Relevance of ID3-TCF3-CCND3 pathway mutations in pediatric aggressive B-cell lymphoma treated according to the non-Hodgkin Lymphoma Berlin-Frankfurt-Münster protocols

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B) Supplemental Methods

Patient samples from pB-ALL cases. Tumor DNA samples from 96 pediatric patients diagnosed with precursor B-cell acute lymphoblastic leukemia (pB-ALL) were kindly provided by the ALL-BFM study center, University of Kiel, Germany. All patients have previously been diagnosed between 2000 and 2006 and were diagnosed and treated according to the ALL-BFM 2000 protocol. Tumor cell content of tumor samples was previously checked to be at least 60%.

Characteristics of pB-ALL patient cohort

Characteristics		ALL patients (n=96)	
Gender	male	59	61%
	female	38	39%
Age (years)	min, max	1, 17	
	median	4	
	mean	5,9	
Leukocyte count (per microliter)	min, max	1500, 284000	
	median	13600	
	mean	27243	
CNS involvement	yes	1	1%
Immunophenotype	pro-B	3	3%
	common	62	65%
	pre-B	31	32%
Risk group	standard	33	34%
	middle	52	54%
	high	11	11%

Tumor DNA isolation in B-NHL cases. Tumor cell DNA was extracted using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany). Tumor cell content of tumor samples was previously checked to be at least 60%.

Primer Pairs

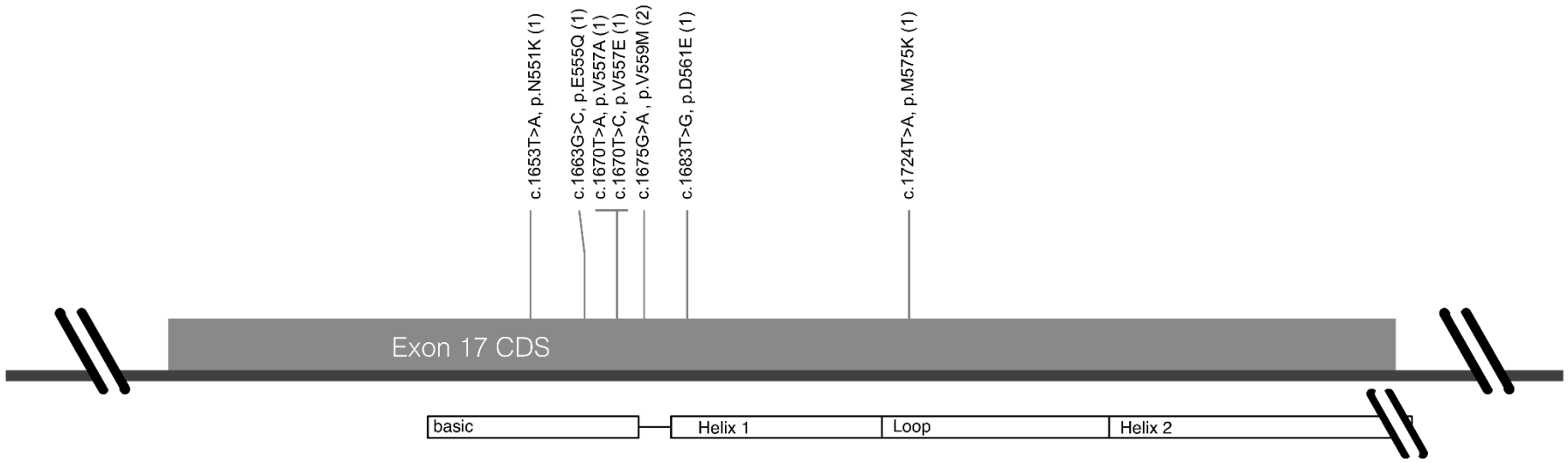
Gene	Accession number	Forward primer	Reverse primer	as published in
ID3	NM_002167.4	5´-TCCAGGCAGGCTCTATAAGTG-3´	5´-CCGAGTGAGTGGCAATTTTT-3´	Richter et al.
TCF3, exon 17	NM_001136139.2	5´-TGCTGTGCCCACCAATGTAAGCCATG-3´	5´-GTGGAGGCTTGTAAAGAAGAGAGTGG-3´	Schmitz et al.
CCND3, coding region exon 5	NM_001760.3	5´-CCATGTGTTGGGAGCTGTC-3	5´-CTGGAGGCAGGGAGGTG-3´	Richter et al.

