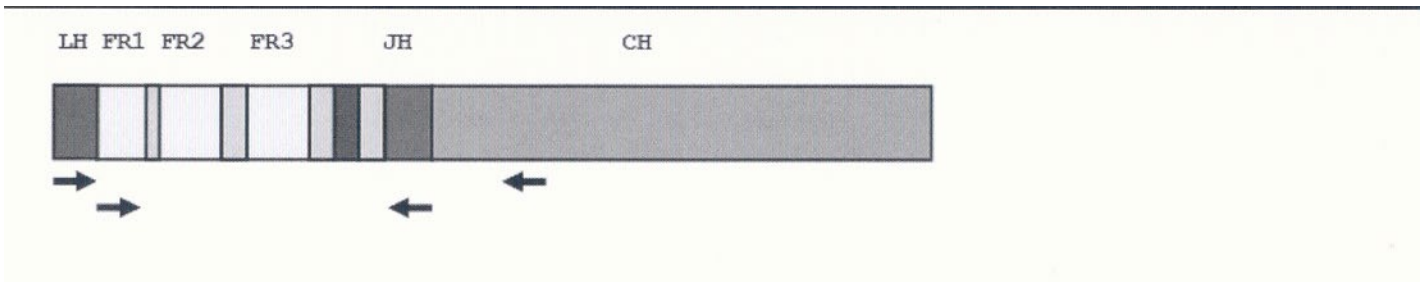
but: non-productive rearrangement can also be amplified



cDNA:

identifies mostly only functional productive rearrangement

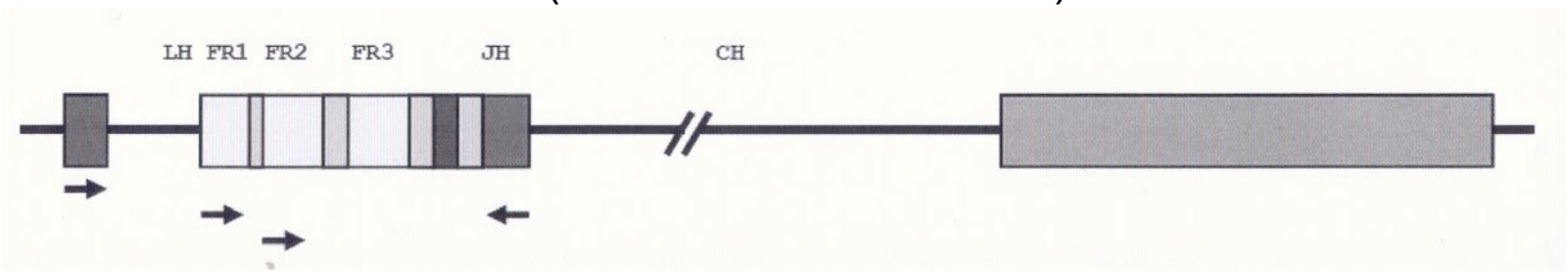
but reverse transcription step necessary



PCR primers

IGHV leader:
accurate SHM level
based on whole IGHV gene
but lower detection rate

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



Primer sets: IGHV leader primers
IGHV FR1 primers: FR1 consensus – JH
FR1 multiplex –JH (BIOMED2)
IGHV FR2 primers: when leader or FR1 are negative ->NO
IGHV FR3 primers: short IGHV sequences -> NO

Sanger Sequencing

Direct, both strands Sequencing of both strands is mandatory for the generation of a single, high quality IGH gene rearrangement sequence!

NGS Lymphotrack workflow

DNA

Amplification

Purification PCR products

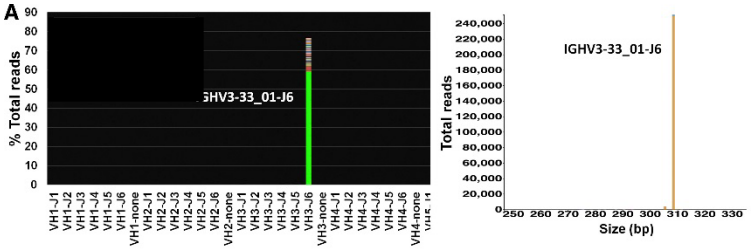
Library quantification

Run prep

FASTQ files

Analysis

Clone identification



Adapted from M.E. Arcile et al. The Journal of Molecular Diagnostics

NGS Lymphotrack software analysis

Fastq_read_summary:

- Contains list with 200 most important unique sequences identified
- The size
- The % of actual total reads
- The percentage of cumulative total reads
- To what extent the reading covers the identified V gene (%), targeted by the primers
- The mutation rate of the partial V gene (%)
- Whether the rearrangement is located inside the frame
- Whether the rearrangement contain a termination codon
- The software also creates a PDF report file, this file includes the top 10 merged sequence reads

LymphoTrack Dx Report for assay IGH_FR1

Sample name: S19-14-220317-0118_S19_L001_001_combined

Total Read Count: 187450

IndexQ30: 87.12

Caution: Do not edit fields and save.

Top 10 Merged Read Summary

Rank	Sequence	Length	Merge count	V-gene	J-gene	% total reads	Cumulative %	Mutation rate to partial V-gene (%)	In-frame (Y/N)	No Stop codon (Y/N)	V-coverage	CDR3 Seq
1	GCCTCTGGATTCA	275	59786	IGHV3-23_04	IGHJ4_02	31,89	31,89	3,52	Y	Y	97,80	GCGGCGAGTTTG
2	GCCTCTGGATTCA	275	38214	IGHV3-23_04	IGHJ4_02	20,39	52,28	3,52	Y	Y	97,80	GCGGCGAGTTTG
3	GCCTCTGGATTCA	275	8142	IGHV3-23_04	IGHJ4_02	4,34	56,62	2,20	Y	Y	97,80	GCGGCGAGTTTG
4	GCCTCTGGATTCA	253	112	IGHV3-13_01	IGHJ5_02	0,06	56,68	0,00	N	N	99,11	not found
5	GCCTCTGGATTCA	260	105	IGHV3-9_01	IGHJ4_02	0,06	56,74	0,00	Y	Y	99,56	not found
6	GCCTCTGGATTCA	266	99	IGHV3-13_01	IGHJ4_02	0,05	56,79	0,00	Y	Y	99,55	not found
7	GCCTCTGGATTCA	261	99	IGHV3-23_04	IGHJ5_02	0,05	56,85	0,00	n/a	N	98,68	not found
8	GCCTCTGGATTCA	273	96	IGHV3-13_01	IGHJ4_02	0,05	56,90	0,00	N	N	96,43	not found
9	GCCTCTGGATTCA	266	95	IGHV3-21_02	IGHJ6_02	0,05	56,95	0,00	Y	Y	98,68	not found
10	GCCTCTGGATTCA	260	93	IGHV3-13_01	IGHJ6_02	0,05	57,00	0,00	n/a	N	91,52	not found

