Critically Appraised Topic

Next-generation sequencing panel for mature lymphoid malignancies

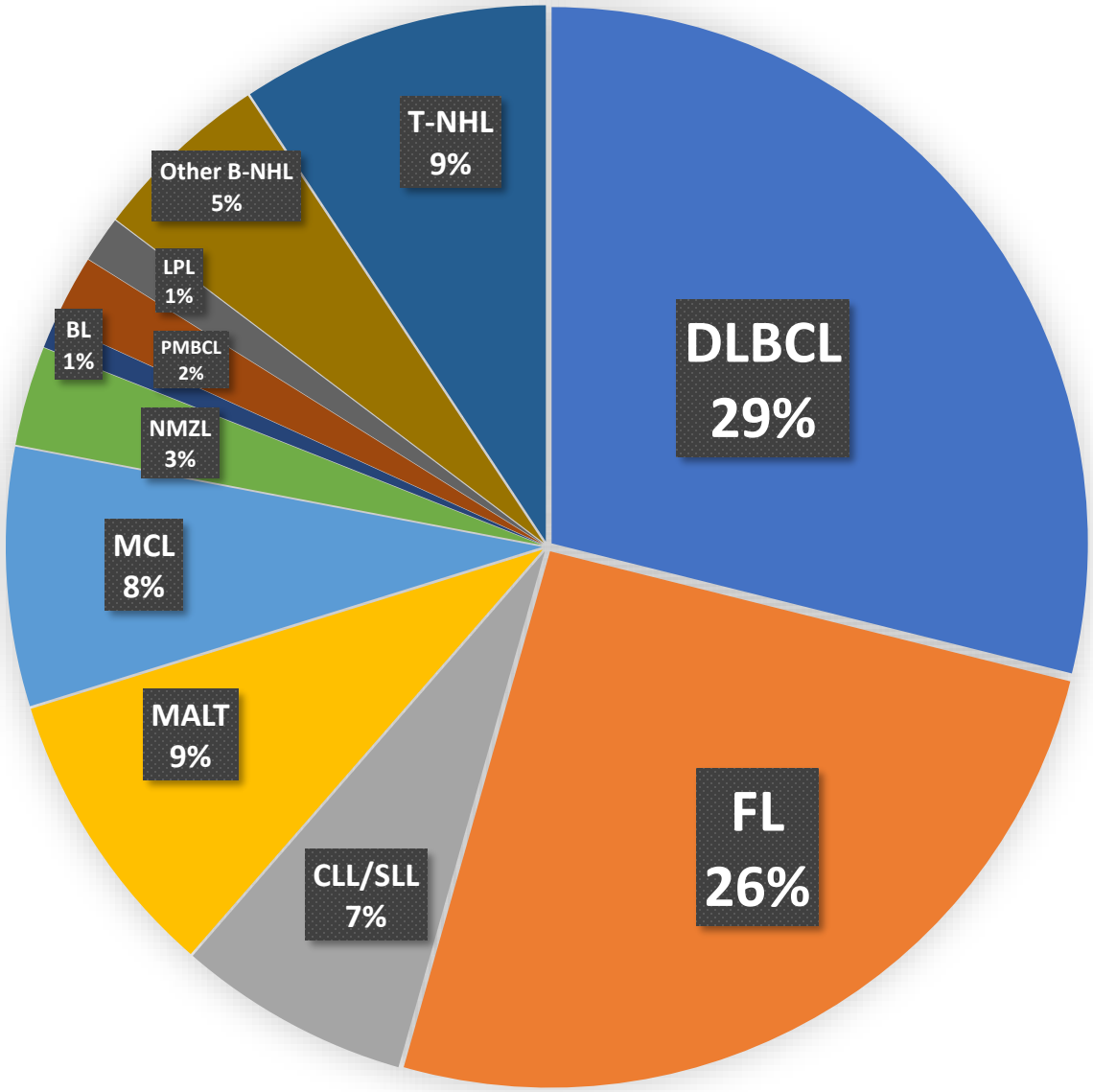
12 May 2020

Louis NEVEJAN

az sint-jan
brugge - oostende av

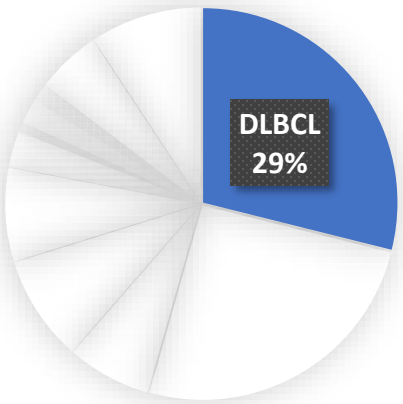
Clinical & diagnostic scenario

Relative frequencies of *non-Hodgkin lymphoma* subtypes in developed regions

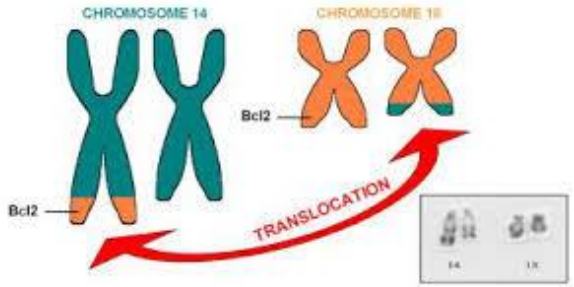


PERRY AM. Haematologica (2016) 101(10):1244-1250

DLBCL, NOS

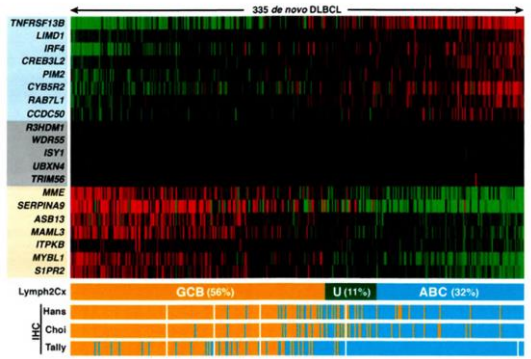


Chromosomal translocations — 1985

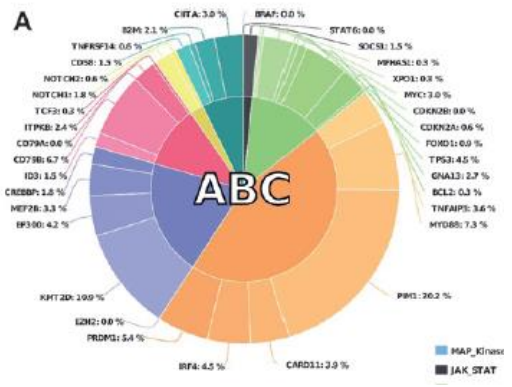


TRANSLOCATION t(14;18)

Gene expression profiling — 2000

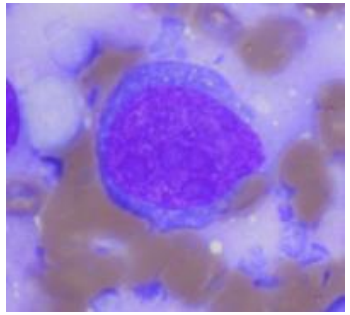


Next-generation sequencing — 2009



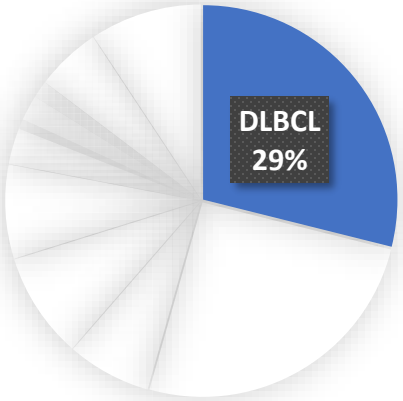
WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues — 2017

2017 WHO Classification	DLBCL-NOS	
Gene Expression Subtypes	ABC	GCB



JARDIN P. Next Generation Sequencing and targeted genotyping as tools for personalized medicine in lymphoma. Presentation BHS 2017
DUBOIS S. Clin Cancer Res (2016), 22(12); 2919–28.

DLBCL, NOS



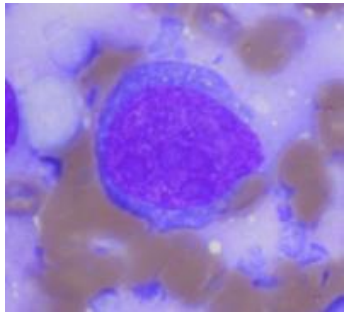
WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

