Low frequency mutations independently predict poor treatment-free survival in early stage chronic lymphocytic leukemia and monoclonal B-cell lymphocytosis

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doi:10.3324/haematol.2014.120238

Supplementary Material

Supplementary Methods

FISH Analysis

FISH analysis was undertaken in all cases using commercially available probes (Abbott Diagnostics, Maidenhead, UK; DakoCytomation, Glostrup, Denmark) to detect deletion of 13q, 11q, 17p and trisomy 12, according to the manufacturers' instructions as previously described (1). Chromosomal analysis was performed and described according to the International System for Human Cytogenetic Nomenclature (2). ZAP70 and CD38 expression were determined as previously described (3) (4), where 10% and 30% positive cells were classed as positive or highly expressive, respectively. IGHV genes were sequenced as previously described (5) and a cut-off of ≥98% germ-line homology was taken to define the unmutated subset.

Mutational screening and sequencing

For each of the seven genes, we aimed to capture the majority of previously reported CLL specific somatic variations. Each genomic DNA sample (gDNA) was subjected to whole genome amplification (WGA) using the Illustra GenomiPhi V2 Amplification Kit® (GE Healthcare) prior to mutational screening. With the exception of the *FBXW7* (direct Sanger Sequencing for all samples), all genes were screened using high-resolution melt (HRM) analysis and subsequent Sanger sequencing as previously described (6, 7). Primer sequences are shown in **Supplementary Table 7.** PCR products showing altered melt patterns were sequenced. All detected changes were sequence validated on genomic DNA (gDNA) from the archival sample. A randomly selected subset of 100 genomic DNA samples that exhibited normal HRM melt profiles on WGA material was directly sequenced for *SF3B1* and *XPO1*. In doing so, we found no additional mutations. For *NOTCH1* we screened a 399bp section of exon 34 (amino acids 2405-2525), where all of the PEST domain mutations have been found (8-11). Further analysis of the c.7544_7545delCT, referred to as 'delCT' variant was performed using PCR-based fragment analysis and KASPar genotyping and is detailed below. We screened exons 14, 15 and 16 of *SF3B1* and exons 15 and 16 of *XPO1* capturing the majority of previously reported disease specific variations (8, 12-14). For *MYD88*, we screened for Exons 3-5 including the known L265P somatic variant by HRM and subsequent sequencing (8). Exons 4 to 10, and exon 18 of *POT1* and Exons 7 and 10 of *BIRC3* were screened using HRM and subsequent Sanger sequencing. For sequentially acquired mutations sample accuracy was confirmed by powerplex testing (Powerplex® 16 System, Promega, Madison, USA). A subset of 31 patients with confirmed trisomy 12 including

sequential samples for 10 patients were screened for mutations in all coding exons of *FBXW7* by Sanger sequencing.

Further analysis of the NOTCH delCT variant

To further assess the sensitivity of the HRM approach for *NOTCH1*, we identified cases with the P2515Rfs*4 (c.7544_7545delCT, referred to as 'delCT') variant using PCR-based fragment analysis [PCR-FA, n= 372] and allele-specific PCR [n= 213]as previously described for the CLL4 cohort (7). For the PCR-FA, the delCT variant was identified using PEST2 domain-specific primers (Forward: GTGACCGCAGCCCAGTTC, reverse: AAAGGAAGCCGGGGTCTC) as previously described (15). PCR products were sized using the LT3500 (Life Technologies) and the 271bp wild-type and 269bp mutant fragments were identified using GeneMapper (v4.1) software (Life Technologies). Allele-specific PCR for the deflect variant was performed by KBiosciences (http://www.kbiosciences.co.uk) using their fluorescence-based competitive allele-specific PCR (KASPar). 106 duplicates were included and the concordance between duplicates was >99%. Sample processing with PCR-FA and KASPar technologies were performed blindly in independent laboratories. Using these two approaches, we confirm the sensitivity of our HRM approach which showed a 100% concordance with our PCR-FA and KASPar analysis.