PCR amplification. PCR was amplified using OneTaq Polymerase 2x MM with Standard Buffer (New England BioLabs, Frankfurt am Main, Germany).

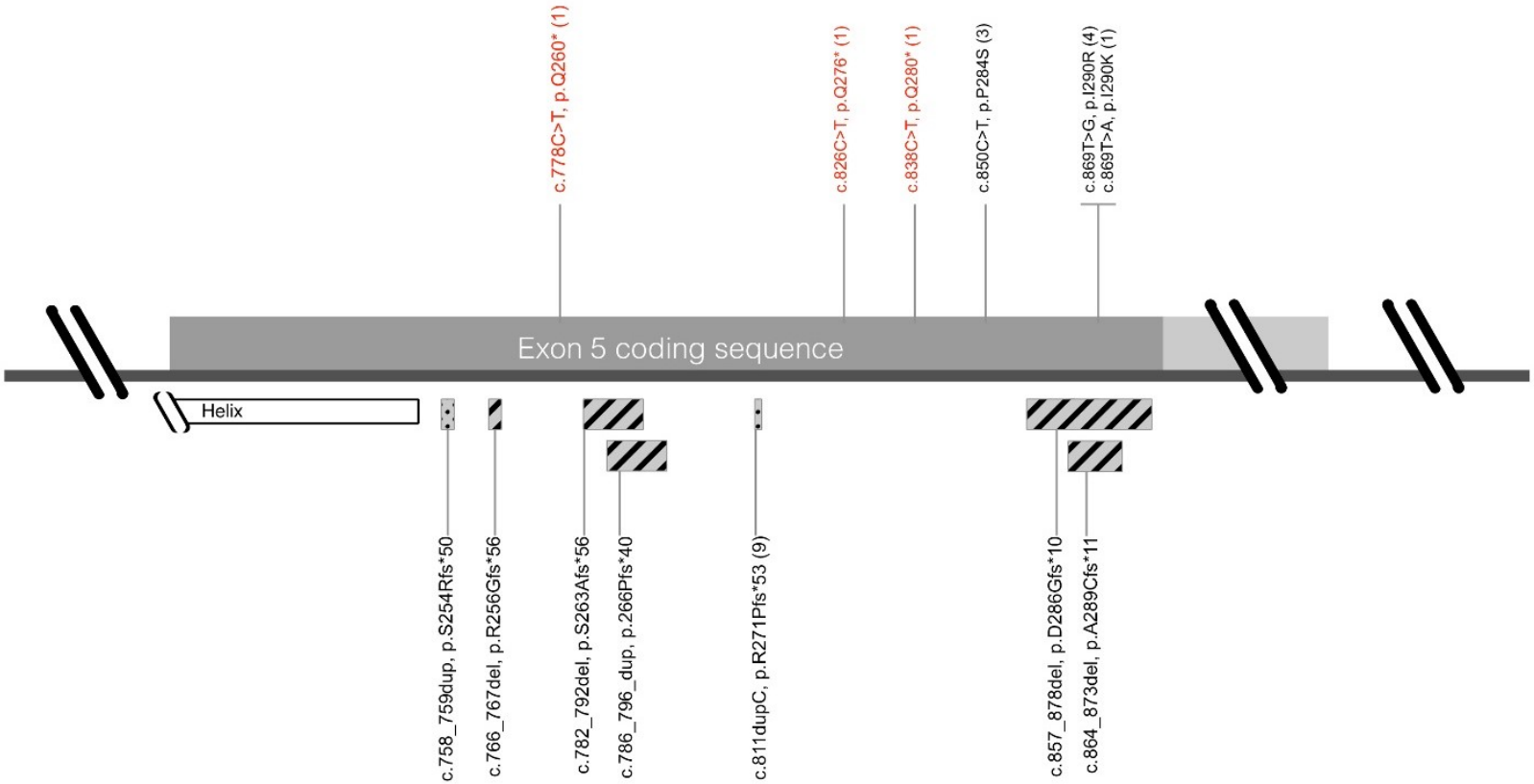
Sanger sequencing. PCR products were subjected to Sanger sequencing using the same primers and the ABI BigDye® Terminator v3.1 Cycle Sequencing Kit. Sequence analysis was performed using an ABI 3130XL sequencer.

