Supplemental Material

Prevalence of *IRF4* Rearrangement in Large B-Cell Lymphomas of the Waldeyer's Ring in Adults

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Supplemental methods

Gene expression profiling (GEP)

Next generation sequencing (NGS-)based gene expression profiling (GEP) was performed to classify the DLBCL samples into GCB, ABC or unclassifiable subtypes using the HTG EdgeSeq System (HTG Molecular Diagnostics Inc., Tucson, AZ, USA). HTG gene expression data was generated using the HTG EdgeSeq DLBCL Cell of Origin Assay IT for Ion Torrent platform (Ion GeneStudio S5 prime, Thermo Fisher Scientific, Waltham, MA, USA). Starting material was tissue sections using 5 µm formalin-fixed paraffin embedded (FFPE) sections according to the manufactures protocol. Target capture, final libraries and semiconductor sequencing were performed with KAPA Library Quantification Kit, the Ion 510 & Ion 520 & Ion 530 Kit – Chef and the Ion 520 Chip Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the HTG recommendations, prepared for sequencing for Ion Torrent platform (F. Hoffman - La Roche AG, Basel/Kaiseraugst, Schweiz). The HTG EdgeSeq DLBCL panel measures the expression of 92 genes associated to B-cell lymphomas. The COO classification was performed using the HTG Edge System software (Version 5.5.823.5747). The HTG algorithm is trained to minimise the unclassifiable subgroup when compared with other methods. [1]

Mutational analysis – targeted Next Generation Sequencing

Targeted mutation analysis was performed by Next Generation Sequencing (Ion GeneStudio S5 prime, Thermo Fisher Scientific) using an AmpliSeq Custom Panel designed for DLBCLs (supplemental Table 1). Amplicon library preparation and semiconductor sequencing was done according to the manufacturers' manuals using the Ion AmpliSeq Library Kit v2.0, the Ion Library TaqMan Quantitation Kit, the Ion 510 & Ion 520 & Ion 530 Kit – Chef, the Ion 520 Chip Kit and the Ion 530 Chip Kit (Thermo Fisher Scientific) as described before [2, 3].

DNA extracted from FFPE material (Maxwell® RSC DNA FFPE Kit and the Maxwell® RSC Instrument (Promega, Madison, WI, USA)) was quantified using the Invitrogen Qubit 3 fluorometer (Thermo Fisher Scientific), according to the manufacturer's protocol. For each sample, two PCR reactions were prepared, using 10 ng of DNA, AmpliSeq HiFi Mix Plus (Thermo Fisher Scientific) and one of the two panel-specific primer pools, containing 95 or 98 amplicons. The PCR run was set up according to the manufacturer's protocol: initial denaturation at 99 °C for 2 minutes, followed by 24 cycles of 99 °C for 15 seconds and 60 °C for 4 minutes. After completing the PCR reaction, an enzymatic partial digestion of the PCR primer was performed using FuPa exonuclease (Thermo Fisher Scientific), followed by an enzymatic ligation of Ion Xpress Barcode Adapters (Thermo Fisher Scientific). The prepared libraries were cleaned up using the HighPrep PCR clean-up system (MagBio Genomics Inc.,

Gaithersburg, MD, USA) and quantified using the Ion Library TaqMan Quantitation Kit (Thermo Fisher Scientific) on a LightCycler 480 qPCR instrument (Roche Molecular Systems). Libraries were pooled and processed to clonal library amplification and enrichment on Ion Sphere particles using the Ion Chef platform (Thermo Fisher Scientific). Sequencing was performed on a 520 or 530 chip on the Ion GeneStudio S5 prime (Thermo Fisher Scientific). The data analysis was performed on the Ion Torrent Software and the Ion Reporter Software (both Thermo Fisher Scientific). The threshold for mutations was set to an allele frequency (VAF) of 5% (6% and 7% in one case each due to the limited integrity of the DNA in these cases). Variant calling of non-synonymous somatic variants compared to the human reference sequence hg19 was performed using Ion Reporter Software (Thermo Fisher Scientific, Version 5.10.1 to Version 5.16.0.2). Variants called by the Ion Reporter Software were visualized using the Integrative Genomics Viewer (IGV; Broad Institute, Cambridge, MA; Version 2.8.0) to exclude panel-specific artefacts. All mutations were verified using the Catalogue of Somatic Mutations in Cancer (COSMIC; Wellcome Sanger Institute, Cambridgeshire, UK), and the reference SNP (rs) report (U.S. National Library of Medicine). Precition scores were created using COSMIC FATHMM, Varsome and CADD.