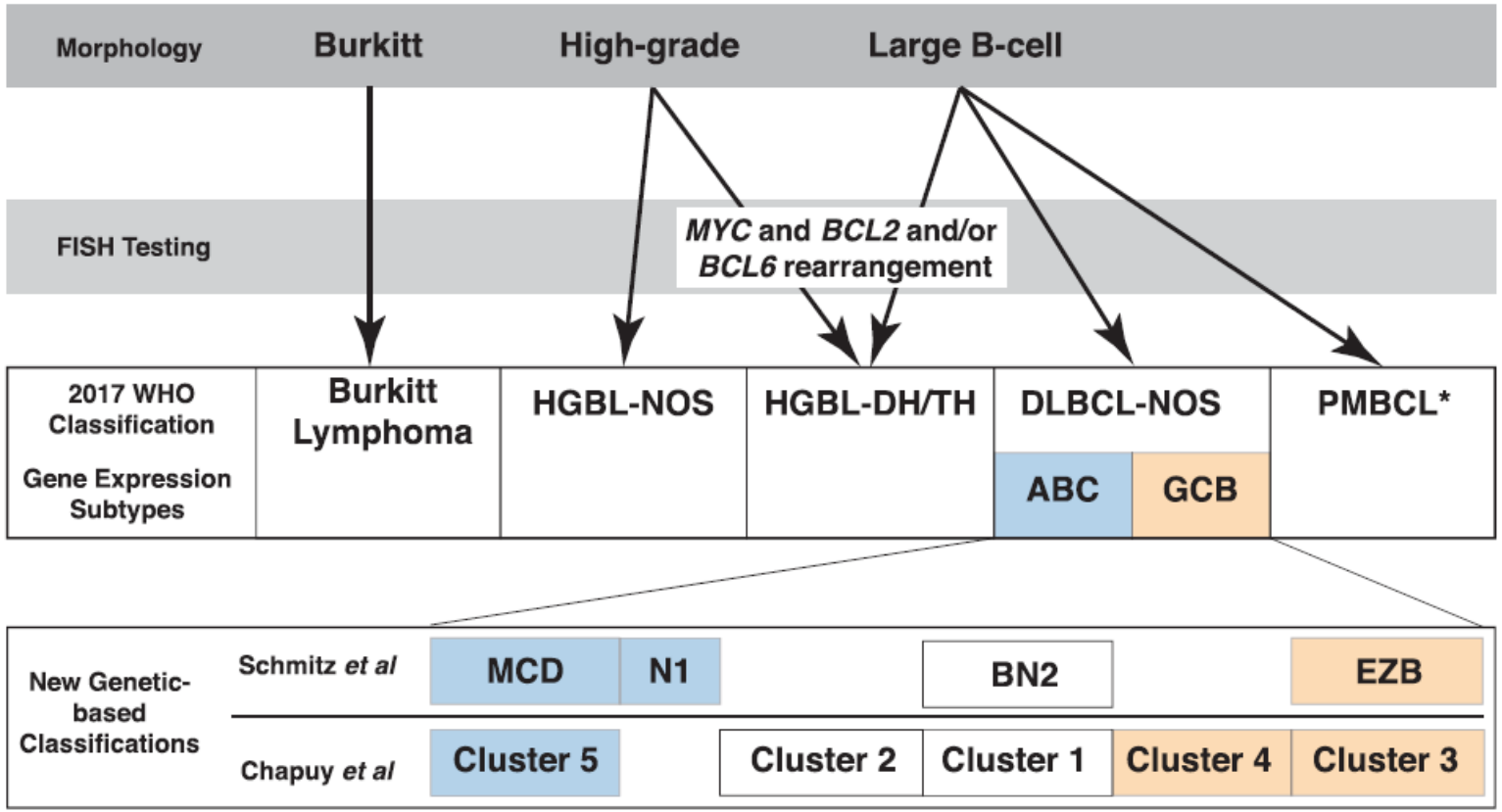
2017

2018

nature medicine

The NEW ENGLAND JOURNAL of MEDICINE

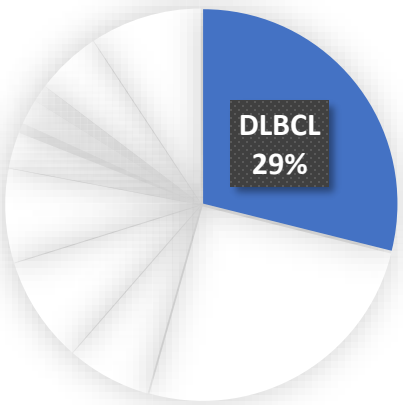




574 DLBCL biopsies

304 DLBCL biopsies

SCOTT DW. Best Pract Res Clin Haematol (2018) 31(3):187-198



1985 **Chromosomal translocations**

2000 **Gene expression profiling**

2009 **Next-generation sequencing**

2017 **WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues**

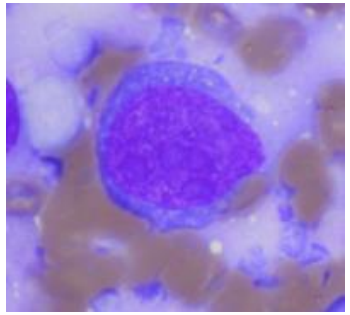
2017 WHO Classification	DLBCL-NOS	
Gene Expression Subtypes	ABC	GCB

CHOP
chemotherapy

R-CHOP
Immuno-chemotherapy

R-CHOP + …
Precision therapy

DLBCL, NOS



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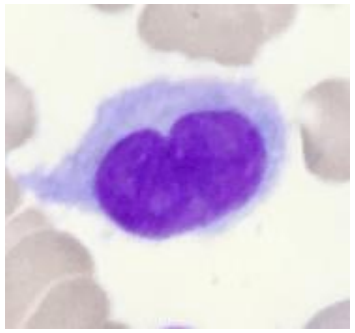
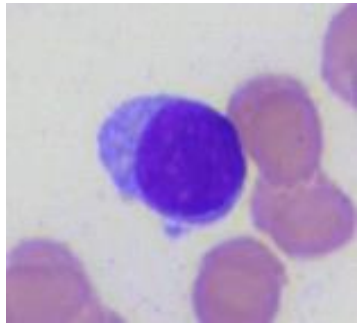
WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

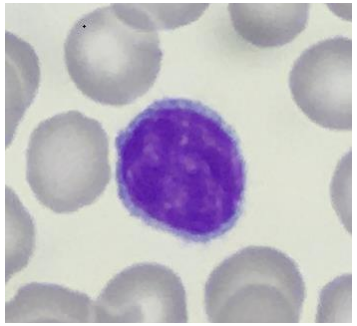
HCL



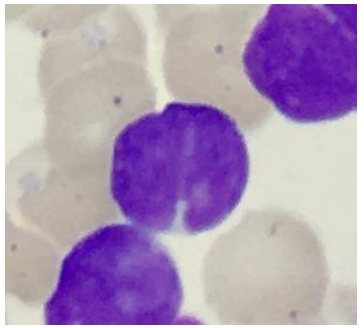
LPL



CLL



FL



BRAF V600E **MYD88 L265P**

TP53 *EZH2*

NOTCH1
SF3B1
BIRC3

Diagnostic **Prognostic** **Therapeutic**

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Trefwoorden invoeren

Home Diensten Artsen Ziekten Opname & bezoek Professional

Home » Professional » Labogids » NGS myeloïd 21-genen panel (Qiaseq): nieuw - vanaf oktober 2018

NGS myeloïd 21-genen panel (Qiaseq): nieuw - vanaf oktober 2018

QIAGEN

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Questions

1.
Which **genes** should be included
in a next-generation sequencing panel
for mature lymphoid malignancies providing
diagnostic, **prognostic** and **therapeutic** information?

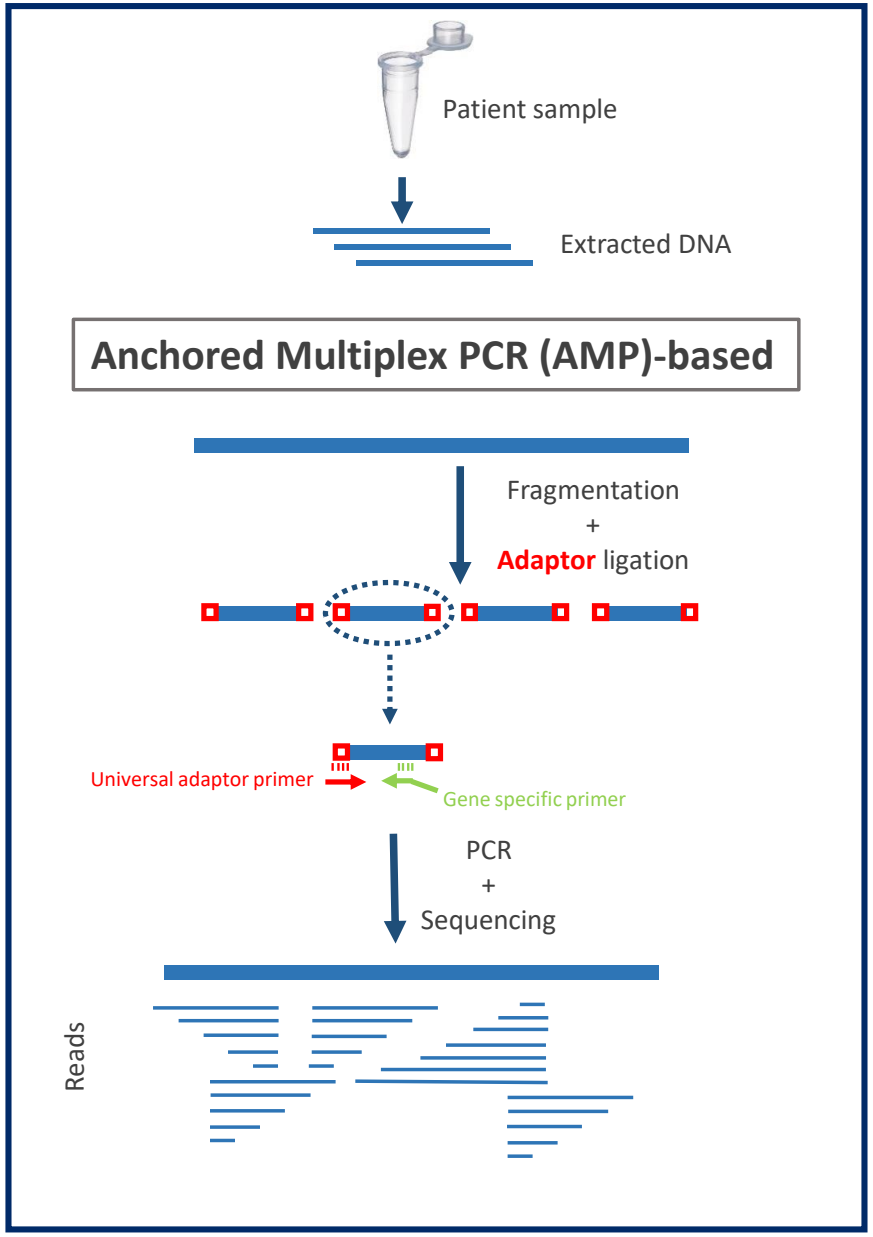
2.
How can such panel be **implemented** in
routine clinical practice?