Table of results

N°		reads total	Rank	Sequence	Length	Raw count	V-gene	J-gene	% total reads	Mut rate partial V-gene (%)	In-frame	No Stop codon	V-coverage
IGH POS		430983	1	GGTCTTCTGCTTGC	490	11303	IGHV1-46_03	IGHJ4_02	2,62	0,00	Y	Y	100,00
			2	GGTCTTCTGCTTGC	490	384	IGHV1-46_03	IGHJ4_02	0,09	0,34	Y	Y	100,00
			3	GGTCTTCTGCTTGC	490	281	IGHV1-46_03	IGHJ4_02	0,07	0,00	Y	Y	100,00
			4	GGTCTTCTGCTTGC	490	276	IGHV1-46_03	IGHJ4_02	0,06	0,34	Y	Y	100,00
NGS NEG		394294	1	GATCCTCTTTTTGG	333	215	IGHV1-3_02	IGHJ6_02	0,05	1,35	n/a	N	50,34
			2	CTCGCCCTCCTCCT	342	215	IGHV5-51_02	IGHJ4_02	0,05	0,34	N	N	32,43
			3	CTCGCCCTCCTCCT	321	187	IGHV5-51_02	IGHJ6_03	0,05	2,03	Y	Y	25,00
			4	CTCGCCCTCCTCCT	342	184	IGHV5-51_02	IGHJ4_02	0,05	0,34	N	N	32,43
SHM POS		573581	1	TTCTCGTGGTGGCA	455	35537	IGHV4-59_08	IGHJ4_02	6,2	11,26	Y	Y	98,63
			2	TTCTCGTGGTGGCA	455	485	IGHV4-59_08	IGHJ4_02	0,08	11,60	Y	Y	98,63
			3	TCTCGTGGTGGCAG	454	392	IGHV4-59_08	IGHJ4_02	0,07	11,26	Y	Y	98,63
			4	TTCTCGTGGTGGCA	455	365	IGHV4-59_08	IGHJ4_02	0,06	11,26	Y	Y	98,63
220121-0061		766237	1	GGTTTTCCTTGTTG	467	25773	IGHV3-21_02	IGHJ6_02	3,36	3,04	Y	Y	98,99
			2	GGTTTTCCTTGTTG	467	15793	IGHV3-21_02	IGHJ6_02	2,06	2,70	Y	Y	98,99
			3	GGTTTTCCTTGTTG	467	15375	IGHV3-21_02	IGHJ6_02	2,01	3,04	Y	Y	98,99
			4	TCCTGCTGGTGGCA	484	14630	IGHV4-31_06	IGHJ6_02	1,91	2,76	n/a	N	79,66

Clones with abundance < 2% of total reads = background

N°		reads total	Rank	Sequence	Length	Raw count	V-gene	J-gene	% total reads	Mut rate partial V-gene (%)	In-frame	No Stop codon	V-coverage
IGH POS		430983	1	GGTCTTCTGCTTGC	490	11303	IGHV1-46_0	IGHJ4_02	2,62	0,00	Y	Y	100,00
			2	GGTCTTCTGCTTGC	490	384	IGHV1-46_0	IGHJ4_02	0,09	0,34	Y	Y	100,00
			3	GGTCTTCTGCTTGC	490	281	IGHV1-46_0	IGHJ4_02	0,07	0,00	Y	Y	100,00
			4	GGTCTTCTGCTTGC	490	276	IGHV1-46_0	IGHJ4_02	0,06	0,34	Y	Y	100,00
NGS NEG		394294	1	GATCCTCTTTTTGG	333	215	IGHV1-3_02	IGHJ6_02	0,05	1,35	n/a	N	50,34
			2	CTCGCCCTCCTCCT	342	215	IGHV5-51_0	IGHJ4_02	0,05	0,34	N	N	32,43
			3	CTCGCCCTCCTCCT	321	187	IGHV5-51_0	IGHJ6_03	0,05	2,03	Y	Y	25,00
			4	CTCGCCCTCCTCCT	342	184	IGHV5-51_0	IGHJ4_02	0,05	0,34	N	N	32,43
SHM POS		573581	1	TTCTCGTGGTGGCA	455	35537	IGHV4-59_08	IGHJ4_02	6,2	11,26	Y	Y	98,63
			2	TTCTCGTGGTGGCA	455	485	IGHV4-59_08	IGHJ4_02	0,08	11,60	Y	Y	98,63
			3	TCTCGTGGTGGCAC	454	392	IGHV4-59_08	IGHJ4_02	0,07	11,26	Y	Y	98,63
			4	TTCTCGTGGTGGCA	455	365	IGHV4-59_08	IGHJ4_02	0,06	11,26	Y	Y	98,63
220121-0061		766237	1	GGTTTTCCTTGTTG	467	25773	IGHV3-21_0	IGHJ6_02	3,36	3,04	Y	Y	98,99
			2	GGTTTTCCTTGTTG	467	15793	IGHV3-21_0	IGHJ6_02	2,06	2,70	Y	Y	98,99
			3	GGTTTTCCTTGTTG	467	15375	IGHV3-21_0	IGHJ6_02	2,01	3,04	Y	Y	98,99
			4	TCCTGCTGGTGGCA	484	14630	IGHV4-31_06	IGHJ6_02	1,91	2,76	n/a	N	79,66

GGTTTTCCTTGTTGCTATTTTAGAAGGTGAATCATGGAAAAGTAGAGAGATTTAGTGTGTGTGGATATGAGTGAGAGAAACGGTGGATGTGTGTGACAGTTTCTGA
CCAATGTCTCTCTGTTTGCAGGTATCCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTC
TGGATTCACCTTCAGTAACTATAACATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCATCCATTATTAGTAGTAGTAGTTACATATACTACG
CAGACTCAGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTGTCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTATATTACTG
TGCGAGAGATATGAACGGTATGGACGTCTGGGGCCAAGGGACCAC