Not entirely clear assessable variants were validated using the Ion Amplicon Library Preparation Fusion Method (Thermo Fisher Scientific) according to the manufacturers protocol. Primers were designed using the primer3 software (supplemental Table 2).

Supplemental tables

Supplemental table 1. AmpliSeq Custom Panel for the analysis of DLBCL.

Gene	Transcript	Position (GRCh37/hg19)	Exon(s)	Amplicons*	Coverage of CDS (%)
EZH2	NM_004456	chr7:148,508,712 - chr7:148,508,789	16	1	-
CD79B	NM_001039933	chr17:62,006,789 - chr17:62,006,840	5	1	-
		chr17:62,006,586 - chr17:62,006,654	6	1	-
MYD88	NM_002468	chr3:38,181,874 - chr3:38,182,064	3	4	-
		chr3:38,182,243 - chr3:38,182,344	4	2	-
		chr3:38,182,618 - chr3:38,182,726	5	2	-
CARD11	NM_032415	chr7:2,946,272 - chr7:2,998,140	CDS	54	95,30
IRF4	NM_002460	chr6:393,153 - chr6:407,598	CDS	18	95,61
BCL2	NM_000633	chr18:60,795,858 - chr18:60,985,899	CDS	9	95,86
TNFAIP3	NM_006290	chr6:138,192,365 - chr6:138,202,456	CDS	29	96,49
PRDM1	NM_001198	chr6:106,534,429 - chr6:106,555,361	CDS	29	91,66
BCL6	NM_001706	chr3:187,440,246 - chr3:187,451,481	CDS	27	92,14
PIM1	NM_002648	chr6:37,138,352 - chr6:37,141,867	CDS	16	90,98

* The amplicon lengths range between 125-175 bp.

CDS: coding sequence. Coverage of the panel in total: 94,23%. Amplicons in total: 193. Amplicons pool 1: 98. Amplicons pool 2: 95.

Supplemental table 2. Custom single amplicons for the NGS targeted sequencing. Primer sequences for targeted resequencing using the Ion Amplicon Library Preparation Fusion Method including the sequences of the A or trP1 adapter and the barcode sequence.

Gene	Primer name	Primer sequence (5´-3´)
EZH2	EZH2 Ex16 646 BC63 AF	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTAGAGTTCGATTATTGCTGGCACCATCTGAC
	EZH2 Ex16 646 BC63 AR	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTAGAGTTCGATTGAATACAGGTTATCAGTGCCTT
	EZH2 Ex16 646 trP1F	CCTCTCTATGGGCAGTCGGTGATTATTGCTGGCACCATCTGAC
	EZH2 Ex16 646 trP1R	CCTCTCTATGGGCAGTCGGTGATTGAATACAGGTTATCAGTGCCTT
	EZH2 Ex16 646 BC64 AF	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGAGTTCCGACGATTATTGCTGGCACCATCTGAC
	EZH2 Ex16 646 BC64 AR	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGAGTTCCGACGATTGAATACAGGTTATCAGTGCCTT
	EZH2 Ex16 646 trP1F	CCTCTCTATGGGCAGTCGGTGATTATTGCTGGCACCATCTGAC
	EZH2 Ex16 646 trP1R	CCTCTCTATGGGCAGTCGGTGATTGAATACAGGTTATCAGTGCCTT
	EZH2 Ex16 646 BC65 AF	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTGGCACATCGATTATTGCTGGCACCATCTGAC
	EZH2 Ex16 646 BC65 AR	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTGGCACATCGATTGAATACAGGTTATCAGTGCCTT
	EZH2 Ex16 646 trP1F	CCTCTCTATGGGCAGTCGGTGATTATTGCTGGCACCATCTGAC
	EZH2 Ex16 646 trP1R	CCTCTCTATGGGCAGTCGGTGATTGAATACAGGTTATCAGTGCCTT
IRF4	IRF4_Ex2_BC91A_F	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGAAGGATGCGATCGGGGCATGAACCTGGAG
	IRF4_Ex2_BC91A_R	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGAAGGATGCGATCGGTTGTAGTCCTGCTTGC
	IRF4_Ex2_trP1_F	CCTCTCTATGGGCAGTCGGTGATCGGGGCATGAACCTGGAG
	IRF4_Ex2_trP1_R	CCTCTCTATGGGCAGTCGGTGATCGGTTGTAGTCCTGCTTGC
	IRF4_A1-35_BC69A_F	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCAATTGGCGATTCTCCCCGCAGTGCAGAG
	IRF4_A1-35_BC69A_R	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCAATTGGCGATTCGTTCTCCCACACCAGC
	IRF4_A1-35_trP1_F	CCTCTCTATGGGCAGTCGGTGATTCTCCCCGCAGTGCAGAG
	IRF4_A1-35_trP1_R	CCTCTCTATGGGCAGTCGGTGATTCGTTCTCCCACACCAGC
	IRF4_35-72_BC69A_F	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCAATTGGCGATGAAGCTCCGCCAGTGG
	IRF4_35-72_BC69A_R	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCAATTGGCGATCTCTGTCTCTGGGCCCTC