Q1.

Composition

Lymphoid gene panel

NGS techniques for DNA variant detection



Q1.

Composition

Lymphoid gene panel

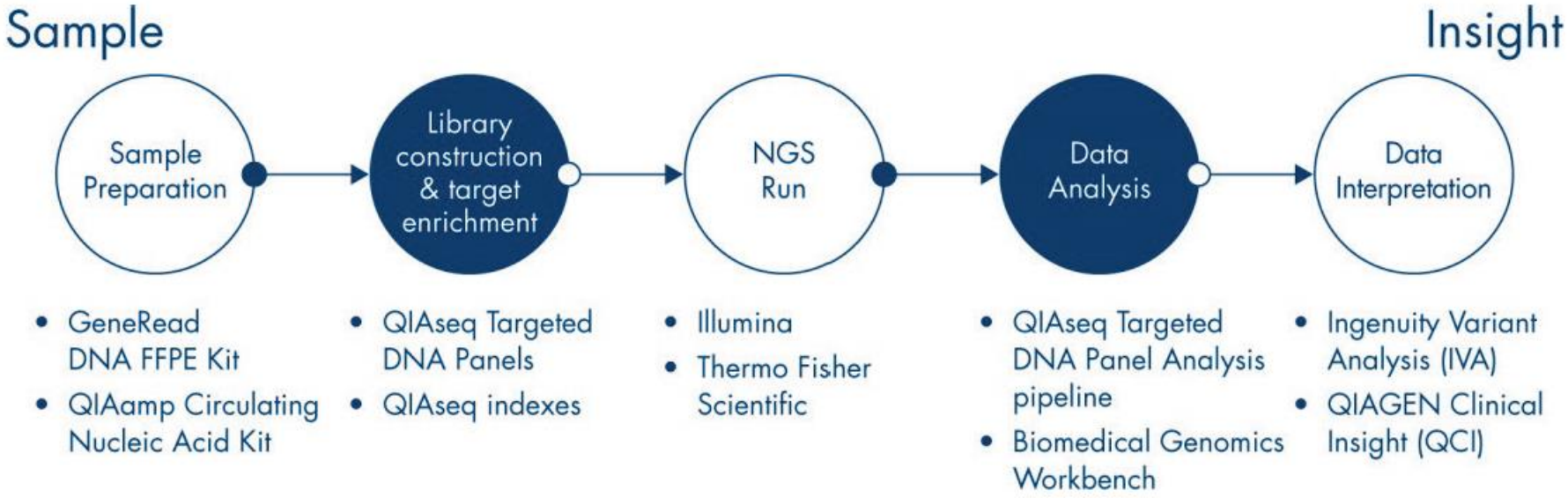


Figure 1. Overview of the sample-to-insight NGS workflow with QIAseq Targeted DNA Panels. The complete sample-to-insight procedure begins with DNA extraction. Next is library construction and target enrichment with QIAseq Targeted DNA Panels. Following NGS, data analysis is performed using the QIAseq Targeted DNA Panel Analysis Software pipeline or Biomedical Genomics Workbench. Ultimately, detected variants can be interpreted with the Ingenuity® Variant Analysis (IVA) tool or with QIAGEN Clinical Insight (QCI™).

Q1.
Composition
Lymphoid gene panel

Literature search

REVIEW ARTICLE

EUROPEAN HEMATOLOGY ASSOCIATION

Ferrata Storti Foundation

Clinical impact of recurrently mutated genes on lymphoma diagnostics: state-of-the-art and beyond

Richard Rosenquist,[1] Andreas Rosenwald,[2] Ming-Qing Du,[3] Gianluca Gaidano,[4] Patricia Groenen,[5] Andrew Wotherspoon,[6] Paolo Ghia,[7] Philippe Gaulard,[8] Elias Campo,[9] Kostas Stamatopoulos,[10] on behalf of the European Research Initiative on CLL (ERIC) and the European Association for Haematopathology (EAHP)

Haematologica 2016
Volume 101(9):1002-1009

European Expert
Group on
NGS-based Diagnostics in
Lymphoma
(EGNL)

HemaSphere
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EUROPEAN HEMATOLOGY ASSOCIATION

Perspective

OPEN ACCESS

The Need for a Consensus Next-generation Sequencing Panel for Mature Lymphoid Malignancies

Pierre Sujobert[1,2], Yannick Le Bris[3,21], Laurence de Leval[4], Audrey Gros[5], Jean Philippe Merlio[5], Cedric Pastoret[6], Sarah Huet[1,2,20], Clémentine Sarkozy[2,7], Frédéric Davi[8], Mary Callanan[9,10], Catherine Thieblemont[11,12,13], David Sibon[14], Vahid Asnafi[15], Claude Preudhomme[16], Philippe Gaulard[17,18], Fabrice Jardin[19], Gilles Salles[2,7], Elizabeth Macintyre[15]

HemaSphere, 2019;3:1. http://dx.doi.org/10.1097/HS9.0000000000000169.

LYmphoma **S**tudy **A**ssociation
(LYSA)

Groupe de
Biologistes
Moléculaires des
Hémopathies
Malignes
(GBMHW)

Q1.

Composition

Lymphoid gene panel

Literature search

HemaSphere
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Perspective
OPEN ACCESS

The Need for a Consensus Next-generation Sequencing Panel for Mature Lymphoid Malignancies

Pierre Sujobert[1,2], Yannick Le Bris[3,21], Laurence de Leval[4], Audrey Gros[5], Jean Philippe Merlio[5], Cedric Pastoret[6], Sarah Huet[1,2,20], Clémentine Sarkozy[2,7], Frédéric Davi[8], Mary Callanan[9,10], Catherine Thieblemont[11,12,13], David Sibon[14], Vahid Asnafi[15], Claude Preudhomme[16], Philippe Gaulard[17,18], Fabrice Jardin[19], Gilles Salles[2,7], Elizabeth Macintyre[15]

Consensus panel **B-cell lymphomas** (33 genes)

Consensus panel **T-cell lymphomas** (11 genes)

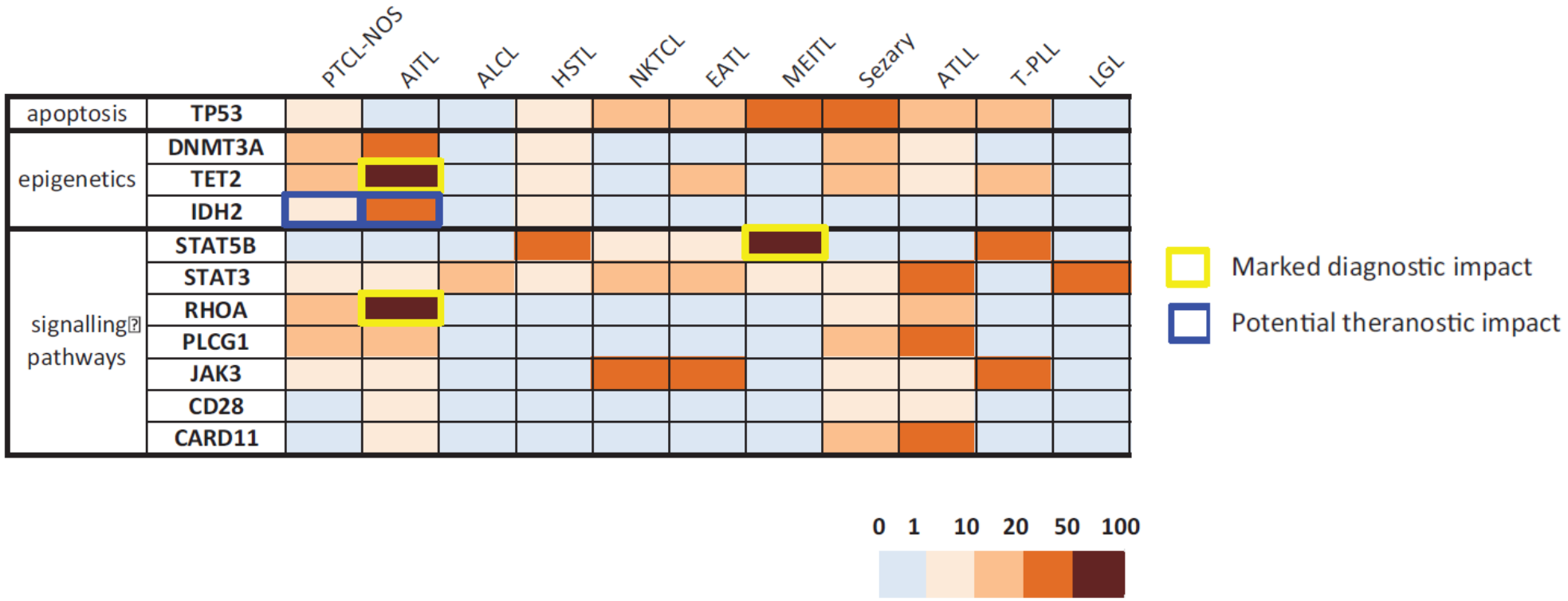


Figure 2. A heatmap representation of the prevalence of gene mutations in mature T lymphoid malignancies from the LYSA/GBMHM consensus panel. The borders of the squares are colored when the alteration has a clinical impact in a particular lymphoma subtype (diagnostic in yellow, theranostic in blue). AITL = angio-immunoblastic T lymphoma, ALCL = anaplastic large cell lymphoma, ATLL = adult T leukemia/lymphoma, EATL = enteropathy associated T lymphoma, HSTL = hepatosplenic T lymphoma, LGL = large granular lymphocytic leukemia, MEITL = monomorphic epitheliotropic intestinal T lymphoma, NKTCL = nasal type NK/T cell lymphoma, PTCL-NOS = peripheral T cell lymphoma, not otherwise specified, PTCL-TFH = nodal peripheral T cell lymphoma derived from T_{FH} cells, Sezary = Sezary syndrome, T-PLL = T-prolymphocytic leukemia.