Data analysis and interpretation. Cases presenting with mutations were confirmed within a repetition experiment. Variants and mutations were described using the reference sequence of the corresponding coding DNA available from NCBI database. Previously published short nuclear polymorphisms were annotated and excluded from analysis using dbSNP (NCBI dbSNP database, Build ID: 137). Change on protein level was determined by using a RNA codon table, amino-acid changes were annotated according to the reference accession numbers: ID3, NP_002158; TCF3, NP_001129611; CCND3, NP_001751.1.

C) Supplemental Figures



Supplemental Figure 1. *TCF3 (E47)* plot with annotated mutations of the study cohort. The coding region of *TCF3* exon 17 is illustrated. Each substitution is labeled with correspondent description on genomic and protein level, as well as the absolute frequency of occurrence in brackets. The functional basic-Helix-Loop-Helix domain is mapped according to the description of the functional sites in UniProt (P15923-2).



Supplemental Figure 2. *CCND3* plot with annotated mutations of the study cohort. The coding region of *CCND3* exon 5 is illustrated, with substitutions on the upper and more complex alterations (insertions, deletions, indels, duplications) on the lower site. Substitutions resulting in a nonsense mutation are depicted in red. Hatched bars delineate deletions and indels, dotted bars characterize insertions and duplications. Each mutation is labeled with correspondent description on genomic and protein level, as well as the absolute frequency of occurrence in brackets. A part of a functional helix domain is mapped according to the description of the functional sites in UniProt (P30281).

D) Supplemental Tables

Supplemental Table 1: Frequency of *ID3* mutations and proportion of pediatric patients with Burkitt lymphoma in previously published studies.

Publication	Frequency of *ID3* mutations in BL cohort	Number of patients with the diagnosis BL	Number of pediatric (<18y) patients in the studied cohort
Love et al.[1]	35%	51*	13† (25%)
Schmitz et al.[2]	58%	78	29‡ (37%)
Richter et al.[3]	68%	53§	36 (68%)
Havelange et al.[4]	65%	24	13 (54%)
Forero-Castro et al.[5]	47%	40	0 (0%)
Current study	78%	64	64 (100%)

Data as given in the publication or derived from appendix data.
*Cell lines not counted, diagnosis "*MYC*-rearrangement positive BL".
†Age not available for 24 cases.
‡Calculated from given relative value.
§ Diagnosis "molecular BL".

Supplemental Table 2. *ID3*, *TCF3* and *CCND3* sequencing results on 84 B-NHL patients from the study cohort, 10 B-NHL patients from the extended cohort and 96 pB-ALL cases.