Gene	Primer name	Primer sequence (5´-3´)
	IRF4_35-72_trP1_F	CCTCTCTATGGGCAGTCGGTGATGAAGCTCCGCCAGTGG
	IRF4_35-72_trP1_R	CCTCTCTATGGGCAGTCGGTGATCTCTGTCTCTGGGCCCTC
CARD11	CARD11 Ex20 871 BC58 AF	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTAGAACACGATAGGGCCTGACTGATTGATAAAT
	CARD11 Ex20 871 BC58 AR	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTAGAACACGATCTGAAGGAGCTGGCCAAAA
	CARD11 Ex20 871 trP1F	CCTCTCTATGGGCAGTCGGTGATAGGGCCTGACTGATTGATAAAT
	CARD11 Ex20 871 trP1R	CCTCTCTATGGGCAGTCGGTGATCTGAAGGAGCTGGCCAAAA
	CARD11 Ex23/24 1046 BC60 AF	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAGCTCTTCGATCTCAGAAGGCAGAAGACGGA
	CARD11 Ex23/24 1046 BC60 AR	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAGCTCTTCGATCATCCAACCTCCCAGTCCC
	CARD11 Ex23/24 1046 trP1F	CCTCTCTATGGGCAGTCGGTGATCTCAGAAGGCAGAAGACGGA
	CARD11 Ex23/24 1046 trP1R	CCTCTCTATGGGCAGTCGGTGATCATCCAACCTCCCAGTCCC
	CARD11_Ex5_BC33A_F	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTCATTGAACGATTGGAGCTGCTAACCTTCCAG
	CARD11_Ex5_BC33A_R	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTCATTGAACGATGTCTCGGCTCCTCATGACC
	CARD11_Ex5_trP1_F	CCTCTCTATGGGCAGTCGGTGATTGGAGCTGCTAACCTTCCAG
	CARD11_Ex5_trP1_R	CCTCTCTATGGGCAGTCGGTGATGTCTCGGCTCCTCATGACC
PIM1	PIM1_Ex4_124_BC71_F	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGGCTCCGACGATAAGAAGGTGAGCTCGGGTTT
	PIM1_Ex4_124_BC71_R	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGGCTCCGACGATTTTTCGTCCTTGATGTCGCG
	PIM1_Ex4_124_trP1_F	CCTCTCTATGGGCAGTCGGTGATAAGAAGGTGAGCTCGGGTTT
	PIM1_Ex4_124_trP1_R	CCTCTCTATGGGCAGTCGGTGATTTTTCGTCCTTGATGTCGCG
BCL2	BCL2_Ex2_192_BC71_F	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGGCTCCGACGATGCCTTCTTTGAGTTCGGTGG
	BCL2_Ex2_192_BC71_R	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGGCTCCGACGATCACCAAGTGCACCTACCCA
	BCL2_Ex2_192_trP1_F	CCTCTCTATGGGCAGTCGGTGATGCCTTCTTTGAGTTCGGTGG
	BCL2_Ex2_192_trP1_R	CCTCTCTATGGGCAGTCGGTGATCACCAAGTGCACCTACCCA

Supplemental table 3. Overview of the NGS analyses performed.