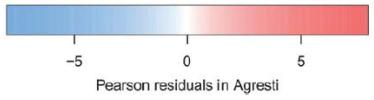
SUJOBERT P et al.
HemaSphere (2019) 3(1):e169

Q1.
Composition
Lymphoid gene panel

Literature search

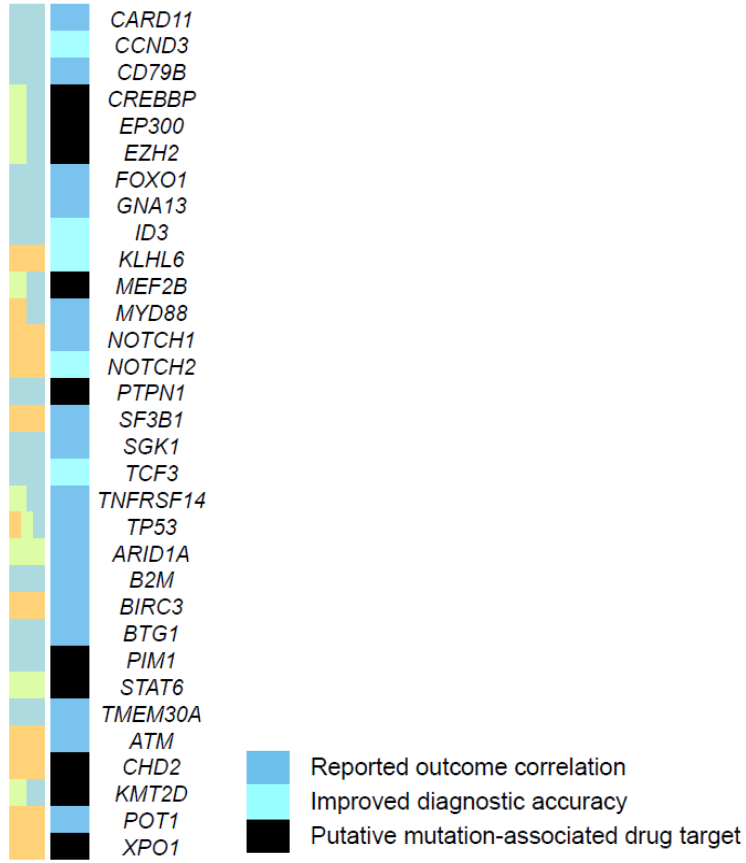
DUBOIS et al.
Lymphopanel (34 genes)
DLBCL

	ABC *n* = 81	GCB *n* = 83	PMBL *n* = 18	other *n* = 33	FDR
STAT6	0%	14%	72%	6%	**6.8e−14**
XPO1	1%	1%	39%	3%	**4.8e−10**
SOCS1	6%	16%	56%	12%	**2.5e−05**
BCL2	1%	24%	0%	3%	**2.5e−05**
CIITA	12%	10%	56%	9%	**3.1e−05**
TNFAIP3	15%	11%	61%	15%	**3.1e−05**
CD79B	25%	2%	0%	3%	**3.2e−05**
PIM1	33%	8%	0%	6%	**3.3e−05**
GNA13	9%	12%	50%	12%	**2.8e−04**
CD58	6%	10%	39%	6%	**1.2e−03**
CREBBP	6%	31%	11%	24%	**1.2e−03**
B2M	9%	18%	50%	24%	**1.2e−03**
EZH2	0%	18%	6%	9%	**1.8e−03**
TNFRSF14	2%	17%	0%	24%	**1.8e−03**
MFHAS1	1%	10%	28%	9%	**3.9e−03**
MYD88	28%	10%	0%	15%	**4.7e−03**
ITPKB	9%	16%	39%	9%	**1.4e−02**
PRDM1	16%	6%	0%	3%	5.1e−02
NOTCH2	2%	10%	0%	15%	7.6e−02
IRF4	14%	5%	11%	0%	8.7e−02
MEF2B	12%	23%	11%	6%	1.4e−01
BRAF	0%	0%	0%	3%	2.1e−01
FOXO1	4%	12%	6%	3%	2.1e−01
KMT2D	41%	46%	17%	42%	2.2e−01
CARD11	14%	7%	0%	9%	3.5e−01
NOTCH1	7%	1%	6%	6%	3.7e−01
CD79A	0%	2%	0%	0%	4.5e−01
TP53	19%	16%	11%	6%	4.6e−01
CDKN2B	0%	1%	0%	3%	5.4e−01
ID3	5%	2%	6%	9%	5.5e−01
MYC	5%	10%	6%	3%	5.5e−01
CDKN2A	2%	1%	0%	0%	7.4e−01
TCF3	1%	2%	0%	0%	7.4e−01
EP300	15%	14%	17%	18%	9.6e−01



DUBOIS et al. Clin Cancer Res. 2016;22(12):2919-28

HUNG et al.
Lymphopanel (32 genes)
CLL - FL - DLBCL



HUNG et al. J Mol Diagnostics (2018);20(2):203-14

Q1.

Composition

Lymphoid gene panel

Literature search

Genetic landscape and deregulated pathways in B-cell lymphoid malignancies

R. Rosenquist[1,2,*], S. Beà[3,*], M.-Q. Du[4], B. Nadel[5] & Q. Pan-Hammarström[6]

Genetic aberrations in small B-cell lymphomas and leukemias: molecular pathology, clinical relevance and therapeutic targets

Agata M. Bogusz & Adam Bagg

Frequent Mutations in Natural Killer/T Cell Lymphoma

Yanjie Zhang Chaoping Li Weili Xue Mingzhi Zhang Zhaoming Li

95 genes

Exclusion of genes based on:
occurence in only one gene panel without any references

BCL6	FGFR3	ITPKB	PRKDC
BCL10	GNAI2	JAK1	SYK
CDH2	IDH1	JAK2	TMEM30A
FAS	IKZF1	KMT2C	
FGFR1	IKZF3	MTOR	
FGFR2	IRF8	PDGFRA	

74 genes

Exclusion of genes based on:
No clear diagnostic/prognostic/theranostic information

BTG1	KIT	POT1	SGK1
CCND3	KLHL6	PRDM1/BLIMP1	ZMYM3
CDKN2B	MAPK7	PTEN	
CHD2	MFHAS1	PTPN1	
CIITA	PIK3CA	PTPN6	
IRF4/MUM1	PIK3CD	SAMHD1	

54 genes

Q1.

Composition

Lymphoid gene panel

Literature search

54 genes

COSMIC
Catalogue Of Somatic Mutations In Cancer

GRCh 37

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COSMIC search results

Your search term "**cxcr4**" was an exact match for the COSMIC gene **CXCR4**.

A search of the whole COSMIC database returned results in **3** sections of the database. More...

Genes (2 hits) | Legacy Mutations (0) | Mutations (810) | SNPs (0) | Cancer (0) | Tumour Site (0) | Samples (0) | Pubmed (30) | Studies (0)

Show 10 entries

Gene	Alternate IDs	Tested samples	Simple Mutations	Fusions	Coding Mutations
CXCR4_ENST00000241393	CXCR4_ENST00000241393,ENST00000241393.3,CXCR4...	52189	646	0	646
CXCR4	CXCR4,ENST00000409817.1,CXCR4...	52189	602	0	602

Showing 1 to 2 of 2 entries

First Previous 1 Next Last

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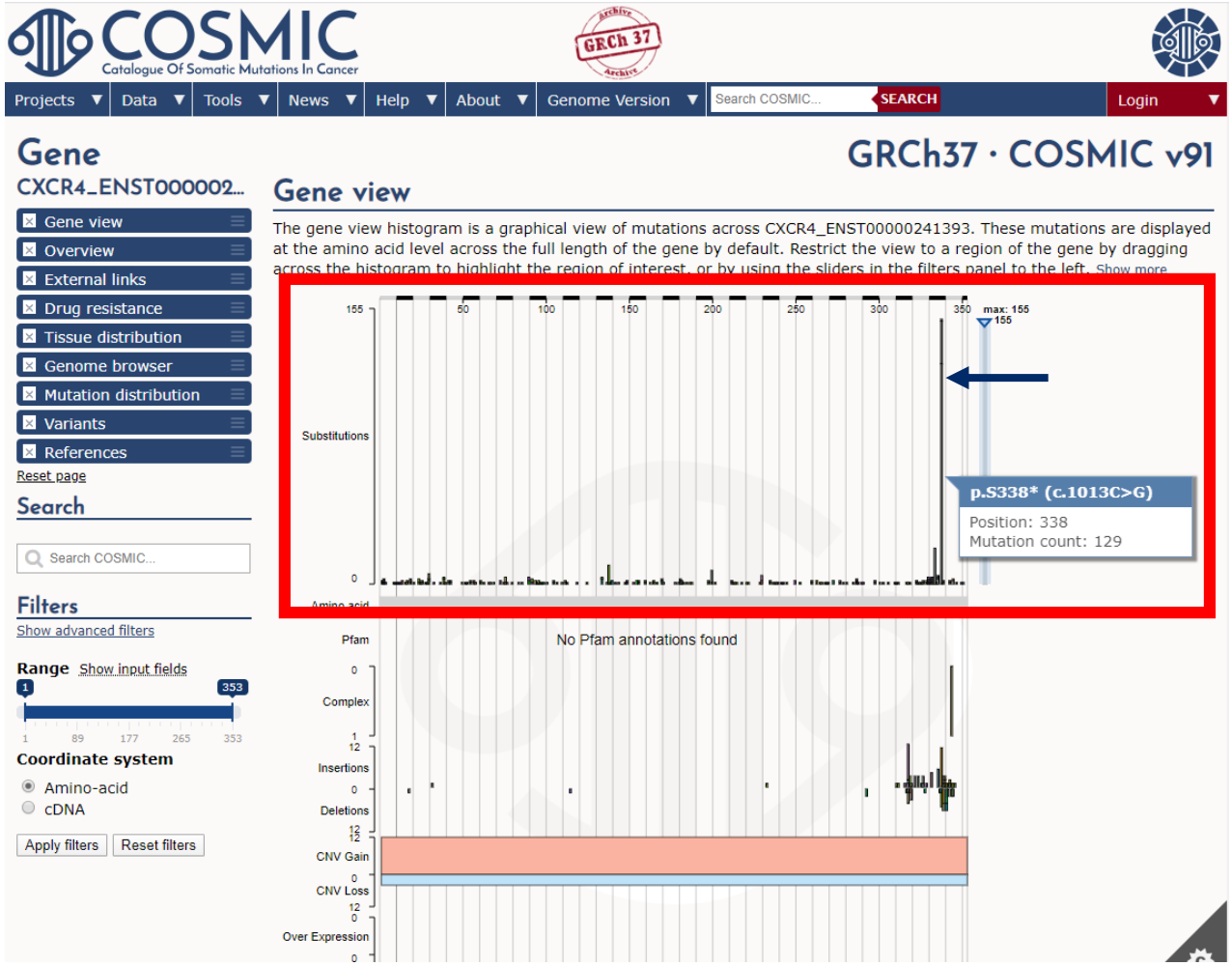
Q1.