Supplementary Tables

Supplementary table 1. Clinical, cytogenetic and molecular characteristics of the complete cohort (n=206) and cMBL (n=90) vs. CLL stage A cases (n=116).

	complete cohort	cMBL	CLL Stage A	cMBL vs. CLL Stage A
variable	**N (%)**	**N (%)**	**N (%)**	**P-Value**
Screened Cases	206(100)	90(44)	116(56)	
Female	89(43)	37(41)	52(45)	
Male	117(57)	53(59)	64(55)	0.3
Age (median)	70	70	70	
IGHV unmutated	64(31)	28(31)	36(31)	
mutated	142(69)	62(69)	80(69)	0.6
CD38 negative (30%)	128(70)	52(63)	76(75)	
CD38 positive (30%)	56(30)	31(37)	25(25)	0.05
ZAP 70 negative	120(71)	54(69)	66(72)	
ZAP 70 positive	50(29)	24(31)	26(28)	0.4
del(11q) negative	176(92)	80(94)	96(91)	
del(11q) positive	15(8)	5(6)	10(9)	0.3
del(13q14) negative	69(38.5)	35(45)	34(34)	
del(13q14)positive (monoallelic)	110(61.5)	44(55)	66(66)	0.1
Trisomy 12 negative	124(76)	47(69)	77(80)	
Trisomy 12 positive	40(24)	21(31)	19(20)	0.08
del(17p) negative	185(97)	81(96)	104(98)	
del(17p) positive [1]	5(3)	3(4)	2(2)	0.4
***NOTCH1* wild type**	192(95)	84(94)	108(95)	
***NOTCH1* mutation**	11(5)	5(6)	6(5)	0.6
***SF3B1* wild type**	183(92)	82(94)	101(90)	
***SF3B1* mutation**	16(8)	5(6)	11(10)	0.2
***POT1* wild type**	190(96)	83(95)	107(96)	
***POT1* mutation**	8(4)	4(5)	4(4)	0.5
***XPO1* wild type**	170(99)	72(99)	98(99)	
***XPO1* mutation**	2(1)	1(1)	1(1)	0.7
***FBXW7* wild type (tri 12 cases only)**	29(93.5)	15(94)	14(93)	
***FBXW7* mutation (tri 12 cases only)**	2(6.5)	1(6)	1(7)	0.7
***MYD88* wild type (exon 3,4,5)**	195(98.5)	86(99)	109(98)	
***MYD88* mutation (exons 3,4,5)**	3(1.5)	1(1)	2(2)	0.6
***BIRC3* wild type (exons 7,10)**	196(99.5)	86(99)	110(100)	
***BIRC3* mutation (exons 7,10)**	1(0.5)	1(1)	0(0)	0.4
total number of mutations	43(21)	18(20)	27(23)	0.3
total number of low frequency mutations (excluding *SF3B1, Notch1* and *MYD88*)	13(6)	7(8)	6(5)	0.4

[1] all the del(17p) cases have mutated *IGHV* genes

Supplementary table 2. Clinical, cytogenetic and molecular characteristics of sequential (n=84) vs. single time point cases (n=122).

	sequential cases	single time point cases	
variable	**N (%)**	**N (%)**	**P-Value**
Screened cases	84(41)	122(59)	
Female	34(41)	55(45)	
Male	50(60)	67(55)	0.3
Age (median)	66	72	
Stage A CLL	49(58)	67(55)	
cMBL	35(42	55(45)	0.4
IGHV unmutated	28(33)	36(30)	
IGHV mutated	56(67)	86(71)	0.3
CD38 negative (30%)	54(70)	74(69)	
CD38 positive (30%)	23(30)	33(31)	0.5
ZAP70 negative	53(69)	67(72)	
ZAP70 positive	24(33)	26(28)	0.4
del(11q) negative	70(91)	106(93)	
del(11q) positive	7(9)	8(7)	0.6
del(13q14) negative	31(40)	38(37)	
del(13q14) positive (monoallelic)	46(60)	64(63)	0.4
Trisomy 12 negative	63(83)	61(69)	
Trisomy 12 positive	13(17)	27(33)	**0.05**
del(17p) negative	76(99)	109(96)	
del(17p) positive	1(1)	4(4)	0.3
***NOTCH1* wild type**	79(95)	113(94)	
***NOTCH1* mutation**	4(5)	7(6)	0.5
***SF3B1* wild type**	69(91)	113(93)	
***SF3B1* mutation**	7(9)	9(7)	0.4
***POT1* wild type**	80(96)	110(96)	
***POT1* mutation**	3(4)	5(4)	0.6
***XPO1* wild type**	67(100)	103(98)	
***XPO1* mutation**	0(0)		0.5
***FBXW7* wild type (tri 12 cases only)**	11(100)	18(90)	
***FBXW7* mutation (tri 12 cases only)**	0(0)	2(10)	0.4
***MYD88* wild type (exon 3,4,5)**	82(99)	113(98)	
***MYD88* mutation (exons 3,4,5)**	1(1)	2(2)	0.6
***BIRC3* wild type (exons 7,10)**	81(99)	115(100)	
***BIRC3* mutation (exons 7,10)**	1(1)	0(0)	0.4