case	diagnosis	MYC rearr.	DNA origin	c.ID3[1]	p.ID3[2]	c.TCF3[3]	p.TCF3[4]	c.CCND3[5]	p.CCND3[6]
1	BL	yes	ascites	c.[198_199insCTAAG];[c.194G>A]	p.[V67fs*16];[S65N]	wt	wt	c.[850C>T]	p.[P284S]
2	BL	yes	tissue	wt	wt	c.[1724T>A]	p.[M575K]	c.[811dupC]	p.[R271Pfs*53]
3	BL	yes	tissue	wt	wt	wt	wt	wt	wt
4	BL	yes	tissue	wt	wt	wt	wt	wt	wt
5	BL	yes	tissue	c.[141C>A(;)144C>T(;)166C>T]	p.[C47*(;)P56S]	wt	wt	c.[758_759dupAG]	p.[S254Rfs*50]
6	DLBCL	N/A	tissue	wt	wt	wt	wt	wt	wt
7	B-AL	yes	tissue	c.[241C>T]	p.[Q81*]	wt	wt	c.[869T>G]	p.[I290R]
8	BL	yes	tissue	c.[144C>G;243G>C];[236_243delACCTGCAG]	p.[Y48*;Q81H];[D79GfsSPGRASPWTP*]	wt	wt	wt	wt
9	BL	yes	tissue	c.[180_190delAGGCACTCAGC]	p.[R60Sfs*]	wt	wt	c.[811dupC]	p.[R271Pfs*53]
10	BL	yes	pleura	c.[153_164delGCGGGAACTGGT];[256_266delGAGCCAGCCCC]	p.[R52_V55del];[E86WfsTP*]	wt	wt	wt	wt
11	BL	yes	ascites	c.[144C>G];[300+1G>C]	p.[Y48*];[splice site]	wt	wt	wt	wt
12	BL	yes	tissue	c.[236_247delACCTGCAGGTAG]	p.[L80PfsGRASPWTP*]	c.[1675G>A]	p.[V559M]	c.[857_878delATGTCACAGCCATACACCTGTA]	p.[D286GfsPGEALWSGH*]
13	BL	yes	ascites	c.[142T>A]	p.[Y48N]	wt	wt	wt	wt
14	B-AL	yes	bm	c.[241C>T]	p.[Q81*]	wt	wt	c.[778C>T]	p.[Q260*]
15	BL	yes	tissue	c.[120delG(;)166C>T]	p.[L40FfsWTT*(;)P56S]	wt	wt	wt	wt
16	B-AL	yes	tissue	c.[211C>T(;)241C>T]	p.[Q71*(;)Q81*]	wt	wt	wt	wt
17	DLBCL	no	tissue	wt	wt	wt	wt	wt	wt
18	DLBCL	N/A	tissue	wt	wt	wt	wt	wt	wt
19	B-NFC	yes	tissue	wt	wt	wt	wt	c.[786_796dup]	p.[A266Pfs*40]
20	BL	yes	tissue	wt	wt	wt	wt	wt	wt
21	DLBCL	no	tissue	wt	wt	wt	wt	wt	wt
22	BL	yes	tissue	wt	wt	wt	wt	wt	wt
23	BL	yes	tissue	c.[236_251delinsC];[300+1G>C]	p.[D79Gfs*?];[splice site]	c.[1663G>C]	p.[E555Q]	wt	wt
24	BL	yes	tissue	c.[190C>T]	p.[L64F]	wt	wt	c.[811dupC]	p.[R271Pfs*53]
25	BL	yes	tissue	c.[143A>G]	p.[Y48C]	wt	wt	wt	wt
26	BL	yes	tissue	c.[189delG]	p.[Q63HfsLARWKSYSASSTTFSTCR*]	wt	wt	wt	wt
27	B-NFC	yes	tissue	c.[152T>C(;)228C>G]	p.[L51P(;)Y76*]	wt	wt	wt	wt
28	DLBCL	no	tissue	wt	wt	wt	wt	wt	wt