Composition

Lymphoid gene panel

Literature search

54 genes



Q1.

Composition

Lymphoid gene panel

Literature search

54 genes

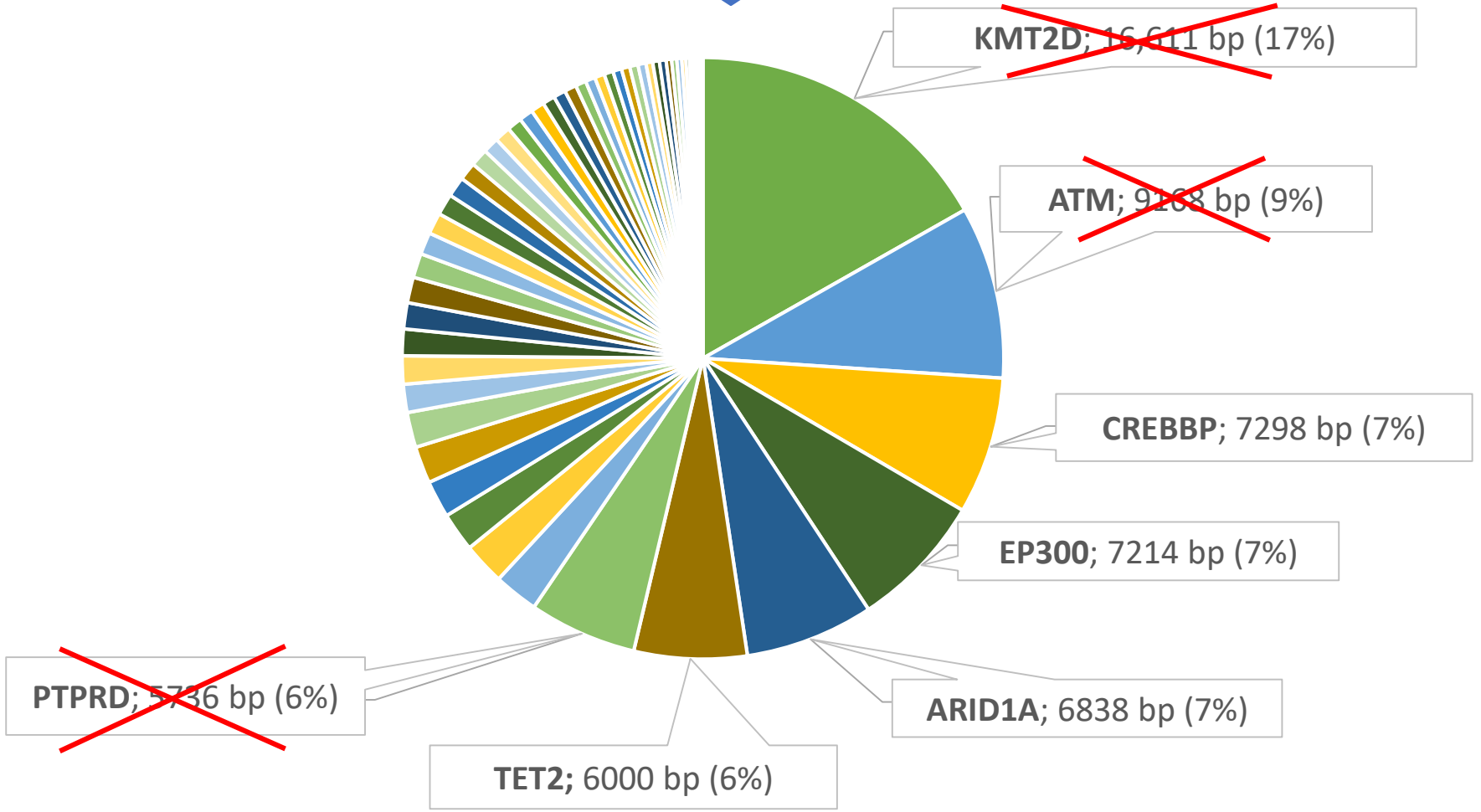
Q1.

Composition

Lymphoid gene panel

Literature search

54 genes = 98,933 base pairs ⟷ *21-gene myeloid panel 21,645 base pairs*



Q1.

Composition

Lymphoid gene panel

Literature search

98,933 base pairs → **54 genes**

67,418 base pairs → **51 genes**

Exclusion of genes based on:
Size of the gene does not outweigh the diagnostic/prognostic/theranostic information

ATM[2]	KMT2D/MLL2	PTPRD[1]

Genes included

ARID1A[1]	DDX3X[2]	MYD88[1,2]	TET2[1,2]
B2M[1]	DNMT3A[1,2]	NFKBIE[1,2]	TNFAIP3[1]
BCL2[1]	EP300[1]	NOTCH1[1,2]	TNFRSF14
BIRC3[1,2]	EZH2[1,2]	NOTCH2[1,2]	TP53[1,2]
BRAF[1,2]	FBXW7	NRAS	TRAF2[1]
BTK[1,2]	FOXO1[1]	PIM1	XPO1[1]
CARD11[1,2]	GNA13	PLCG1[1]	
CCND1[1]	ID3[1,2]	PLCG2[1,2]	
CD28[1]	IDH2[1,2]	RHOA[1,2]	
CD58	JAK3[1]	SF3B1[1,2]	
CD79A[1]	KLF2[1,2]	SOCS1	
CD79B[1,2]	KRAS	STAT3[1,2]	
CDKN2A[1]	MAP2K1	STAT5B[1]	
CREBBP[1]	MEF2B[1]	STAT6[1]	
CXCR4[1,2]	MYC[1]	TCF3[1,2]	

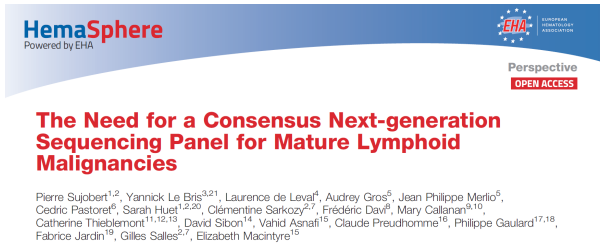
[1] included in panel of SUJOBERT et al.
[2] Included in panel of ROSENQUIST et al.


REVIEW ARTICLE

Clinical impact of recurrently mutated genes on lymphoma diagnostics: state-of-the-art and beyond

Richard Rosenquist,[1] Andreas Rosenwald,[2] Ming-Qing Du,[3] Gianluca Gaidano,[4] Patricia Groenen,[5] Andrew Wotherspoon,[6] Paolo Ghia,[7] Philippe Gaulard,[8] Elias Campo,[9] Kostas Stamatopoulos,[10] on behalf of the European Research Initiative on CLL (ERIC) and the European Association for Haematopathology (EAHP)

Haematologica 2016
Volume 101(9):1002-1009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Perspective
OPEN ACCESS

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Q1.

Composition

Lymphoid gene panel

Literature search

Genes included			
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B2M[1]	DNMT3A[1,2]	NFKBIE[1,2]	TNFAIP3[1]
BCL2[1]	EP300[1]	NOTCH1[1,2]	TNFRSF14
BIRC3[1,2]	EZH2[1,2]	NOTCH2[1,2]	TP53[1,2]
BRAF[1,2]	FBXW7	NRAS	TRAF2[1]
BTK[1,2]	FOXO1[1]	PIM1	XPO1[1]
CARD11[1,2]	GNA13	PLCG1[1]	
CCND1[1]	ID3[1,2]	PLCG2[1,2]	
CD28[1]	IDH2[1,2]	RHOA[1,2]	
CD58	JAK3[1]	SF3B1[1,2]	
CD79A[1]	KLF2[1,2]	SOCS1	
CD79B[1,2]	KRAS	STAT3[1,2]	
CDKN2A[1]	MAP2K1	STAT5B[1]	
CREBBP[1]	MEF2B[1]	STAT6[1]	
CXCR4[1,2]	MYC[1]	TCF3[1,2]	

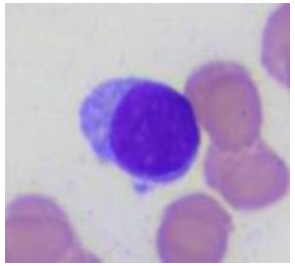
[1] included in panel of SUJOBERT et al.
[2] Included in panel of ROSENQUIST et al.