Sequence analysis

Lymphotrack Invovoscribe

http://imgt.cines.fr/IMGT_vquest/vquest

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

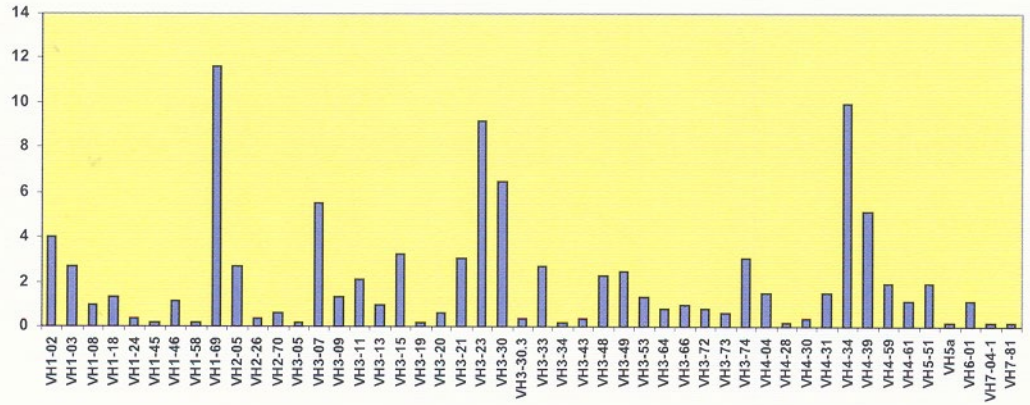
Somatic hypermutations

Mostly point mutations

Transitions (e;g; C>T, G>A) are more frequent than transversions (e.g. C>A or G, G>C or T)

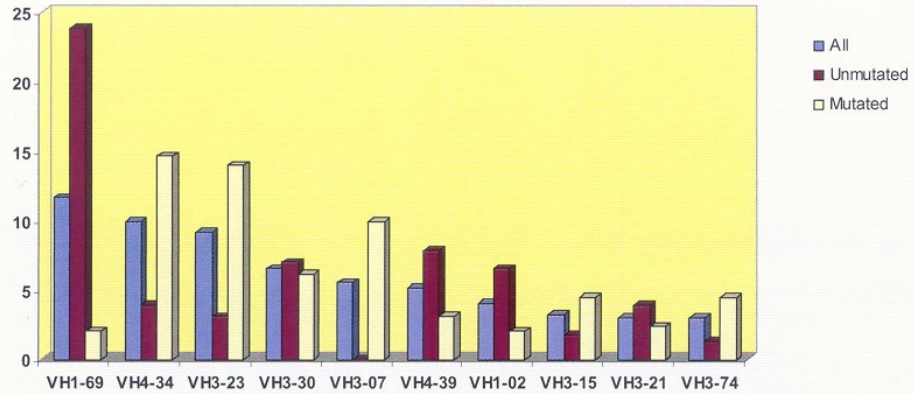
IGHV repertoire in CLL

(Ghia, *Blood 2004*)



IGHV repertoire in CLL

(Ghia, *Blood 2004*)



More unmutated then mutated cases

Result NGS

D:\Runs_MiSeq\BMH\RUN20170215\IGH_FR1_output\combined_fastq\161124-0058_S16_L001_001_combined.fastq_read_summary.tsv
Sample 161124-0058
Total count 391.356

Rank	Sequence	Length	Merge count	V-gene	J-gene	% total reads	Cumulative %	Mutation rate to partial V-gene (%)	In-frame (Y/N)	No Stop codon (Y/N)	V-coverage
1	CTTCTGGATACAC	283	277774	IGHV1-8_01	IGHJ5_02	709773199,00	709773199,00	5,75	Y	Y	98,67
2	CTTCTGGAGGCAC	337	694	IGHV1-69_13	IGHJ6_02	0,1773321	711546520,00	4,87	Y	Y	99,56
3	GCCTCTGGATTCA	310	382	IGHV3-13_01	IGHJ6_02	0,0976093	712522614,00	0,00	N	N	100,00
4	GCCTCTGGATTCA	296	376	IGHV3-15_05	IGHJ4_02	0,0960762	713483376,00	7,73	Y	Y	98,71
5	CACTGTCTCTGGT	297	290	IGHV4-39_05	IGHJ6_02	0,0741013	714224389,00	7,86	Y	Y	100,00
6	GCCTCTTGATTCA	199	259	IGHV3-13_01	IGHJ6_02	0,0661802	714886191,00	2,68	n/a	N	48,21
7	CTTCCGGATACAC	267	245	IGHV1-45_02	IGHJ4_02	0,0626028	715512219,00	5,31	n/a	N	92,92
8	GCCTCTGGATTCA	260	214	IGHV3-15_07	IGHJ5_02	0,0546817	716059036,00	7,30	Y	Y	100,00
9	GCCTCTGGATTCA	299	206	IGHV3-23_04	IGHJ6_02	0,0526375	716585411,00	0,00	Y	N	100,00
10	GCCTCTGGATTCA	278	202	IGHV3-30-3_01	IGHJ4_02	0,0516154	717101565,00	0,00	Y	Y	100,00

Rank
Sequence
Length
Merge count
V-gene
J-gene
% total reads
Cumulative %
Mutation rate
In frame (Y/N)
No stop codon
V-coverage

N°	reads total	Rank	Sequence	Length	Raw count	V-gene	J-gene	% total reads	Cumulative %
58	391356	1	CTTCTGGATACAC GTTCACCAATTAT GATATTAACTGGG TGCGACAGGCCAC TGGACAAGGGCTT GAGTGGGTGGGAT GGATGAATCCTGA CAGTGGTAACACA GACTATGCACAGG AGTTCCAGGGCAG AGTCACCATGACC AGGGACACCTTCA AAAGCACAGCCTA CATGGAGCTGAAC AGCCTGAGATTTG AGGACACGGCCGT GTATTACTGTGCG AGGGGCCCGTATG AGACGATTCTTGG AGGGAACTGGTTC GACCCCTGGGGCC AGGGAACCCT	283	176424	IGHV1-8_01	IGHJ5_02	450801827,00	450801827,00

Mut rate partial V-gene (%)	In-frame	No Stop codon	V-coverage	Résultat FRI	Taille frag FRI	% HMIGH	V-gene HMIGH	In Frame HMIGH	NO Stop Codon HMIGH
5,75	Y	Y	98,67	Clonal	332 bp	95,49	IGHV1-8*01f	+	+

Sequence interpretation

Sequence alignment

IMGT/V-QUEST (https://www.imgt.org/IMGT_vquest/input)

Adjustable parameter: search for insertions/deletions.