Supplementary Table 3. Mutation characteristics and mutation prediction

No.	Chromosome (forward strand)	Gene Symbol (Hugo)	Mutation	Nature of Mutation	COSMIC variation	Somatic in CLL	conservation data(USCSC)	dbsnp/ 1000 genomes	SIFT	SIFT_INT	Polyphen	Polyphen_INT
1	2:198267490	*SF3B1*	32327A>AG:623Y>Y/C	missense variant	COSM1651682	yes	conserved to zebrafish	no known snp	0	deleterious	0.98	probably damaging
2	2:198266611	*SF3B1*	33205G>GA:742G>G/D	missense variant	COSM145923	yes	conserved to zebrafish	no known snp	0.12	tolerated	0.77	possibly damaging
3	2:198266834	*SF3B1*	32982A>AG:700K>K/E	missense variant	COSM84677	yes	conserved to zebrafish	no known snp	0.01	deleterious	0.96	probably damaging
4	2:198267361	*SF3B1*	32455A>AG:666K>K/E	missense variant	COSM110694	yes	conserved to zebrafish	no known snp	0	deleterious	0.98	probably damaging
5	2:198267369	*SF3B1*	32447C>CT:663T>T/I	missense variant	COSM145921	yes	conserved to zebrafish	no known snp	0	deleterious	1	probably damaging
6	2:198266709	*SF3B1*	33107G>GC:741K>K/N	missense variant	COSM572730	yes	conserved to zebrafish	no known snp	0	deleterious	0.99	probably damaging
7	2:198267484	*SF3B1*	32332C>CT:625R>R/C	missense variant	COSM255276	yes	conserved to zebrafish	no known snp	0	deleterious	1	probably damaging
8	2:198267545	*SF3B1*	32269G>GA:604A>A/T	missense variant	no data	unknown	conserved to zebrafish	no known snp	no data		no data	
9	2:198266795	*SF3B1*	33021G>GA:713A>A/T	missense variant	COSM1691803	yes	conserved to zebrafish	no known snp	0	deleterious	1	probably damaging
No.	**Chromosome (forward strand)**	**Gene Symbol (Hugo)**	**Mutation**	**Nature of Mutation**	**COSMIC variation**	**Somatic in CLL**	**conservation data(USCSC)**	**dbsnp/ 1000 genomes**	**SIFT**	**SIFT_INT**	**Polyphen**	**Polyphen_INT**
10	9:139390912	*NOTCH1*	c.7275delCAGCGG:p.S2426Hfs*128-deletion contains a known snp	frameshift variant	no data	unknown	conserved to rhesus	rs370722609	0.3	tolerated	0.09	benign
11	9:139390637-139390646	*NOTCH1*	c.7544_7545delCT P2515Rfs	frameshift variant	COSM13071	yes	conserved to zebrafish	no known snp	no data		no data	
12	9:139391076	*NOTCH1*	7115G>G/A:2372R>R/Q	missense variant	no data	unknown	conserved to x-tropicalis	rs373119531	0.22	tolerated	0.76	possibly damaging