Functie gen	Gen	Type	pathologie	mutatie frequentie (%)	CNV frequentie (%)	Diagnostisch belang	Prognostisch belang	Therapeutisch belang	Referentie
Signalisatie pathways									
andere	**CXCR4**	OC	WM	27		1	0	2 (respons ibrutinib afh van mutatie MYD88 en CXCR4)	Sujobert 2018, Rosenquist 2016
			DLBCL	4		?	?	?	Rosenquist 2017

Q1.
Composition
Lymphoid gene panel

Literature search

LPL

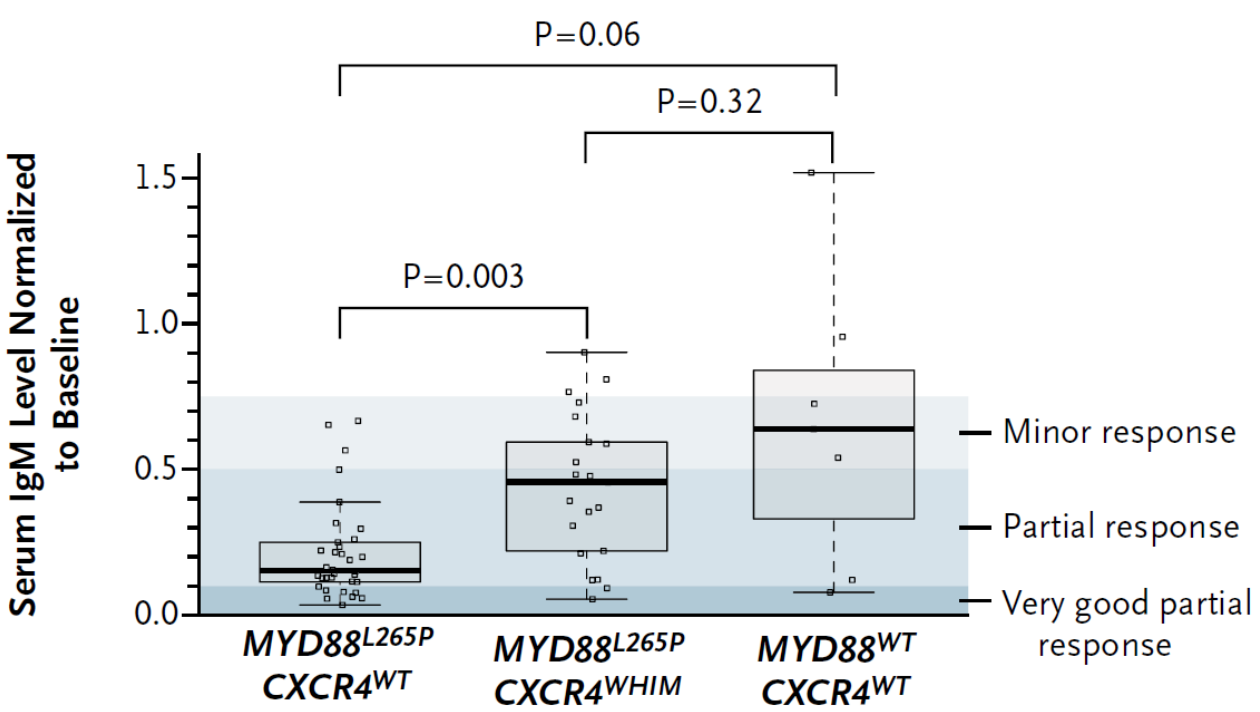


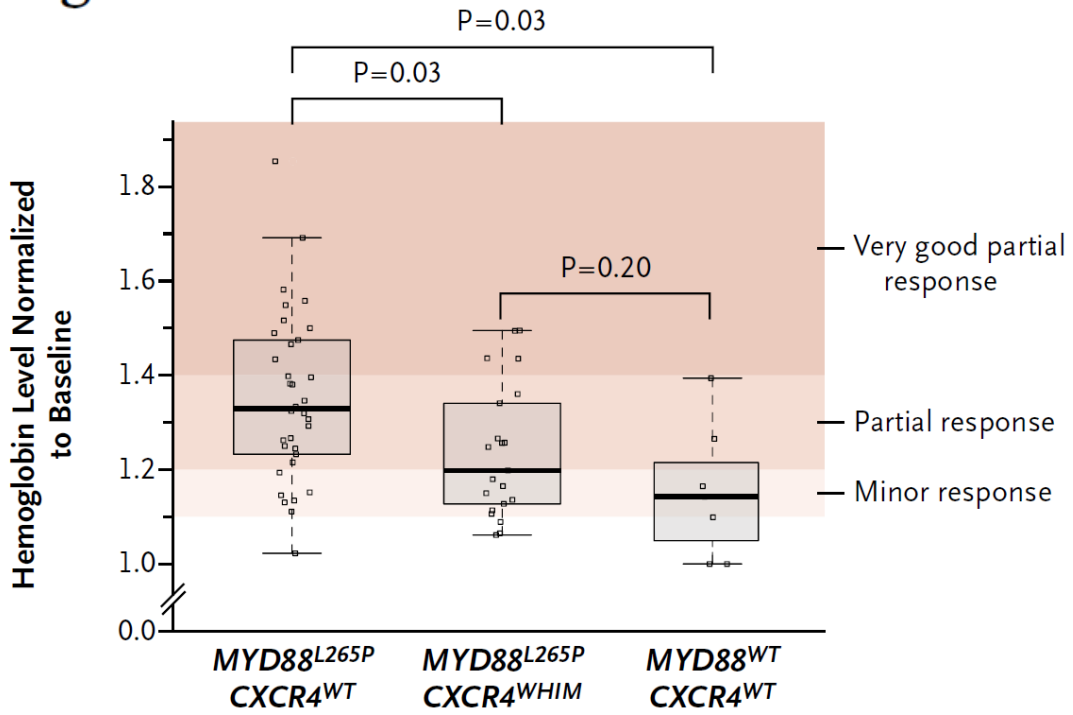
MYD88^{L265P}	triggers tumor-cell growth through Bruton's tyrosine kinase (target ibrutinib)
CXCR4^{WHIM}	in vitro resistance ibrutinib

The NEW ENGLAND JOURNAL of MEDICINE

Ibrutinib in Previously Treated Waldenström's Macroglobulinemia

A





Treon SP et al. NEJM (2015) 372:1430-40.

Q1.

Composition

Lymphoid gene panel

Literature search

Gene	Exon	transcript	refseq
ARID1A	1-20	ENST00000324856.7	NM_006015
B2M	1-3	ENST00000558401.1	NM_004048
BCL2	1-2	ENST00000398117.1	NM_000633
BIRC3	4-10	ENST00000532808.1	NM_182962
BRAF	15	ENST00000288602.6	NM_004333
BTK	15	ENST00000308731.7	NM_000061
CARD11	4-10	ENST00000396946.4	NM_032415
CCND1	1	ENST00000227507.2	NM_053056
CD28	1-4	ENST00000324106.8	NM_001243077
CD58	1-6	ENST00000369489.5	NM_001779
CD79a	4-5	ENST00000221972.3	NM_001783
CD79b	5-6	ENST00000392795.3	NM_001039933
CDKN2A	1-2	ENST00000361570.3	NM_058195
CREBBP	1-31	ENST00000262367.5	NM_004380
CXCR4	2	ENST00000241393.3	NM_003467
DDX3X	1-17	ENST00000399959.2	NM_001193416
DNMT3A	8-23	ENST00000264709.3	NM_17562
EP300	1-31	ENST00000263253.7	NM_001429
EZH2	2-20	ENST00000320356.2	NM_001203247
FBXW7	8-10	ENST00000281708.4	NM_033632
FOXO1	1-2	ENST00000379561.5	NM_002015
GNA13	1-4	ENST00000439174.2	NM_006572
ID3	1-2	ENST00000374561.5	NM_002167
IDH2	4	ENST00000330062.3	NM_002168
JAK3	10-19	ENST00000458235.1	NM_000215
KLF2	1-3	ENST00000248071.5	NM_016270

Gene	Exon	transcript	refseq
KRAS	2-5	ENST00000311936.3	NM_004985
MAP2K1	2-3	ENST00000307102.5	NM_002755
MEF2B	2-9	ENST00000424583.2	NM_001145785
MYC	1-3	ENST00000377970.2	NM_002467
MYD88	3-5	ENST00000396334.3	NM_002468
NFKBIE	1-6	ENST00000275015.5	NM_004556
NOTCH1	26-27,34	ENST00000277541.6	NM_017617
NOTCH2	34	ENST00000256646.2	NM_024408
NRAS	2-3	ENST00000369535.4	NM_002524
PIM1	1-6	ENST00000373509.5	NM_001243186
PLCG1	1,11,29	ENST00000373272.2	NM_002660
PLCG2	19,20,22,24,26,27	ENST00000359376.3	NM_002661
RHOA	2-3	ENST00000418115.1	NM_001664
SF3B1	13-16	ENST00000335508.6	NM_012433
SOCS1	2	ENST00000332029.2	NM_003745
STAT3	19-21	ENST00000264657.5	NM_139276
STAT5B	14-17	ENST00000293328.3	NM_012448
STAT6	12-18	ENST00000300134.3	NM_001178078
TCF3	16-18	ENST00000344749.5	NM_001136139
TET2	3-11	ENST00000380013.4	NM_001127208
TNFAIP3	2-9	ENST00000237289.4	NM_001270507
TNFRSF14	1-8	ENST00000355716.4	NM_003820
TP53	2-11	ENST00000269305.4	NM_000546
TRAF2	2-11	ENST00000247668.2	NM_021138
XPO1	15,19	ENST00000401558.2	NM_003400

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brugge - oostende av

Questions

1.
Which **genes** should be included
in a next-generation sequencing panel
for mature lymphoid malignancies providing
diagnostic, **prognostic** and **therapeutic** information?