V, D, J gene

Functionality: Productive or unproductive

Identity: Gene identified

Homology: Percentage. (<98% HMS mutated; ≥98% unmutated)

Check In frame/ no stop codon, pseudogenes, must include Cys 104 and Trp 118

www.imgt.org/IMGT_vquest/input

Different sequences at the same time possible

[Search for insertions and deletions in V-REGION](#) YES (low V-region identity)

Different sequences can be entered at the same time

Sequence: 1 2023

Analysed sequence length: 490.
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)

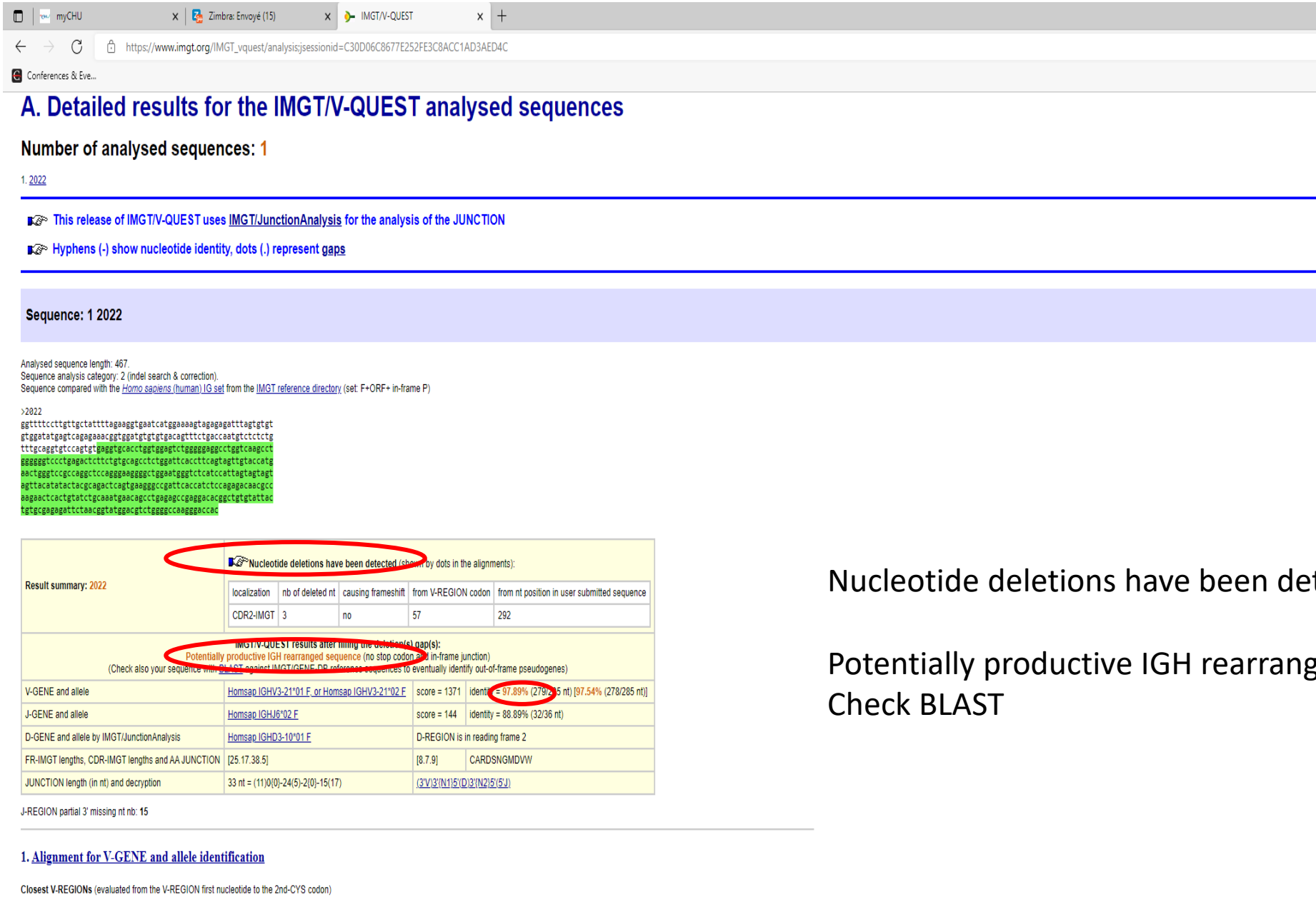
>2023
ggtcttctgcttgctggctgtagctccaggtaaagggccaactggttccagggctgagga
agggatttttttccagtttagaggactgtcattctctactgtgtcctctccgcaggtgctc
actcc[illegible]

- V, D, J gene
- Productive
- % of homology
- In frame/ no stop codon

Pseudogenes
insertions/deletions
Cys 104 and Trp 118

Result summary: 2023	Productive IGH rearranged sequence (no stop codon and in-frame junction)		
V-GENE and allele	Homsap IGHV1-46*01 F or Homsap IGHV1-46*03 F	score = 1440	identity = 100.00% (288/288 nt)
J-GENE and allele	Homsap IGHJ4*02 F	score = 155	identity = 100.00% (31/31 nt)
D-GENE and allele by IMGT/JunctionAnalysis	Homsap IGHD2-2*01 F	D-REGION is in reading frame 2	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[25.17.38.5]	[8.8.20]	CARDLTGCISTSCYPPNYFDYW
JUNCTION length (in nt) and decryption	66 nt = (5)-6(15)-6(22)-3(7)0(17)	(3'V)3'(N1)5'(D)3'(N2)5'(5'J)	