No.	Chromosome (forward strand)	Gene Symbol (Hugo)	Mutation	Nature of Mutation	COSMIC variation	Somatic in CLL	conservation data(USCSC)	dbsnp/ 1000 genomes	SIFT	SIFT_INT	Polyphen	Polyphen_INT
13	7:124892275	*POt1*	37710A>T:K39I	missense variant	COSM131380	yes for cancers	conserved to mammals	no known snp	0	deleterious	0.06	benign
14	7:124826283	*POt1*	UTR g.104749T>G	intron variant	no data	unknown	conserved to rhesus	rs113233510	no data		no data	
15	7:124853116	*POt1*	76868A>AG:242Y>Y/C	missense variant	COSM1659034	yes for cancers	conserved to mammals	no known snp	0	deleterious	0.97	probably damaging
16	7:124503424	*POt1*	526G>GA:176G>G/R (known variant: 176G>G/V)	missense variant	COSM1547942	unknown	conserved to x-tropicalis	no known snp	0.01	deleterious	0.34	benign
17	7:124537282	*POt1*	in between two polymorphic regions in intron 4, chr7:124537282 T>A	intron variant	no data	no data	conserved to rhesus	no known snp	no data		no data	
18	7:124824015-124824016	*POt1*	c.1458_1459del:p.486_487del and c.1851_1852del:p.617_618del	frameshift variant, feature truncation	TMP_ESP_7_124464069_124464070	no data	conserved to rhesus	no known snp	no data		no data	
19	7:37338304	*POt1*	UTR g.23777T>G	intron variant	no data	no data	conserved to humans	no known snp	no data		no data	
20	7:124537197	*POt1*	UTR g.32906A>G	intron variant	no data	no data	conserved to mammals	no known snp	no data		no data	
No.	**Chromosome (forward strand)**	**Gene Symbol (Hugo)**	**Mutation**	**Nature of Mutation**	**COSMIC variation**	**Somatic in CLL**	**conservation data(USCSC)**	**dbsnp/ 1000 genomes**	**SIFT**	**SIFT_INT**	**Polyphen**	**Polyphen_INT**
21	2:61492337	*XPO1*	46290G>GA:571E>E/K	missense variant	COSM96797	yes	conserved to rhesus	no known snp	0	deleterious	1	probably damaging
No.	**Chromosome (forward strand)**	**Gene Symbol (Hugo)**	**Mutation**	**Nature of Mutation**	**COSMIC variation**	**Somatic in CLL**	**conservation data(USCSC)**	**dbsnp/ 1000 genomes**	**SIFT**	**SIFT_INT**	**Polyphen**	**Polyphen_INT**
22	4:152326215	*FBXW7*	208806C>C/G:479R>R/G	missense variant	COSM3127988	yes	conserved to rhesus	no known snp	0	deleterious	1	probably damaging
23	4:152332710	*FBXW7*	202312A>AC:291Y>Y/S (291 Y>Y/N is a known snp)	missense variant	no data	no data	conserved to rhesus	rs369187069 SNP	0.18	tolerated	0.07	benign

No.	Chromosome (forward strand)	Gene Symbol (Hugo)	Mutation	Nature of Mutation	COSMIC variation	Somatic in CLL	conservation data(USCSC)	dbsnp/ 1000 genomes	SIFT	SIFT_INT	Polyphen	Polyphen_INT
24	3:38140534	*MYD88*	c.649G>T, p.V217F	missense variant	COSM85941	yes for cancers	conserved to mammals	no known snp	0.36	tolerated	0.62	possibly damaging
25	3:38181970-38181972	*MYD88*	c.594_596delAAA, p.N199del	missense variant	no data	no data	conserved to rhesus	no known snp	no data		no data	
26	3:38141150	*MYD88*	ENSP00000379625.3:p.Leu265Pro, , c.794T>C, p.L265P	missense variant	no data	yes	conserved to mammals	rs387907272	0	deleterious	1	probably damaging
No.	**Chromosome (forward strand)**	**Gene Symbol (Hugo)**	**Mutation**	**Nature of Mutation**	**COSMIC variation**	**Somatic in CLL**	**conservation data(USCSC)**	**dbsnp/ 1000 genomes**	**SIFT**	**SIFT_INT**	**Polyphen**	**Polyphen_INT**
27	11:102207657	*BIRC3*	g.19464delC, c.1639delc, p.Q547Nfs*21, comment: close to COSM1627718, L548L	missense variant	no data	no data	conserved to zebrafish	no known snp	no	data	no data	no data

Supplementary table 4: Univariate Cox proportional hazard analysis of treatment-free and overall survival.