2.
How can such panel be **implemented** in
routine clinical practice?

Variant analysis

cancers

MDPI

Project Report

Standardization of Somatic Variant Classifications in Solid and Haematological Tumours by a Two-Level Approach of Biological and Clinical Classes: An Initiative of the Belgian ComPerMed Expert Panel

Guy Froyen [1,*,†], Marie Le Mercier [2,†], Els Lierman [3,†], Karl Vandepoele [4,†], Friedel Nollet [5,†], Elke Boone [6,†], Joni Van der Meulen [7,†], Koen Jacobs [8], Suzan Lambin [9], Sara Vander Borght [10], Els Van Valckenborgh [11], Aline Antoniou [12] and Aline Hébrant [11]

Cancers **2019**, *11*, 2030; doi:10.3390/cancers11122030

Variant analysis

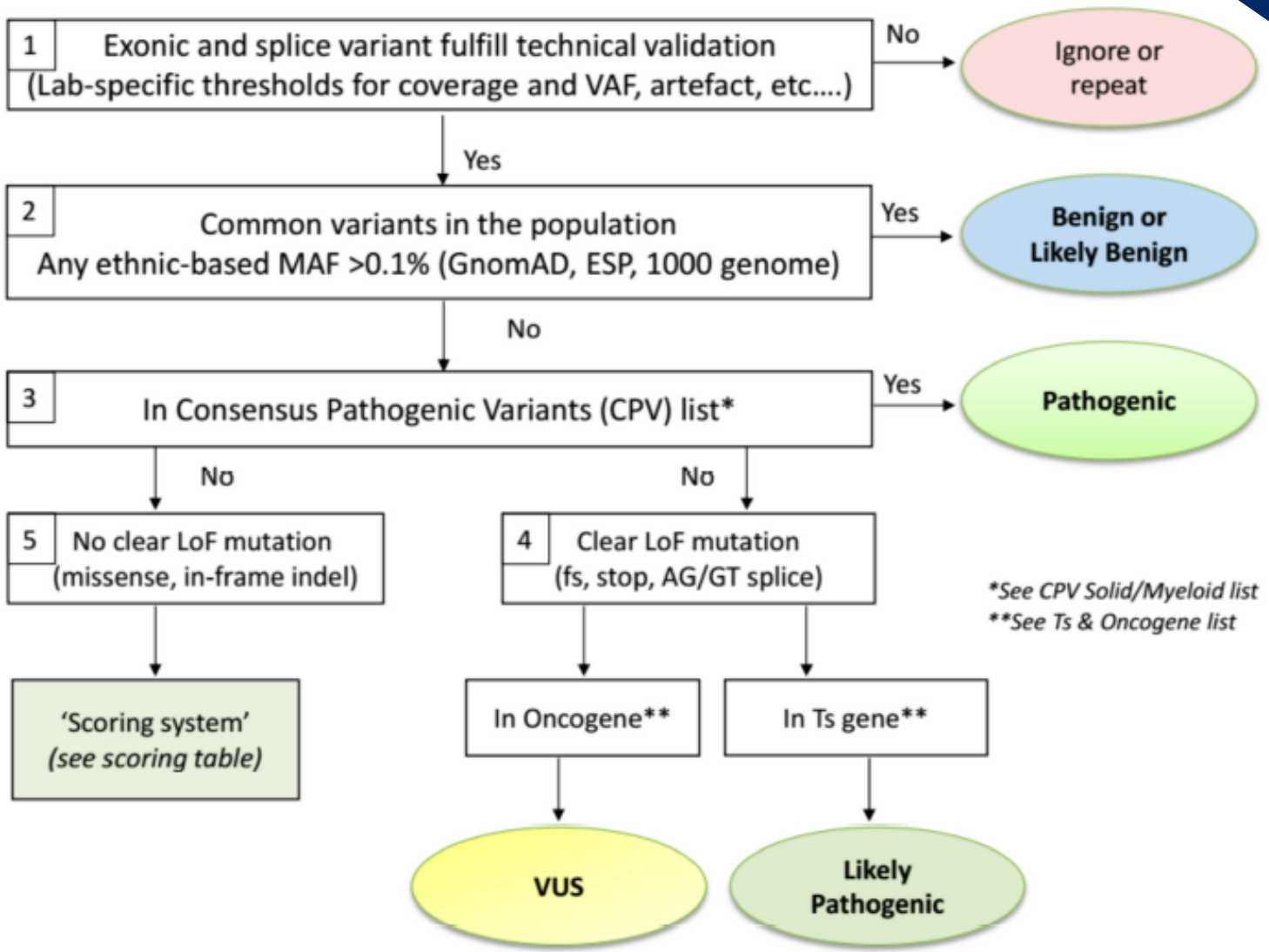


Figure 1. ComPerMed workflow for the biological classification of somatic variants.

Variant analysis

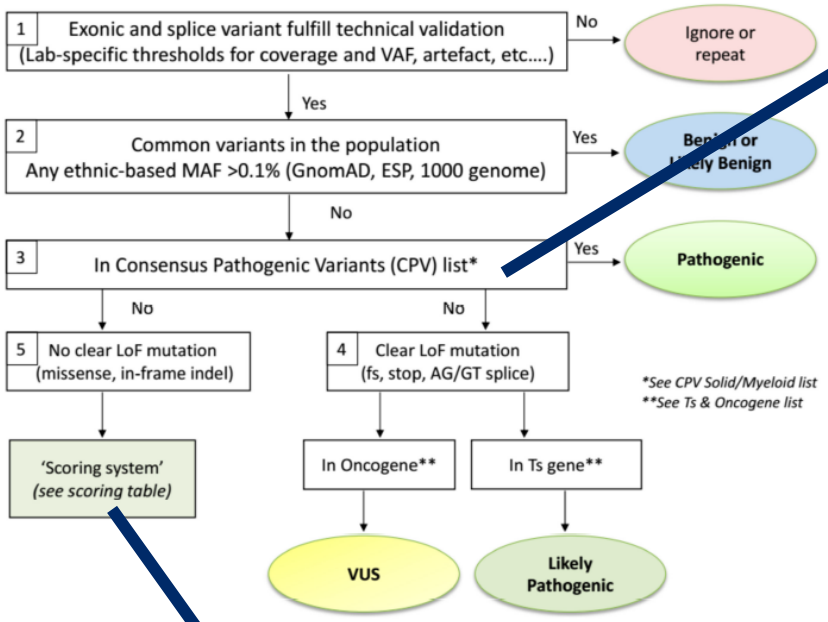


Figure 1. ComPerMed workflow for the biological classification of somatic variants.

Table 2. Consensus Pathogenic Variant (CPV) list of the ComPerMed genes selected for myeloid tumours.

Gene	Transcript ID	Hs1	Hs2	Hs3	Hs4	Hs5	Hs6
ASXL1	NM_015338.5	none					
CALR	NM_004343.3	ex9of-del	ex9of-ins				
CEBPA	NM_004364.3	none					
CSF3R	NM_156039.3	T618I					
DNMT3A	NM_175629.2	R882C/H					
EZH2	NM_004456.4	Y646F/H/N/S					
FLT3	NM_004119.2	ex14if-dup	D835A/E/H/V/Y				
IDH1	NM_005896.3	R132C/G/H/L/S					
IDH2	NM_002168.3	R140L/Q/W	R172K/M/S				
JAK2	NM_004972.3	ex12 if-del/if-dup	V617F				
KIT	NM_000222.2	*see CPV Solid list*					
MPL	NM_005373.2	S505N	W515any ms				
NPM1	NM_002520.6	ex11of-ins					
RUNX1	NM_001754.4	none					
SETBP1	NM_015559.3	D868N	G870S				
SF3B1	NM_012433.3	E622D	R625C/H	H662Q	K666N/R/T	K700E	G742D
SRSF2	NM_003016.4	P95H/L/R	P95_R102del				
TET2	NM_001127208.2	none					
TP53	NM_000546.5	R175H	Y220C	G245S	R248Q/W	R273C/H	R282W
U2AF1	NM_006758.2	S34F/Y	Q157P/R				
WT1	NM_024426.5	none					

Hs: Hotspot; if-del: inframe deletion; if-dup: inframe duplication; of-del: out of frame deletion; of-ins: out of frame insertion; any ms: any missense variant; none: no consensus pathogenic variants present.

Table 3. Scoring Table for the biological variant classification of non-loss-of-function (LoF) variants.

Parameter	Score +2	Score +1	Score +0.5	Score 0	Score −1
Total # of entries of that particular AA change at that position in COSMIC	Solid: ≥50 Hemato: ≥10	50 > x > 10 10 > x > 5	/ /	≤10 ≤5	/ /
In silico prediction tools SIFT and MutationTaster	/	/	Both damaging and deleterious	Other	/
Harmful in functional studies (PubMed, JAX-CKB, MDA, MCG)	/	/	Yes	Not reported	No
Described in at least one genomic db (CIVIC, ClinVar, OncoKb, VarSome)	/	/	As (Likely) Pathogenic	Not described/unknown	As (Likely) Benign

Variants with a score ≥2 will be classified as "Likely Pathogenic". Variants with a score <2 are classified as "VUS".