J-REGION partial 3' missing nt nb: **16**



Nucleotide deletions have been det

Potentially productive IGH rearrang
Check BLAST

IMGT/V-QUEST 'Detailed view':
Result summary table

http://imgt.cines.fr

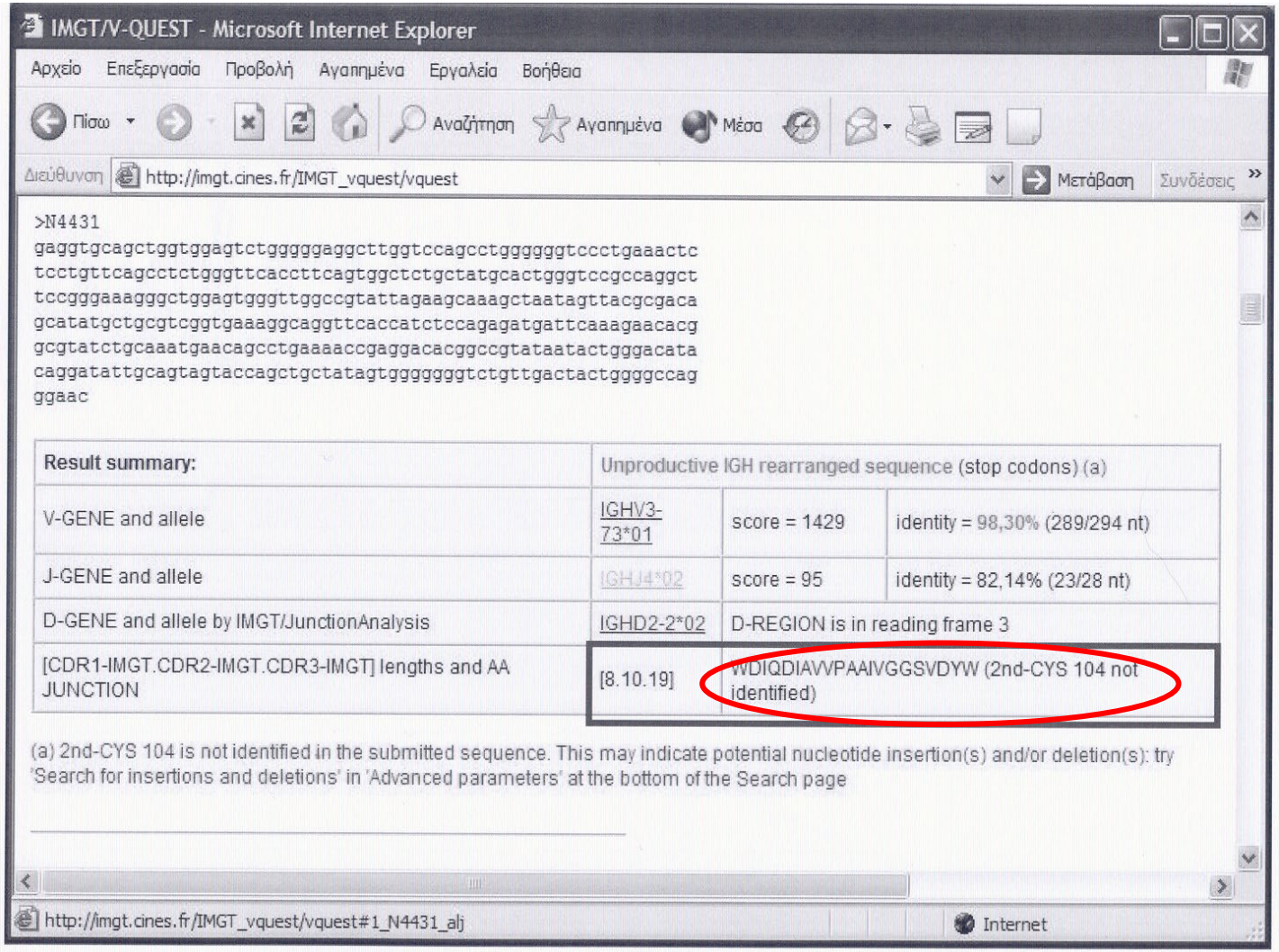
Sequence number 2: seq_2

Sequence compared with the human IG set from the IMGT reference directory

```
>seq_2
gaggtgcagctggtggagtctgggggaggcttggtaaagccgggggggtccctgagactc
tcccgtgcagcctctggattcaccttcagtgactactacatgaactgggtccgccaggct
ccagggaaggggctggagtggggctcatccattactagtagtagtactatatattacgca
gactctgtgaagggccgattcaccatctccagagacaacgccaagaactaactgtatctg
caaatgaagagcctgagagttgaggacacggctgtgcattactgttcgagagataaggtc
gagtaggtattacgatttttggagtggttattttacgaagaaactggttcgacccctggg
gccagggaaccctggtcaccgtctcctcag
```

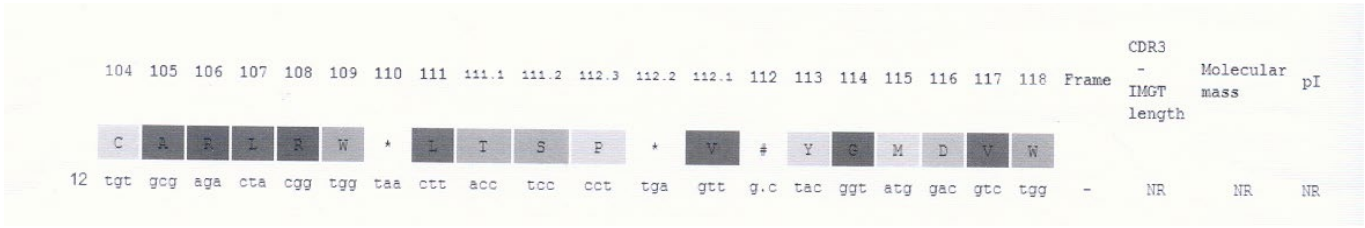
Result summary:	Unproductive IGH rearranged sequence (stop codons,out-of-frame junction)		
V-GENE and allele	IGHV3-h*01(P)	score = 1321	identity = 96.14% (274/285 nt)
J-GENE and allele	IGHJ5*02	score = 237	identity = 96.08% (49/51 nt)
D-GENE and allele by IMGT/JunctionAnalysis	IGHD3-3*01	D-REGION is in reading frame 3	
[CDR1-IMGT.CDR2-IMGT.CDR3-IMGT] lengths and AA JUNCTION	[8.7.X]	CSRDKVE*VLRFLEWLFYE#NWFDPW	