Case	Panel Analysis	Single Amplicons
1	AmpliSeq Custom DLBCL Panel	EZH2 Ex16 646 IRF4 Ex2 35-72
2	AmpliSeq Custom DLBCL Panel	CARD11 Ex20 871 EZH2 Ex16 646 IRF4 Ex2 35-72
5	AmpliSeq Custom DLBCL Panel	EZH2 Ex16 646 IRF4 Ex2 35-72
6	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72 EZH2 Ex16 646
7	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72
8	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72
9	AmpliSeq Custom DLBCL Panel	CARD11 Ex5 215 IRF4 Ex2 35-72
10	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72
11	AmpliSeq Custom DLBCL Panel	CARD11 Ex23 1046 IRF4 Ex2 35-72
13	AmpliSeq Custom DLBCL Panel	CARD11 Ex23 1046 IRF4 Ex2 1-35 IRF4 Ex2 35-72
15	AmpliSeq Custom DLBCL Panel	CARD11 Ex23 1046 EZH2 Ex16 646 IRF4 Ex2 35-72
16	AmpliSeq Custom DLBCL Panel	CARD11 Ex23 1046 IRF4 Ex2 35-72
17	AmpliSeq Custom DLBCL Panel	CARD11 Ex23 1046 IRF4 Ex2 35-72
19	AmpliSeq Custom DLBCL Panel	EZH2 Ex16 646 IRF4 Ex2 35-72
20	AmpliSeq Custom DLBCL Panel	BCL2 Ex2 53 IRF4 Ex2 35-72 EZH2 Ex16 646
21	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72

Case	Panel Analysis	Single Amplicons
		EZH2 Ex16 646
22	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72
23	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72
24	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72
25	AmpliSeq Custom DLBCL Panel	EZH2 Ex16 646 IRF4 Ex2 35-72
26	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72 BCL2 Ex2 192
27	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72
29	AmpliSeq Custom DLBCL Panel	EZH2 Ex16 646 IRF4 Ex2 35-72
30	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72
31	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72
32	AmpliSeq Custom DLBCL Panel	EZH2 Ex16 646 IRF4 Ex2 35-72
33	AmpliSeq Custom DLBCL Panel	EZH2 Ex16 646 IRF4 Ex2 35-72 PIM1 Ex4 124
34	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 35-72
35	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 1-35 IRF4 Ex2 35-72
36	AmpliSeq Custom DLBCL Panel	IRF4 Ex2 1-35 IRF4 Ex2 35-72

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Supplemental table 4. List of all mutations detected.

#	VAF Threshold	Gene	Transcript	Coordinates	VAF	Coverage	cDNA change	Protein change	Prediction		
									COSMIC FATHMM	Varsome	CADD
2	5%	*CD79B*	NM_001039933	chr17:62006798	29%	2076	c.590A>C	p.Y197S	Pathogenic (score 0.94)	Likely pathogenic	24.5
5	5%	*TNFAIP3*	NM_001270507	chr6:138198338	10%	10385	c.931G>T	p.E311*	–	Pathogenic	38
8	5%	*BCL2*	NM_000633	chr18:60985692	45%	2488	c.208T>G	p.S70A	–	Uncertain significance	17.07
		EZH2	NM_004456	chr7:148508728	14%	190	c.1936T>A	p.Y646N	Pathogenic (score 0.99)	Likely pathogenic	32
9	5%	*PIM1*	NM_002648	chr6:37139097	19%	33595	c.437G>A	p.S146N	Pathogenic (score 0.78)	Uncertain significance	21.9
		CARD11	NM_032415	chr7:2983885	10%	5299	c.645G>C	p.K215N	–	Uncertain significance	22
10	5%	*MYD88*	NM_002468	chr3:38182292	50%	18078	c.728G>A	p.S243N	–	Pathogenic	28.7
		PIM1	NM_002648	chr6:37138354	14%	3582	c.3G>A	p.M1I	–	Pathogenic	24.1
		PIM1	NM_002648	chr6:37138423	13%	3699	c.72G>C	p.K24N	–	Uncertain significance	18.57
11	5%	*PRDM1*	NM_001198	chr6:106536223 - chr6:106536233	50%	34216	c.190_200dup	p.D68Tfs	–	Pathogenic	N/A
13	5%	*PRDM1*	NM_001198	chr6:106547183	51%	7353	c.420delC	p.R141Efs	–	Pathogenic	31
		IRF4	NM_002460	chr6:393187	23%	1217	c.35T>G	p.F12C	–	Uncertain significance	23.3