case	diagnosis	MYC rearr.	DNA origin	c.ID3[1]	p.ID3[2]	c.TCF3[3]	p.TCF3[4]	c.CCND3[5]	p.CCND3[6]
29	BL	no	tissue	wt	wt	wt	wt	wt	wt
30	B-NFC	yes	tissue	wt	wt	wt	wt	wt	wt
31	DLBCL	yes	ascites	c.[166C>T(;)211C>T]	p.[P56S(;)Q71*]	wt	wt	c.[811dupC]	p.[R271Pfs*53]
32	BL	yes	tissue	c.[190C>T]	p.[L64F]	c.[1653T>A]	p.[N551K]	wt	wt
33	BL	yes	tissue	c.[190C>T]	p.[L64F]	c.[1675G>A]	p.[V559M]	c.[766_767delAG]	p.[R256Gfs*56]
34	BL	yes	tissue	wt	wt	wt	wt	wt	wt
35	BL	yes	tissue	c.[167C>T];[181_209delGGCACTCAGCTTAGCCAGGTGGAAATCCT]	p.[P56L];[G61WfsKSYSASSTTFSTCR*]	wt	wt	wt	wt
36	BL	yes	pleura	c.[167C>T(;)190C>T]	p.[P56L(;)L64F]	wt	wt	wt	wt
37	BL	yes	tissue	c.[166_167delinsTT]	p.[P56F]	wt	wt	wt	wt
38	BL	yes	tissue	wt	wt	wt	wt	c.[869T>G]	p.[I290R]
39	BL	yes	pleura	c.[191_195delTTAGC]	p.[L64PfsGGNPTARHRLHSRPAGSPGRASPWTP*]	wt	wt	wt	wt
40	B-AL	yes	bm	c.[300+1G>T]	p.[splice site]	wt	wt	c.[782_792delCCAGCTCCAGC]	p.[S263Afs*56]
41	DLBCL	no	tissue	wt	wt	wt	wt	wt	wt
42	B-NFC	no	tissue	wt	wt	wt	wt	wt	wt
43	BL	yes	tissue	c.[27_40delCTGCTACGAGGCGG;202delG];[202delG];[27_40delCTGCTACGAGGCGG]	p.[C10VfsLPVGTQSGHRPGPREGPGS*;E68KfsSYSASSTTFSTCR*];[E68KfsSYSASSTTFSTCR*];[C10VfsLPVGTQSGHRPGPREGPGS*]	wt	wt	c.[811dupC]	p.[R271Pfs*53]
44	B-AL	yes	bm	c.[137A>C(;)241C>T]	p.[H45P(;)Q81*]	wt	wt	c.[869T>A]	p.[I290K]
45	BL	yes	tissue	c.[190C>T(;)233T>C]	p.[L64F(;)L78P]	wt	wt	wt	wt
46	DLBCL	no	tissue	wt	wt	wt	wt	wt	wt
47	BL	yes	tissue	c.[241C>T]	p.[Q81*]	wt	wt	wt	wt
48	B-AL	yes	bm	c.[181_270del]	p.[G61_G90del]	wt	wt	c.[850C>T]	p.[P284S]
49	BL	yes	tissue	c.[116_delG]	p.[S36Tfs*5]	wt	wt	wt	wt
50	B-AL	yes	tissue	c.[166C>T(;)209T>C]	p.[P56F(;)L70P]	wt	wt	c.[869T>G]	p.[I290R]
51	DLBCL	no	tissue	wt	wt	wt	wt	wt	wt
52	B-AL	yes	bm	wt	wt	c.[1670T>C]	p.[V557A]	wt	wt
53	BL	yes	tissue	c.[134_140dupACCACTG];[209T>C]	p.[C48*];[L70P]	wt	wt	wt	wt
54	BL	yes	pleura	c.[214_243del;(214_243del)]	p.[R72_Q81del;(R72_Q81del)]	wt	wt	c.[811dupC]	p.[R271Pfs*53]
55	BL	yes	tissue	c.[144C>G;(144C>G)]	p.[Y48*;(Y48*)]	wt	wt	c.[811dupC]	p.[R271Pfs*53]