Done

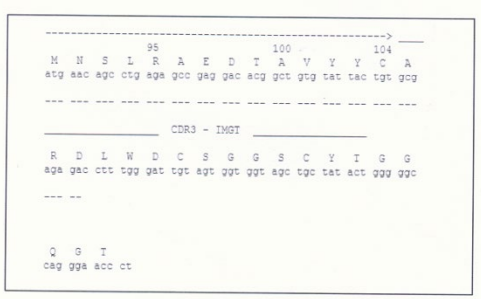

IMGT/V-QUEST - Microsoft Internet Explorer
Αρχείο Επεξεργασία Προβολή Αγαπημένα Εργαλεία Βοήθεια
Πίσω
Αναζήτηση
Αγαπημένα
Μέσα
Διεύθυνση
http://imgt.cines.fr/IMGT_vquest/vquest
Μετάβαση
Συνδέσεις
>N4431
gaggtgcagctggtggagtctgggggaggcttggtccagcctgggggtccctgaaactc
tcctgttcagcctctgggttcaccttcagtggctctgctatgcactgggtccgccaggct
tccgggaaagggctggagtgggttggccgtattagaagcaaagctaatagttacgcgaca
gcatatgctgcgtcggtgaaaggcaggttcaccatctccagagatgattcaaagaacacg
gcgtatctgcaaatgaacagcctgaaaaccgaggacacggccgtataatactgggacata
caggatattgcagtagtaccagctgctatagtgggggggtctgttgactactggggccag
ggaac
Result summary:
Unproductive IGH rearranged sequence (stop codons) (a)
V-GENE and allele
IGHV3-73*01
score = 1429
identity = 98,30% (289/294 nt)
J-GENE and allele
IGHJ4*02
score = 95
identity = 82,14% (23/28 nt)
D-GENE and allele by IMGT/JunctionAnalysis
IGHD2-2*02
D-REGION is in reading frame 3
[CDR1-IMGT.CDR2-IMGT.CDR3-IMGT] lengths and AA JUNCTION
[8.10.19]
WDIQDIAVVPAAIVGGSVDYW (2nd-CYS 104 not identified)
(a) 2nd-CYS 104 is not identified in the submitted sequence. This may indicate potential nucleotide insertion(s) and/or deletion(s): try 'Search for insertions and deletions' in 'Advanced parameters' at the bottom of the Search page
http://imgt.cines.fr/IMGT_vquest/vquest#1_N4431_alj
Internet

Single unproductive rearrangement

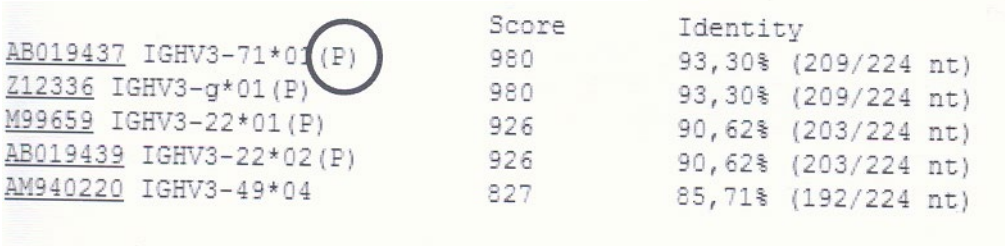
- Out of frame – deletions/insertions



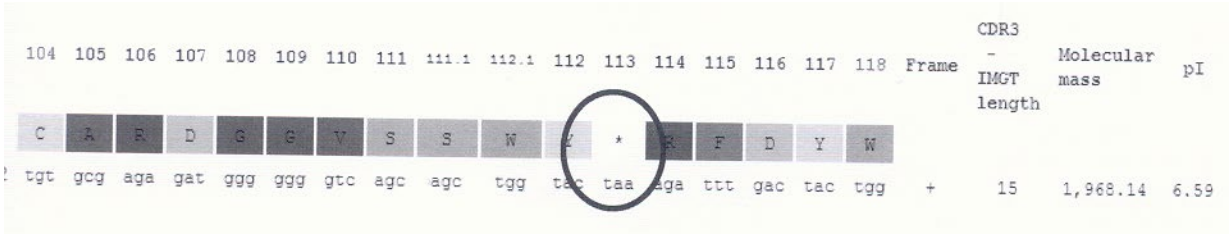
- Absent CDR3 anchors mutation at C104 or W118



- IGHV pseudogenes



- Stop codons



Functionality

IGHV-IGHD-IGHJ gene rearrangements
can be rendered unproductive if they

- carry pseudogenes
- out-of-frame VDJ junctions
- stop codons
- indels leading to frameshifts within the coding part of the sequence

Single unproductive rearrangement

CLL cells are mature B cells that should express functional IG molecules on their surface.
Identification of a single unproductive IGH rearrangement is rare (<0.1% of all CLL)
Detect the productive IGHV-IGHD-IGHJ gene rearrangement (on the other allele of the IGH locus).

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

Unproductive rearrangement: NO clinical association possible

Double rearrangement 10.5%

One productive and one non-productive (90%)	Mutational status defined by the productive rearrangement, irrespective of the IGHV mutation status of the unproductive rearrangement.
Double productive	
Concordant IGHV mutation status	Consider as mutated or unmutated, according to the IGHV mutation status.
	Check immunophenotype for the presence of 2 clonal populations.
Discordant IGHV mutation status	Recommend to the physician that it is safer to consider as U-CLL; close follow-up.

Double rearrangement

Multiple (>2) productive rearrangements	Perform NGS to assess the relative frequency of each clonotype and consider the predominant clonotype, if it is clearly identified.

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

Sequence analysis

IMGT/V-QUEST
(https://www.imgt.org/IMGT_vquest/input)

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

Assignment to stereotyped subsets #2 and #8

Homology + interpretation

Percentage identity

Count from IMGT codon 1 – 104 (automatically by IMGT/V-QUEST)

Codon 105-107 eventuel mutations have minor effect

Cut-off 98% (-> borderline 97-99%)

CLL cases with homology close to cut-off of 98%
have varying clinical outcome

Result by using FR1 primers -> reanalyse with leader primers

Stereotype

Stereotyped B cell receptor immunoglobulin (BcR IG) are classified into subsets.