#	VAF Threshold	Gene	Transcript	Coordinates	VAF	Coverage	cDNA change	Protein change	Prediction		
									COSMIC FATHMM	Varsome	CADD
		IRF4	NM_002460	chr6:393190	23%	1218	c.38G>C	p.G13A	–	Uncertain significance	25
		IRF4	NM_002460	chr6:393208	22%	3827	c.56G>T	p.C19F	–	Uncertain significance	28.9
		IRF4	NM_002460	chr6:393252	28% †	61399	c.100A>G	p.S34G	–	Uncertain significance	27
		IRF4	NM_002460	chr6:393260	42% †	61720	c.108G>T	p.K36N	Pathogenic (score 0.91)	Uncertain significance	24.8
		IRF4	NM_002460	chr6:393295	26% †	64929	c.143G>A	p.S48N	–	Uncertain significance	25
		IRF4	NM_002460	chr6:393332	38% †	65135	c.180G>C	p.Q60H	Pathogenic (score 0.87)	Uncertain significance	24.9
		IRF4	NM_002460	chr6:393342	41% †	65920	c.190C>T	p.R64C	–	Uncertain significance	32
15	5%	*PIM1*	NM_002648	chr6:37139062 - chr6:37139070	89%	4100	c.402_410del	p.E135_G137del	–	Uncertain significance	N/A
		CD79B	NM_001039933	chr17:62006798	42%	4652	c.590A>T	p.Y197F	Pathogenic (score 0.94)	Uncertain significance	24
		BCL2	NM_000633	chr18:60985889	14%	4434	c.11C>T	p.A4V	Pathogenic (score 0.92)	Uncertain significance	22.7
17	5%	*BCL2*	NM_000633	chr18:60985743	89%	2300	c.157C>T	p.P53S	Neutral (score 0.04)	Uncertain significance	11.12
		BCL2	NM_000633	chr18:60985594	58%	5600	c.306C>A	p.D102E	Neutral (score 0.01)	Uncertain significance	21.2

#	VAF Threshold	Gene	Transcript	Coordinates	VAF	Coverage	cDNA change	Protein change	Prediction		
									COSMIC FATHMM	Varsome	CADD
		BCL2	NM_000633	chr18:60985590	58%	5600	c.310T>A	p.F104I	–	Uncertain significance	22.2
		CARD11	NM_032415	chr7:2977614	51%	11300	c.1070A>T	p.D357V	Pathogenic (score 0.94)	Uncertain significance	28.2
		CARD11	NM_032415	chr7:2979453	46%	3970	c.794A>C	p.Q265P	–	Uncertain significance	21.6
		BCL2	NM_000633	chr18:60985890	45%	2300	c.10G>C	p.A4P	Neutral (score 0.44)	Uncertain significance	22.2
		BCL2	NM_000633	chr18:60985886	45%	2300	c.14G>A	p.G5E	–	Uncertain significance	25.3
		PIM1	NM_002648	chr6:37139210	36%	13990	c.550C>G	p.L184V	Pathogenic (score 0.94)	Uncertain significance	27.3
19	5%	*MYD88*	NM_002468	chr3:38182641	84%	5946	c.794T>C	p.L265P	Pathogenic (score 0.96)	Pathogenic	32
		PRDM1	NM_001198	chr6:106534471	61%	837	c.42+1G>C	p.?	–	Pathogenic	35
		PRDM1	NM_001198	chr6:106554273	59%	2282	c.1801C>T	p.R601W	–	Uncertain significance	32
		PIM1	NM_002648	chr6:37138802 - chr6:37138804	38%	3128	c.235_237delinsAAA	p.E79K	Pathogenic (score 0.97)	Uncertain significance	23.4
20	7%	*MYD88*	NM_002468	chr3:38182641	52%	9706	c.794T>C	p.L265P	Pathogenic (score 0.96)	Pathogenic	32
21	5%	*BCL2*	NM_000633	chr18:60985840	33%	5426	c.60T>G	p.H20Q	N/A	Uncertain significance	21.9