	NOTCH1 (all PEST terminating mutations)						*SF3B1 (Exon 14-16)*					*Low Frequency Mutations*				
variable	**status**	**total(%)**	**Wild-type (%)**	**Mutated (%)**	**OR**	**P-Value**	**total (%)**	**Wild-type (%)**	**Mutated (%)**	**OR**	**P-Value**	**total (%)**	**Wild-type (%)**	**Mutated (%)**	**OR**	**P-Value**
Screened Cases		203(99)	192 (95)	11(5)			199(100)	183(92)	16(8)			163 (79)	156(96)	7(4)		
Gender	Female	87(43)	83(95)	4(5)			87(44)	81(93)	6(7)			72(44)	69(96)	3(4)		
	Male	116(57)	109(94)	7(6)	1.3	0.45	112(56)	102(91)	10(9)	1.3	0.4	91(56)	87(96)	4(4)	1.0	0.6
Median Age		70	70	72			70	70	69			70	70	74		
Disease Stage	cMBL	89(44)	84(94)	5(6)			87(44)	82(94)	5(6)			73(45)	69(95)	4(5)		
	Stage A CLL	114(56)	108(95)	6(5)	1.1	0.57	112(56)	101(90)	11(10)	0.6	0.4	90(55)	87(97)	3 (3)	1.7	0.4
IGHV	unmutated	62(30)	54(87)	8(13)			63(32)	54(86)	9(14)			42(26)	38(90.5)	4(9.5)		
	mutated	141(70)	138(98)	3(2)	**6.8**	**0.004**	136(63)	129(95)	7(5)	**3.1**	**0.03**	121(74)	118(97.5)	3(2.5)	4.1	0.07
CD38 (30%)	negative	128(70)	126(98)	2(2)			123(70)	118(96)	5(4)			111(75.5)	107(96)	4(4)		
	positive	54(30)	47(87)	7(13)	**9.4**	**0.003**	54(31)	45(83)	9(17)	**4.7**	**0.012**	36(24.5)	33(92)	3(8)	2.4	0.2
ZAP70	negative	119(71)	116(97.5)	3(2.5)			114(70)	109(96)	5(4)			101(75)	99(98)	2(2)		
	positive	49(29)	44(90)	5(10)	**4.4**	**0.047**	49(30)	42(86)	7(14)	**3.6**	**0.03**	34(25)	30(88)	4(12)	**6.6**	**0.04**
del(11q)	absent	174(92)	165(95)	9(5)			169(92)	160(95)	9(5)			143(94)	137(96)	6(4)		
	present	15(8)	15(100)	0(0)	0.95	0.47	15(8)	9(60	6(40)	**11.8**	**<0.001**	9(6)	8(89)	1(11)	2.9	0.4
del(13q14)	absent	69(39)	63(91)	6(9)			67(39)	62(92.5)	5(7.5)			54(37)	52(96)	2(4)		
	present	108(61)	104(96)	4(4)	0.4	0.31	105(61)	96(91)	9(9)	1.2	0.5	86(63)	82(95)	4(5)	1.3	0.6
Trisomy 12	absent	123(76)	120(98)	3(2)			118(75)	105(89)	13(11)			97(76)	94(96)	3(4)		
	present	39(24)	33(85)	6(15)	**7.3**	**0.007**	39(25)	37(95)	2(5)	0.4	0.3	30(24)	28(93)	2(7)	2.2	0.4
del(17p)	absent	183(97)	175(96)	8(4)			178(97)	164(92)	14(8)			148(97)	141(95)	7(5)		
	present	5(3)	5(100)	0(0)	0.9	0.8	5(3)	4(80	1(20)	3.0	0.4	4(3)	4(100)	0(0)	0.95	0.8
Progressive Disease	absent	138(68)	135(98)	3(2)			133(67)	127(95.5)	6(5.5)			117(72)	116(99)	1(1)		
	present	65(32)	57(88)	8(12)	**6.3**	**0.005**	66(33)	56(85)	10(15)	**3.8**	**0.012**	46(28)	40(87)	6(13)	**17.4**	**0.002**
Treatment	no treatment	138(68)	135(98)	3(2)			133(67)	128(96)	5(4)			118(72)	117(99)	1(1)		
	treatment	65(32)	57(88)	8(12)	**6.3**	**0.005**	66 (33)	55(83)	11(17)	**5.1**	**0.003**	45(28)	39(87)	6(13)	**18.0**	**0.002**