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

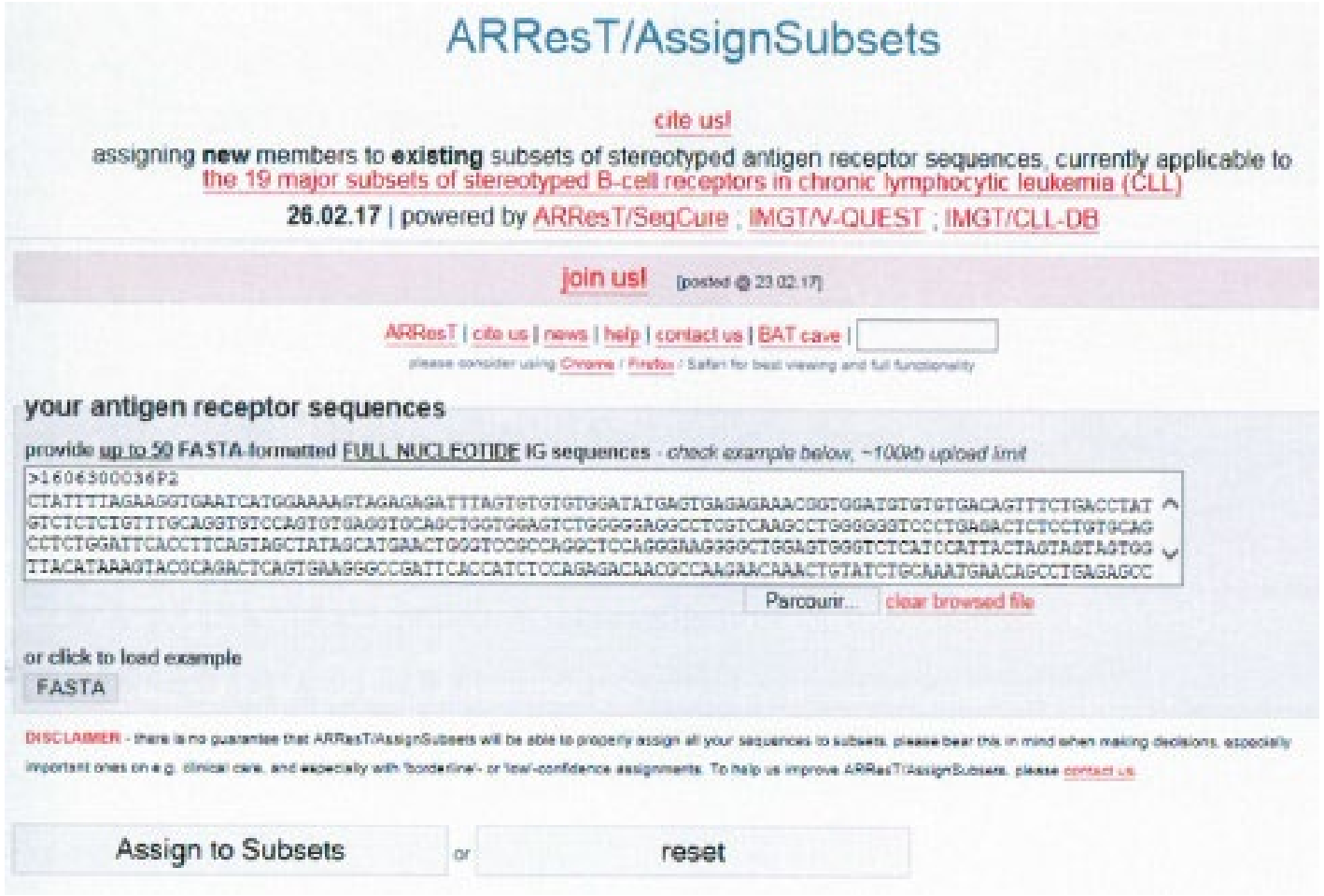
Subset #2 was found to be an independent prognostic marker for shorter TTFT, time-to-next-treatment (TTNT), and PFS, irrespective of the SHM status.

Subset #8 associated with aggressive disease (risk of transformation to Richter).

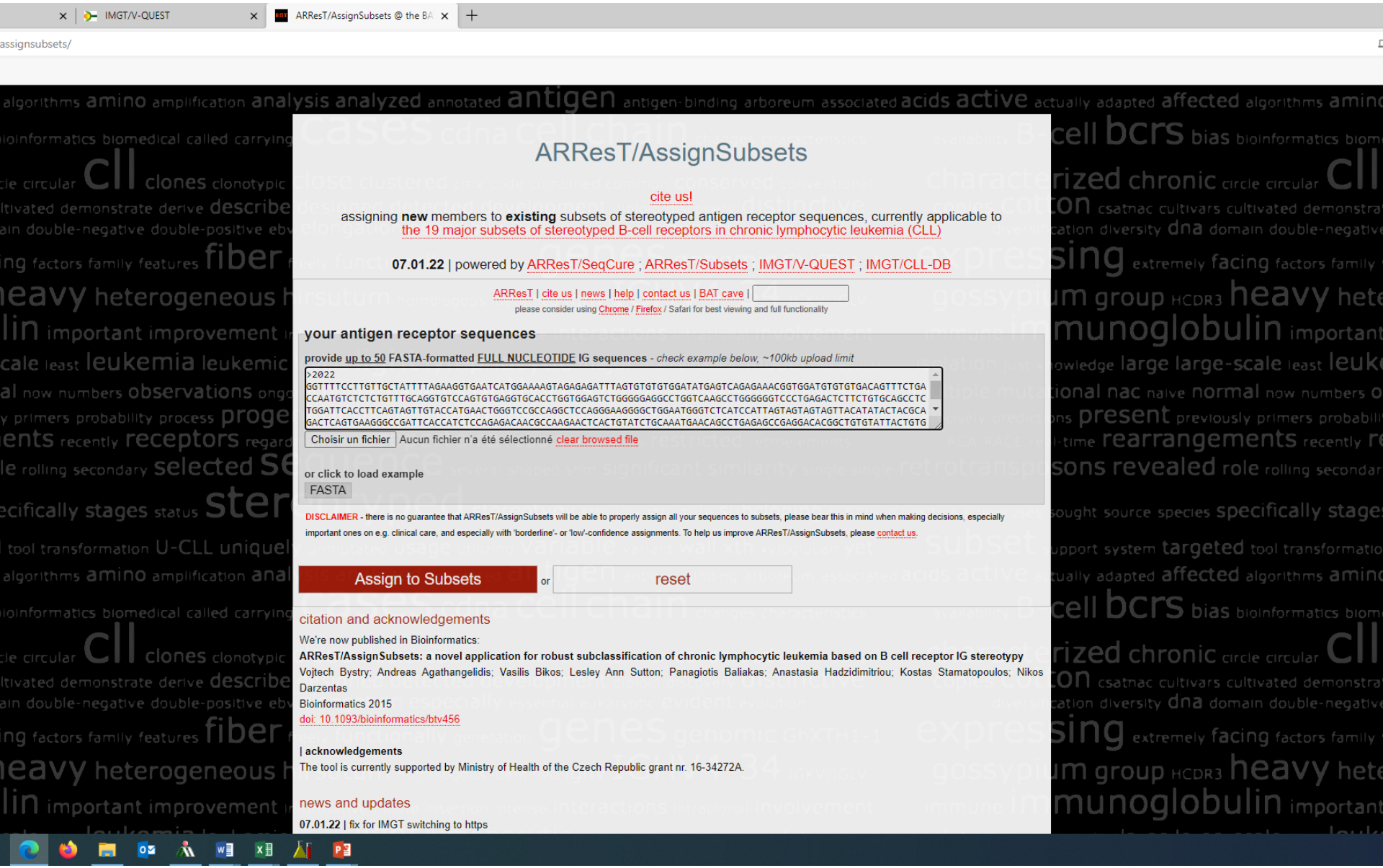
.