#	VAF Threshold	Gene	Transcript	Coordinates	VAF	Coverage	cDNA change	Protein change	Prediction		
									COSMIC FATHMM	Varsome	CADD
		CARD11	NM_032415	chr7:2977614	30%	8364	c.1070A>T	p.D357V	Pathogenic (score 0.94)	Uncertain significance	29.8
		BCL6	NM_001706	chr3:187444538	30%	10326	c.1689C>G	p.S563R	–	Uncertain significance	28.8
23	5%	*MYD88*	NM_002468	chr3:38182032	28%	11828	c.656C>G	p.S219C	Pathogenic (score 0.96)	Uncertain significance	29.1
24	5%	*PIM1*	NM_002648	chr6:37138769	35%	1057	c.202C>T	p.H68Y	Pathogenic (score 0,97)	Uncertain significance	22.4
		BCL2	NM_000633	chr18:60985549	7%	2238	c.351C>G	p.S117R	Neutral (score 0,03)	Uncertain significance	14.26
		BCL2	NM_000633	chr18:60985562	8%	2211	c.338C>T	p.A113V	Neutral (score 0,03)	Uncertain significance	14.07
		BCL2	NM_000633	chr18:60985575	8%	2216	c.325C>G	p.R109G	–	Uncertain significance	24.7
		BCL2	NM_000633	chr18:60985635	32%	658	c.265G>A	p.V89M	–	Uncertain significance	22.4
		BCL2	NM_000633	chr18:60985644	32%	644	c.256C>G	p.L86V	Neutral (score 0,24)	Uncertain significance	17.47
25	5%	*CARD11*	NM_032415	chr7:2958135	55%	347	c.2597G>A	p.R866Q	Neutral (score 0.22)	Uncertain significance	12.47
26	6%	*PIM1*	NM_002648	chr6:37138365	35%	1027	c.14A>G	p.K5R	–	Uncertain significance	24.1
		PIM1	NM_002648	chr6:37138367	35%	1027	c.16A>G	p.I6V	–	Uncertain significance	18.74