case	diagnosis	MYC rearr.	DNA origin	c.ID3[1]	p.ID3[2]	c.TCF3[3]	p.TCF3[4]	c.CCND3[5]	p.CCND3[6]
56	B-NFC	yes	tissue	c.[137A>C(;)161T>A(;)300+44T>C(;)300+85C>T]	p.[H45P(;)L54Q]	wt	wt	wt	wt
57	BL	N/A	tissue	c.[190C>T(;)230T>A]	p.[L64F(;)I77N]	wt	wt	c.[811dupC]	p.[R271Pfs*53]
58	DLBCL	no	tissue	wt	wt	wt	wt	wt	wt
59	BL	yes	tissue	c.[167C>G]	p.[P56R]	wt	wt	c.[850C>T]	p.[P284S]
60	DLBCL	N/A	tissue	wt	wt	wt	wt	c.[864_873delAGCCATACAC]	p.[A289CfsSPGEALWSGH*]
61	BL	yes	pleura	c.[141C>A(;)190C>T]	p.[C47*(;)L64F]	wt	wt	wt	wt
62	DLBCL	no	tissue	wt	wt	wt	wt	wt	wt
63	BL	yes	ascites	wt	wt	c.[1683T>G]	p.[D561E]	wt	wt
64	B-AL	yes	bm	c.[167C>G]	p.[P56R]	wt	wt	c.[869T>G]	p.[I290R]
65	BL	yes	tissue	wt	wt	wt	wt	wt	wt
66	B-AL	yes	bm	c.[135C>G(;)166C>T(;)190C>T]	p.[N45K(;)P56S(;)L64F]	c.[1670T>A]	p.[V557E]	wt	wt
67	BL	yes	tissue	c.[142T>G(;)166C>G(;)190C>G]	p.[Y48D(;)P56A(;)L64V]	wt	wt	wt	wt
68	B-NFC	no	pleura	c.[20T>A(;)164T>A]	p.[V7E(;)V55E]	wt	wt	wt	wt
69	BL	yes	tissue	c.[190C>T]	p.[L64F]	wt	wt	wt	wt
70	DLBCL	N/A	tissue	c.[152_174dup(;)209T>G]	p.[P59Cfs*32(;)L70R]	wt	wt	wt	wt
71	BL	yes	ascites	c.[122_140delTGGACGACATGAACCACTG_insC;(122_140delTGGACGACATGAACCACTG_insC)]	p.[L41Pfs*73;(L41Pfs*73]	wt	wt	c.[826C>T]	p.[Q276*]
72	BL	yes	ascites	c.[242dupA]	p.[V82Gfs*11]	wt	wt	wt	wt
73	BL	yes	pleura	c.[81delC];[247_248insTCTACAGCGCGTCATCGACTACATTCTCGACCTGCAGGTAG]	p.[R28EfsGRARQLRSR*];[L84Yfs*12]	wt	wt	wt	wt
74	BL	yes	pleura	wt	wt	wt	wt	c.[838C>T]	p.[Q280*]
75	BL	yes	tissue	c.[166C>T]	p.[P56S]	wt	wt	wt	wt
76	B-AL	yes	bm	c.[167_182dupCCGGAGTCCCGAGAGG]	p.[T62RfsSPERHSA*]	wt	wt	c.[811dupC]	p.[R271Pfs*53]
77	BL	yes	tissue	wt	wt	wt	wt	wt	wt
78	B-AL	yes	bm	c.[189dedelG;193A>T];[190C>T]	p.[Q63HfsFARWKSYSASSTTFSTCR*];[L64F]	wt	wt	wt	wt
79	B-AL	yes	bm	c.[160C>G(;)164T>C]	p.[L54V(;)V55A]	wt	wt	wt	wt
80	B-NFC	N/A	tissue	c.[160C>G];[165_166insG]	p.[L54V];[P56AfsRSPERHSA*]	wt	wt	wt	wt
81	B-AL	yes	bm	c.[166C>G];[190C>G;206T>C;229A>G]	p.[P56A];[L64V(;)I69T(;)I77V]	wt	wt	wt	wt

case	diagnosis	MYC rearr.	DNA origin	c.ID3[1]	p.ID3[2]	c.TCF3[3]	p.TCF3[4]	c.CCND3[5]	p.CCND3[6]
82	BL	no	tissue	wt	wt	wt	wt	wt	wt
83	BL	yes	tissue	c.[166C>T(;)190C>T]	p.[P56S(;)L64F]	wt	wt	wt	wt
84	BL	yes	tissue	c.[157delG(;)166C>G(;)209T>A]	p.[P56A(;)E53NfsWYPESREALSLARWKSYSASSTTFSTCR*(;)L70Q]	wt	wt	wt	wt
extended cohort (cases 85-94)									
case ID	**diag.**	**MYC rearr.**	**tumor origin**	**c.ID3[1]**	**p.ID3[2]**	**c.TCF3[3]**	**p.TCF3[4]**	**c.CCND3[5]**	**p.CCND3[6]**
85	BL	yes	ascites	c.[190C>T(;)300+1G>A]	p.[L64F(;)splice site]	wt	wt	wt	wt
86	BL	yes	tissue	wt	wt	wt	wt	wt	wt
87	BL	yes	tissue	c.[166C>T(;)202lG]	p.[P56S(;)E68KfsSYSASSTTFSTCR*]	wt	wt	wt	wt
88	B-AL	yes	pleura	wt	wt	wt	wt	wt	wt
89	BL	yes	ascites	c.[167C>G;(167C>G)]	p.[P56R;(P56R)]	wt	wt	wt	wt
90	BL	yes	tissue	c.[241C>T(;)300G>A(;)310C>T]	p.[Q81*(;)Q100Q(;)L103F]	c.[1675G>A]	p.[V559M]	wt	wt
91	BL	yes	tissue	c.[92_dupG;(92_dupG)]	p.[G31fsPGS*;(G31fsPGS*)]	wt	wt	c.[856_857dupGA]	p.[D286Efs*18]
92	B-NFC	yes	tissue	c.[122_130lTGGACGACinsA;(122_130lTGGACGACinsA)]	p.[L41Hfs*21;(L41Hfs*21)]	wt	wt	wt	wt
93	B-AL	yes	bm	c.[45C>A(;)202G>T]	p.[C15*(;)E68*]	wt	wt	wt	wt
94	B-AL	yes	tissue	c.[190C>T(;)298C>T]	p.[L64F(;)Q100*]	wt	wt	c.[811dupC]	p.[R271Pfs*53]