IGHV3-21

Homologie with IGHV3-21 (but also all other rearrangements) analyse by 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)
26.02.17 | powered by ARResT/SeqCure ; IMGT/V-QUEST ; IMGT/CLL-DB
join us! [posted @ 23.02.17]
ARResT | cite us | news | help | contact us | BAT cave |
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
>1606300036P2
CTATTTIAGAAGGTGAATCATGGAAAAGTAGAGAGATTTAGTGTGTGTGGATATGAGTGAGAGAAACGGTGGATGTGTGTGACAGTTTCTGACCTAT
GTCTCTCTGTTTGCAGGTGTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTCGTCAAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAG
CCTCTGGATTCACCTTCAGTAGCTATAGCATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCATCCATTACTAGTAGTAGTGG
TTACATAAAGTACGCAGACTCAGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACAAACTGTATCTGCAAATGAACAGCCTGAGAGCC
Parcourir...
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

Bat.infspire.org/arrest/assignsubsets/



IMGT/V-QUEST | ARResT/AssignSubsets @ the BA

/assignsubsets_results/0007313994.html

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...

(=) 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

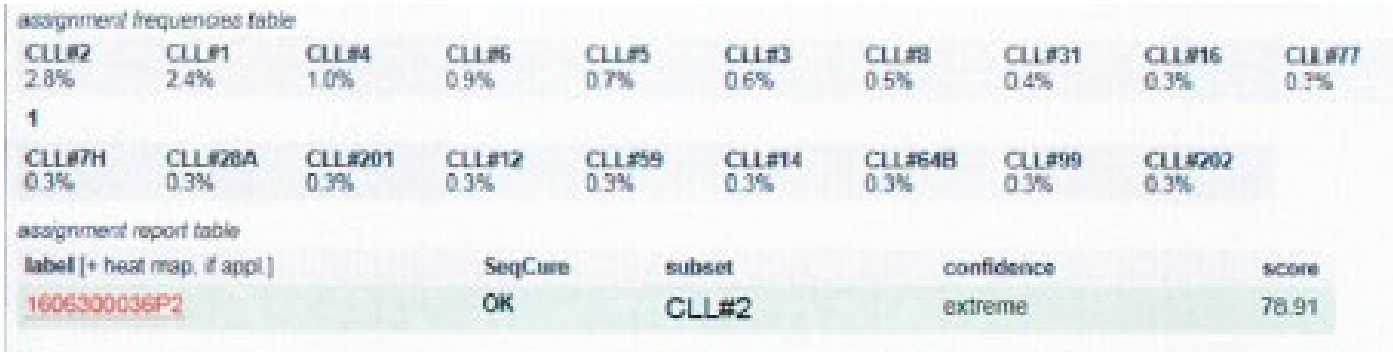
CLL#7H	CLL#28A	CLL#201	CLL#12	CLL#59	CLL#14	CLL#64B	CLL#99	CLL#202
0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%

assignment report table

label [+ heat map, if appl.]	SeqCure	subset	confidence	score
2022	warning	CLL#2	extreme	75.12

hosted at the Bioinformatics Analysis Team / BAT

IGHV3-21-subset



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
1606300036P2	OK	CLL#2	extreme	78.91

This CLL sample displays a productive IGHV3-21 mutated rearrangement (<98% homology) with stereotyped BcR Ig with subset #2.

Amongst CLL cases expressing clonotypic BcR Ig encoded by the IGHV3-21, those belonging to subset#2 are associated with adverse prognosis, regardless of their SHM status (Baliakas et al. Blood 2015).

Clinical report

• Basic data: patient data
tissue type
sample arrival date
reference

• Technique: direct sequencing, subcloning
gDNA or cDNA
PCR primers (BIOMED2)
bioinformatics tools for SHM status assessment, and stereotypy analysis.

• Results: % identity to the germline to 2 decimal points as reported by IMGT + cut-off

IGHV/IGHD/IGHJ gene usage
functionality - productive/unproductive
► SHM status only for productive rearrangements;
if the rearrangement is unproductive, mention reasons for that
(e.g., IG pseudogene, out-of-frame junction, stop codon, large indel).

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

• Subset identification/BcR IG stereotypy: For subsets with
well-established prognostic value (currently, subsets #2 and #8).

No clear interpretation possible -> prognostic implication cannot
be determined

1. Example of the IG report, IG - mutated

Name of the Hospital/Lab

Determination of IGHV gene SHM status

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.

Name of the Hospital/Lab

Determination of IGHV gene SHM status

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 rearrngements 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

Name of the Hospital/Lab

Determination of IGHV gene SHM status

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 rearrngements 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

Name of the Hospital/Lab

Determination of IGHV gene SHM status

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 rearrngements 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 typical case I

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

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

Example typical case II

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

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

Example borderline

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

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

Not if FR1 primers were used!

Example special case I

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

IGHV3-21 mutated rearragement with a stereotyped CDR3 (9 codons).
The presence of a mutated IGHV3-21 with stereotype #2 have been associated with a poor clinical outcome.

Example special case II (rare)

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 (rare)

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 rearragements 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 rearragements and a productive IGHV unmutated rearrangement.
It is safer to consider as U-CLL; close follow-up.

Example difficult case II

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

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

Advice

Follow:

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)

Baliakas P et al. Not all IGHV3-21 chronic lymphocytic leukemias are equal: prognostic considerations. Blood 2015; 125:856-859.

Difficult cases: ERIC helps www.ERICLL.org

www.ericll.org

- 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)
- 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)

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