Frequencies of *NOTCH1, SF3B1* and flow frequency mutations with biologic and clinical characteristics in Binet Stage A CLL and cMBL (n=206): *NOTCH1*-, *SF3B1*- and low frequency mutations are all associated with progressive disease and need for therapy

Supplementary table 5. Sequential data of patients with a *POT1* mutation (n=8)

ID	diagnosis	gender	age at diagnosis	IGHV	year 1	cytogenetics at diagnosis	FISH 1	*POT1* mutation	disease stability	year 2	cytogenetics 2nd timepoint	FISH2	treatment	clonal evolution
50	stage A CLL	female	59ys	mutated	1985	normal	normal	g.104749T>G	stable	2004	normal	13q loss +/-	no	yes
335	cMBL	male	77ys	unmutated	1994	normal	normal	g.76868A>G,c.1321A>G,p.Y242C	stable	2002	normal	normal	no	no
568	cMBL	male	79ys	unmutated	1997	normal	normal	UTR g.32906A>G	stable	2004	normal	13q loss +/-	no	yes
2938	stage A CLL	male	72ys	unmutated	2005	46, XY, del (13) q14q22	13q loss +/-	g.37710A>T;p.K39I	progressive	2011	same as 2005	13q loss +/-	yes	no
3069	cMBL	female	63ys	mutated	2003	normal	normal	124503424C>T;c.526G>A;p.G176R	progressive	2007	normal	normal	yes	no
3439	stage A CLL	female	69ys	unmutated	2004	normal	13q loss +/-	c.1458_1459del:p.486_487del and c.1851_1852del:p.617_618del	progressive	2007	normal	13q loss +/-	yes	no
3873	stage A CLL	female	n/a	unmutated	2008	normal	normal	UTR g.23777T>G	progressive	2010	not done	normal	yes	no
4286	cMBL	male	59ys	mutated	2008	normal	13q loss +/-	intron between two polymorphic regions in intron 4, chr7:124537282 T>A	stable	2011	n/a	n/a	no	n/a

Supplementary table 6. Clinical data from patients with mutations in *SF3B1* and *XPO1*, detected at the second time point only (n=5).

Gene	ID	diagnosis	gender	age	IGHV	cytogenetics and molecular prognostic factor analysis at diagnosis	acquired mutation	disease stability	treatment	clonal evolution	follow up data
SF3B1	338	cMBL	male	76ys	unmutated	trisomy 12, ZAP70 positive, 7544_7545delCT NOTCH1 mutation	33205G>GA:742G>G/D	stable	no	n/a	died 36 months after diagnosis independent from disease
	656	CLL Binet A	male	82ys	mutated	normal, no initial mutations	32982A>AG:700K>K/E	progressive	yes	yes	died 7 months after detection of mutation in progressive disease
	948	cMBL	male	78ys	mutated	normal, no initial mutations	32455A>AG:666K>K/E	stable	no	no	died 17 years after diagnosis independent from disease
	3439	CLL Binet A	female	69ys	unmutated	n/a	18771A>AG:626N/S	progressive	yes	n/a	progression and treatment from 36 months after diagnosis
XPO1	306	cMBL	male	n/a	unmutated	n/a	46290G>GA:571E>E/K	progressive	yes	n/a	progression and treatment from 3 months after diagnosis

Supplementary table 7. Primer Sequences.