Q2.
Implementation
Lymphoid gene panel

az sint-jan brugge - oostende av

AZ SINT-JAN BRUGGE-OOSTENDE AV
Laboratoriumgeneeskunde
Ruddershove 10, 8000 Brugge
Dr.B.Cauwelier, Dr.H.Devos,Dr.J.Emmerechts,Dr.K.Floré
Prof.Dr.M.Langlois,Dr.E.Nulens, Dr.M.Reynders
Dr.S.Roggeman, Dr.T.Vanwynsberghe, Prof.Dr.M.Vercammen

Tel: 050/45.99.00 Fax: 050/45.26.19

03783024

Patientnr 5102223580

Geboortedatum: 22/02/1951
Bloedgroep : A Positief
Voorschrift: 09/01/20
Ontvangst: 09/01/20 om 16:10
Afname: 09/01/20 om 16:00

Hematologie
Voorschrijvende arts: Snauwaert Sylvia

Selectief SJ JA09 1463
van 23/04/20 om 11:30 **Pagina 1 / 4**

Materiaal: 1 x BM Cytogenetica (PCG), 1 x BM op EDTA (BED), 1 x Beenmerg
Klinische geg.: Vermoeden therapie-gerelateerde acute leukemie

Naam	Resultaat	Vorig resultaat	Eenheid	Referentiewaarden	
Beenmergonderzoek					
NGS myeloid panel					
Genen panel en toestel	QiaSeq myeloid custom DNA panel v1.0 (Qiagen) op MiSeq Achtergrond: Volgende genen worden via targeted NGS resequencing onderzocht: ASXL1, CALR, CEBPA, CSF3R, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, MPL, NPM1, RUNX1, SETBP1, SF3B1, SRSF2 (=SFRS2), TET2, TP53, U2AF1 en WT1. Enkel varianten tot een waargenomen allel frequentie van 2% worden gerapporteerd. De gevoeligheid van de analyse is minstens 5% allel frequentie voor substitutievarianten en kleine indels (<25 bp, zoals bv. NPM1 type A,B,D hotspot en CALR typ 10^{-2} mutaties). Grote indels (zoals bv. CALR typ 10^{-1} mutaties) vertonen een verminderde gevoeligheid, alsook de detectie van grote ITD mutaties. Daarom wordt volgens indicatie een Flt3-ITD PCR-fragmentanalyse ter bevestiging uitgevoerd. Deze test kan geen onderscheid maken tussen een verworven en een germline mutatie. Voor meer informatie over de doelwitregios en de gebruikte transcripten zie http://www.azsintjan.be/labogids. Gedetailleerde informatie over de analyse is in het laboratorium beschikbaar.				
Variant-1	DNMT3A c.2645G>A p.(Arg882His) 42% VAF				(BCP)
Variant-1 classificatie	Pathogeen, gekende hotspot mutatie				(BCP)
Besluit	Een pathogene mutatie in het DNMT3A gen werd waargenomen. Het waargenomen mutatieprofiel past bij de diagnose van therapy related myeloïd neoplasm.				(BCP)
Klinische info pathogene mutaties **DNMT3A** mutaties komen voornamelijk voor bij AML (20-30%) en MDS (10-15%) en in mindere mate bij CMML en andere myeloproliferatieve aandoeningen. Ook bij gezonde oudere personen worden mutaties van het DNMT3A gen waargenomen (Clonal Hematopoiesis of Indeterminate Potential - CHIP, 10% op de leeftijd van 80j). Mutaties in dit gen zouden prognostisch ongunstig zijn.					(BCP)
NGS VUS varianten Volgende variant van onbekende betekenis (VUS) werd vastgesteld: DNMT3A, c.1648G>A p.(Gly550Arg), 46% VAF					(BCP)

Section test results

HGVS-nomenclature *(Human Genome Variation Society)*

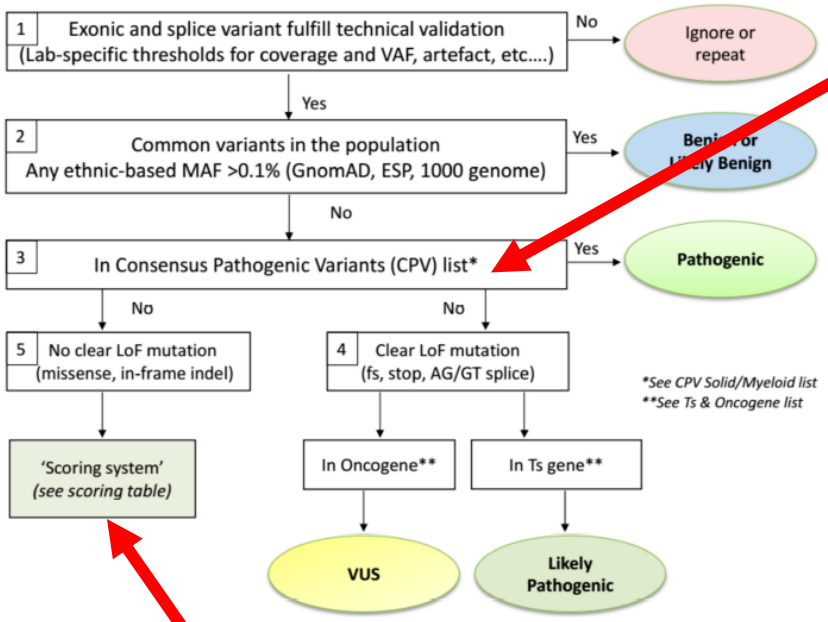
c.2645G>A p.(Arg882His)

- Coding DNA sequence
- Guanine-to-adenine at position 2645
- protein
- Arginine-to-Histidine at codon 882

Section conclusion & interpretation

VUS (Likely) benigne variants must **not** be reported

Variant analysis



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WT1	NM_024426.5	none					

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Variants with a score ≥2 will be classified as "Likely Pathogenic". Variants with a score <2 are classified as "VUS".

Total costs per sample

	Price per sample (€) 51-gene Lymphoid Panel
Extraction	3.63
Concentration calculation	0.66
QiaSeq (custom panel + index set)	82.24
Sequencing	127.87
Instruments	18.89
Bioinformatic software	8.84
Maintenance contracts	10.87
Working time	64.68
validation & quality control	42.96
Total	**360.65**
21% VAT + 56,6% overhead costs	680.76

Price per sample (€) *21-gene Myloid Panel*
239.52
452.12

Clinical indications

Genes included			
ARID1A[1]	DDX3X[2]	MYD88[1,2]	TET2[1,2]
B2M[1]	DNMT3A[1,2]	NFKBIE[1,2]	TNFAIP3[1]
BCL2[1]	EP300[1]	NOTCH1[1,2]	TNFRSF14
BIRC3[1,2]	EZH2[1,2]	NOTCH2[1,2]	TP53[1,2]
BRAF[1,2]	FBXW7	NRAS	TRAF2[1]
BTK[1,2]	FOXO1[1]	PIM1	XPO1[1]
CARD11[1,2]	GNA13	PLCG1[1]	
CCND1[1]	ID3[1,2]	PLCG2[1,2]	
CD28[1]	IDH2[1,2]	RHOA[1,2]	
CD58	JAK3[1]	SF3B1[1,2]	
CD79A[1]	KLF2[1,2]	SOCS1	
CD79B[1,2]	KRAS	STAT3[1,2]	
CDKN2A[1]	MAP2K1	STAT5B[1]	
CREBBP[1]	MEF2B[1]	STAT6[1]	
CXCR4[1,2]	MYC[1]	TCF3[1,2]	

[1] included in panel of SUJOBERT et al.
[2] Included in panel of ROSENQUIST et al.

2020

1. **Inconclusive** tentative **diagnosis**

   [+ lymphomas of very rary entities]

   decision: **MOC**

...

2. New guidelines/targeted therapies?

Clinical indications

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ARID1A[1]	DDX3X[2]	MYD88[1,2]	TET2[1,2]
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CD28[1]	IDH2[1,2]	RHOA[1,2]	
CD58	JAK3[1]	SF3B1[1,2]	
CD79A[1]	KLF2[1,2]	SOCS1	
CD79B[1,2]	KRAS	STAT3[1,2]	
CDKN2A[1]	MAP2K1	STAT5B[1]	
CREBBP[1]	MEF2B[1]	STAT6[1]	
CXCR4[1,2]	MYC[1]	TCF3[1,2]	

[1] included in panel of SUJOBERT et al.
[2] Included in panel of ROSENQUIST et al.

B-cell lymphomas

T-cell lymphomas

2020

1. **Inconclusive** tentative **diagnosis**

   [+ lymphomas of very rary entities]

   decision: **MOC**

...

2. New guidelines/targeted therapies?

Clinical indications

Genes included			
ARID1A[1]	DDX3X[2]	MYD88[1,2]	TET2[1,2]
B2M[1]	DNMT3A[1,2]	NFKBIE[1,2]	TNFAIP3[1]
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BIRC3[1,2]	EZH2[1,2]	NOTCH2[1,2]	TP53[1,2]
BRAF[1,2]	FBXW7	NRAS	TRAF2[1]
BTK[1,2]	FOXO1[1]	PIM1	XPO1[1]
CARD11[1,2]	GNA13	PLCG1[1]	
CCND1[1]	ID3[1,2]	PLCG2[1,2]	
CD28[1]	IDH2[1,2]	RHOA[1,2]	
CD58	JAK3[1]	SF3B1[1,2]	
CD79A[1]	KLF2[1,2]	SOCS1	
CD79B[1,2]	KRAS	STAT3[1,2]	
CDKN2A[1]	MAP2K1	STAT5B[1]	
CREBBP[1]	MEF2B[1]	STAT6[1]	
CXCR4[1,2]	MYC[1]	TCF3[1,2]	

[1] included in panel of SUJOBERT et al.
[2] Included in panel of ROSENQUIST et al.

2020

TO DO – verification study

1. **Inconclusive** tentative **diagnosis**

   [+ lymphomas of very rary entities]

   decision: **MOC**

...

2. New guidelines/targeted therapies?

...

TO DO – cell free DNA panel

Critically Appraised Topic

Next-generation sequencing panel for mature lymphoid malignancies

az sint-jan
brugge - oostende av

Helena DEVOS, MD
Friedel NOLLET, PhD