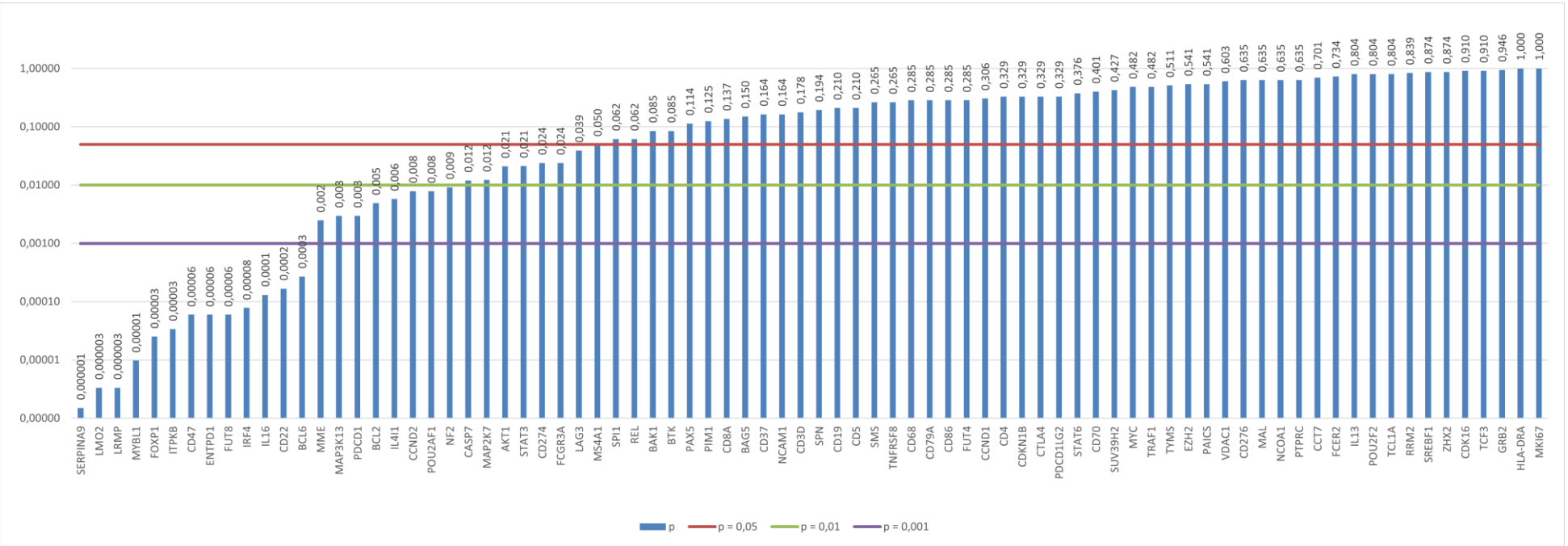
#	VAF Threshold	Gene	Transcript	Coordinates	VAF	Coverage	cDNA change	Protein change	Prediction		
									COSMIC FATHMM	Varsome	CADD
		PIM1	NM_002648	chr6:37138901	30%	2088	c.241C>T	p.P81S	Pathogenic (score 0,97)	Uncertain significance	22.8
		EZH2	NM_004456	chr7:148508727	30% ‡	5985	c.1937A>G	p.Y646C	Pathogenic (score 0,99)	Likely pathogenic	25.2
		BCL2	NM_000633	chr18:60985644	34%	1002	c.256C>T	p.L86F	Neutral (score 0,29)	Uncertain significance	19.64
		BCL2	NM_000633	chr18:60985326	9% ‡	256782	c.574A>C	p.N192H	Neutral (0,10)	Uncertain significance	28,2
27	5%	*BCL2*	NM_000633	chr18:60795869	30%	9448	c.709G>A	p.G237S	–	Uncertain significance	24.5
		BCL2	NM_000633	chr18:60985840	24%	4187	c.60T>A	p.H20Q	N/A	Uncertain significance	21.9
31	5%	*CD79B*	NM_001039933	chr17:62006793	62%	9807	c.594+1G>C	p.?	–	Pathogenic	32
33	5%	*PIM1*	NM_002648	chr6:37139078	13% ‡	152732	c.418C>T	p.Q140*	N/A	Pathogenic	37
		PIM1	NM_002648	chr6:37139063	11% ‡	152732	c.403G>T	p.E135*	–	Pathogenic	37
35	5%	*IRF4*	NM_002460	chr6:393172	31%	903	c.20G>T	p.G7V	–	Uncertain significance	22
		IRF4	NM_002460	chr6:393201	11%	911	c.49G>C	p.V17L	–	Uncertain significance	22.6
		IRF4	NM_002460	chr6:393206	11%	906	c.54C>A	p.S18R	–	Uncertain significance	22.6
		IRF4	NM_002460	chr6:393262	21% †	2285	c.110A>T	p.Y37F	–	Uncertain significance	28.4

#	VAF Threshold	Gene	Transcript	Coordinates	VAF	Coverage	cDNA change	Protein change	Prediction		
									COSMIC FATHMM	Varsome	CADD
		IRF4	NM_002460	chr6:393283	21% †	2444	c.131A>G	p.N44S	–	Uncertain significance	27
		IRF4	NM_002460	chr6:393295	16% †	2310	c.143G>C	p.S48T	–	Uncertain significance	21.1
		IRF4	NM_002460	chr6:393298	23% †	2496	c.146T>C	p.I49T	–	Uncertain significance	25.4
		IRF4	NM_002460	chr6:393330 - chr6:393332	23% †	2575	c.178_180delinsTTC	p.Q60F	–	Uncertain significance	N/A

† Covered only in the single amplicon, frequency therefore of the single amplicon.

‡ Covered in both single amplicon and panel, frequency of single amplicon.

Supplemental figures



Supplemental Fig. 1: p values of Mann Whitney U test of the distribution of gene expression levels across the GEP subtype (GCB – ABC) (log10 y-Axis). Each bar on the x-axis represents the p-value of one statistical test. The red, green, and purple horizontal lines represent the significance levels of p = .05, p = .005 and p = .001. At the significance level p ≤ 0.001, a total of 13 of the genes examined resp. the corresponding mRNAs showed significant differences in gene expression between the subgroups. For the significance level p ≤ 0.01, this was the case for a total of 21 of the genes resp. mRNAs examined (including the 13 genes resp. mRNAs significant at p ≤ 0.001), for p ≤ 0.05 for a total of 29 genes resp. mRNAs.

(Supplemental Figure 1 was created using Microsoft Excel version 2210.)

Supplemental references

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