	Primers and sequences	
Gene name	**Primer name**	**Primer sequence (5'-3')**
SF3B1	SF3B1_Ex14F	CCAACTCATGACTGTCCTTTCTT
	SF3B1_Ex14R	GGGCAACATAGTAAGACCCTGT
	SF3B1_Ex14R_Seq*	CAAGATGGCACAGCCCATAA
	SF3B1_Ex15F	TTGGGGCATAGTTAAAACCTG
	SF3B1_Ex15R	AAATCAAAAGGTAATTGGTGGA
	SF3B1_Ex16F	TCTTCATTAAAGTTAAGGCGACA
	SF3B1_Ex16R	TGTTAGAACCATGAAACATATCCA
NOTCH1	NOTCH1_Ex34_1F	AGCAAACATCCAGCAGCAG
	NOTCH1 _Ex34_1R	GCTCTCCTGGGGCAGAATA
	NOTCH1_Ex34_2F	GAGCTTCCTGAGTGGAGAGC
	NOTCH1_Ex34_2R	GTGAGGAAGGGGTGCTCAG
	NOTCH1_Ex34_3F	CACTATTCTGCCCCAGGAGA
	NOTCH1_Ex34_3R	CAGTCGGAGACGTTGGAATG
	NOTCH1_Ex34F_Seq*	GCCACAAAACTTACAGATGC
	NOTCH1_Ex34R_Seq*	CGCCGTTTACTTGAAGG
XPO1	XPO_Ex15_16F	TTAGGAAATGTACTTGTAGTTTCTA
	XPO_Ex15_16R	GGGTCTCTAACAAGACAAAAACAT
	XPO1_Ex15F_Seq*	GCTCATTATTTGCATTTGAAACC
	XPO1_Ex15R_Seq*	TCTAATTCATACCTATCCCTTGCAT
	XPO1_Ex16F_Seq*	TGCAAGGGATAGGTATGAATTAGA
	XPO1_Ex16R_Seq*	TTTTGTCCTGGACTCCATCAT
POT1	POT1_Ex4F_HRM**	TGCAATGTAATTAGAGAATAAAAGCTG
	POT1_Ex4R_HRM**	ATTATACGTATTTTGGTGATTGATTCA
	POT1_EX4F_Seq*	AAGTGCAATATCTGCCAAGT
	POT1_Ex4R_Sseq*	TCCAAACAATGACAAAATCA
	POT1_Ex5F_HRM**	CACATGTATCTATGTGTGTGGCATA
	POT1_Ex5R_HRM**	AGCATGTAATCACATTGGAGGTT
	POT1_Ex5F_Seq*	TCAGCAGATATTCCAGACAA
	POT1_Ex5R_Seq*	AGCTTAGACAACTTTGCACAT
	POT1_Ex6F_HRM**	AAACTCCACCAGTTTTAATACCTACC
	POT1_Ex6R_HRM**	TACATGGATTTGCTGCTAATATGAT
	POT1_Ex6F_Seq*	AGCCAAAGAATATGCATCAG
	POT1_Ex6R_Seq*	CCATTTATAAACAAAGTTCTAAGGA