BL indicates Burkitt lymphoma; B-AL, Burkitt leukemia; DLBCL, Diffuse large B cell lymphoma; B-NFC, B cell lymphoma with features intermediate between BL and DLBCL; wt, wildtype; bm, bone marrow; MYC rearr.: MYC-rearrangement; and N/A, not available.

[1]Annotated according to NM_002167.4

[2]Annotated according to NP_002158

[3]Annotated according to NM_001136139.2

[4]Annotated according to NP_001129611

[5]Annotated according to NM_001760.3

[6]Annotated according to NP_001751.1

Supplemental Table 3. Clinical characteristics of 61 patients with reference diagnosis "BL/B-AL" and positive MYC rearrangement regarding *ID3*, *TCF3* and *CCND3* mutation status

Characteristics		***ID3*^{mutated}**		***ID3*^{wt}**		***P***	***TCF3*^{mutated}**		***TCF3*^{wt}**		***P***	***CCND3*^{mutated}**		***CCND3*^{wt}**		***P***
All		49		12			8		53			22		39		
Gender	male	41	84%	12	100%		6	75%	47	89%		17	77%	36	92%	
	female	8	16%	0	0%	.13	2	25%	6	11%	.29	5	23%	3	8%	.09
Age	< 10 y	30	61%	6	50%		7	88%	29	55%		15	68%	21	54%	
	10-14 y	13	27%	5	42%		1	13%	17	32%		4	18%	14	36%	
	> 14 y	6	12%	1	8%	.58	0	0%	7	13%	.20	3	13%	4	10%	.35
Stage of	I	1	2%	1	8%		0	0%	2	4%		0	0%	2	6%	
disease	II	7	16%	3	25%		4	50%	6	12%		5	23%	5	14%	
	III	23	51%	6	50%		2	25%	27	55%		7	32%	22	63%	
	IV	1	2%	1	8%		0	0%	2	4%		2	9%	0	0%	
	B-AL	13	29%	1	8%	.39	2	25%	12	25%	.11	8	36%	6	17%	.04
BM involvement	yes	13	27%	1	8%	.18	2	25%	12	23%	0.88	8	36%	6	15%	.06
CNS involvement	yes	6	12%	1	8%	.70	1	13%	6	11%	0.92	5	23%	2	5%	.04
LDH	< 500 U/l	15	31%	8	67%		4	50%	19	36%		7	32%	16	41%	
	500-1000 U/l	10	20%	0	0%		2	25%	8	15%		4	18%	6	15%	
	> 1000 U/l	24	49%	4	33%	.04	2	25%	26	49%	.44	11	50%	17	44%	.78
Diagnosis	BL	36	74%	11	92%		6	75%	41	77%		14	64%	33	85%	
	B-AL	13	26%	1	8%	.18	2	25%	12	23%	.88	8	36%	6	15%	.06
Outcome	pEFS (2y)	86 ± 5%		100%		.18 (LR)	88 ± 12%		89 ± 4%		.87 (LR)	82 ± 8%		92± 4%		.25 (LR)
	pOS (2y)	88 ± 5%		100%		.21 (LR)	88 ± 12%		91 ± 4%		.74 (LR)	87 ± 7%		92 ± 4%		.50 (LR)

Wt indicates wildtype; y, years; BM, bone marrow; CNS, central nervous system; LDH, lactate dehydrogenase serum level; BL, Burkitt lymphoma; B-AL, Burkitt leukemia; DLBCL, Diffuse large B cell lymphoma; B-NHL nfc; B cell Non-Hodgkin-lymphoma with features intermediate between BL and DLBCL; pEFS, probability of event free survival; pOS, probability of overall survival; and LR, log-rank.

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