POT1	POT1_Ex7F_HRM**	TTCTCTTCAAATAAATATAAGTTCTAGAC
(continued)	POT1_Ex7R_HRM**	GGTTTGGTGTTTTGAAGTAAGCA
	POT1_Ex7F_Seq*	GCAGTGGTTTGTTCAAATG
	POT1_Ex7R_Seq*	TTGCAGTGTGTATTGAAAGC
	POT1_Ex8F_HRM1**	TGGTGCTAACTTATAATTCCCAGTATT
	POT1_Ex8R_HRM1**	CCTTACGTGTTTGGGCATCT
	POT1_Ex8F_HRM2**	AGATGCCCAAACACGTAAGG
	POT1_Ex8R_HRM2**	CTGTTTTCTACTTTGCCCTACTTTC
	POT1_Ex8F_Seq*	CCACACAAATCTCATGTCAA
	POT1_Ex8R_Seq*	TCACCCAGTAAATCTCTTTAGC
	POT_Ex9F_HRM**	TCAGAGATCTTGCCACATGAA
	POT1_Ex9R_HRM**	TTATGGCAGGTATGGGATGG
	POT1_Ex9F_Seq*	CATTTTACAACTTAAAAATCAAAGA
	POT1_Ex9R_Seq*	TTCCACATTACCCATATTTCA
	POT1_Ex10F_HRM**	TCGGCTTAATCGATACCTTATTTAC
	POT1_Ex10R_HRM**	TTTTTCCCCACTTTCTAAATAACAA
	POT1_Ex10F_Seq*	ATTTGTTTCATTTGGCTCAT
	POT1_Ex10R_Seq*	CCATGCAGCTGATATTCAA
	POT1_Ex18F_HRM**	TCAAGTAAAAGAAGTGTGGGATTG
	POT1_Ex18R_HRM**	AAGGACAAATTCTTCCAGATTCC
	POT1_Ex18F_Seq*	TTTGACTGCAGGAATTATGA
	POT1_Ex18R_Seq*	GATTTTGGAGTTGAGACCAG
BIRC3	BIRC3_Ex7F_HRM**	TTCCATATAGTTATCCATTTTGAACCT
	BIRC3_Ex7R_HRM**	ACATACTTGATTCTTTTTCCTCAGTTG
	BIRC3_Ex7F_Seq*	TGCCTATACATTTTGTTGGTT
	BIRC3_Ex7R_Seq*	AAAAACCTGACTGGATTGAG
	BIRC3_Ex10F_HRM**	TGAAGAAGCAAACTGCCTTTTATT
	BIRC3_Ex10R_HRM**	AAAGTTTAGACGATGTTTTGGTTCT
	BIRC3_Ex10F_Seq*	CCACAGAAGATGTTTCAGGT
	BIRC3_Ex10R_Seq*	GTGCTACCTCTTTTTCGTTC
MYD88	MYD88_Ex3F_HRM**	TCTGACCACCACCCTTGTG
	MYD88_Ex3R_HRM**	GGCCTTCTAGCCAACCTCTT
	MYD88_Ex3F_Seq*	GGCACTTTCTCTGAGGAGTA
	MYD88_Ex3R_Seq*	GACAGTGCACAGCTAGGAG
	MYD88_Ex4F_HRM**	GCTGAACTAAGTTGCCACAGG
	MYD88_Ex4R_HRM**	CCAGAGCAGGGTTGAGCTT
	MYD88_Ex4F_Seq*	CAGGGGATATGCTGAACTAA
	MYD88_Ex4R_Seq*	GATCTTCAGCAGTTCTTTGG
	MYD88_Ex5F_HRM**	CAGGTGCCCATCAGAAGC
	MYD88_Ex5R_HRM**	GGTTGGTGTAGTCGCAGACA
	MYD88_Ex5F_Seq*	GCAGAAGTACATGGACAGGCAGACAGATAC
	MYD88_Ex5R_Seq*	GTTGTTAACCCTGGGGTTGAAG

FBXW7	FBXW7_Ex2F	ATTTTCCCCTGCAGAATGTG
	FBXW7_Ex2R	TTTAGTAATACAAAGACTGTGAGGAAA
	FBXW7_Ex3F	TGACTCAAGATTTGATAGTTAGACGA
	FBXW7_Ex3R	AAACTAAAACACTTTCAGAATCAACTC
	FBXW7_Ex4F	TCTTTGCTTTCACTTTTGTTTTT
	FBXW7_Ex4R	GCAGCAATTAAGTGAGGCATT
	FBXW7_Ex4Fseq	ACCATGTTCAGCAACACCAA
	FBXW7_Ex5F	GCCTGTAATTTGGGACATCTG
	FBXW7_Ex5R	CAAACTGACAATACCGAATACCA
	FBXW7_Ex6F	TCAAGTATCTCATCCTGTGGAGAA
	FBXW7_Ex6R	TTCGGCTCATCTGAATGTGT
	FBXW7_Ex7F	TGGTTTTGAGCAGAGAGATGG
	FBXW7_Ex7R	TTTCTTTCTACAGAAGAGGAGTGTCA
	FBXW7_Ex8F	TGTTCCTGTTTATGCCTTCATTT
	FBXW7_Ex8R	CCAGTTGCTACTTGCAATGAT
	FBXW7_Ex9F	TCACTTTTCCTTTCTACCCAAAA
	FBXW7_Ex9R	CTACACAGAAAGGGCCCAAA
	FBXW7_Ex10F	AAAAATTCTAAACGTGGGTTTTT
	FBXW7_Ex10R	TGGATCAGCAATTTGACAGTG
	FBXW7_Ex11F	TCCTCTTCCCCCTTTCCTAC
	FBXW7_Ex11R	TTTTGTGATGCTAAGGCTCCAT
	FBXW7_Ex12F	TTTCAAATGTTGCATTTATTGTATG
	FBXW7_Ex12R	CAACATCCTGCACCACTGAG

* Theses primers were used as a sequencing primers only

** These primers were used as High Resolution Melt primers only

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