Table S1. Cases with aberration in the *TERT-CLPTM1L* locus

Case	Diagnosis	Age[a]	Sex	Studied tissue	Karyotype	FISH/Array CGH results in 5p15.33	Aberrant cells by FISH [%]
1	CLL	71	m	PB	46,XY	Translocation, break in *CLPTM1L*, juxtaposition of *TERT-CLPTM1L* and *IGH*, FISH on metaphases indicating a t(5;14)	91
2	CLL	77	m	PB	47,XY,der(2)t(2;14)(p11;q32), der(8)t(2;8)(p1?6;p21),+12, add(12)(p11)x2,add(14)(q32), i(17)(q10)	Translocation and deletion, break between *TERT* and *CLPTM1L,* juxtaposition of *TERT* and *IGH*, FISH on metaphases indicating a t(5;14)	46
3	CLL	50	m	PB	No metaphases	Translocation, break centromeric of *TERT*, juxtaposition of *TERT* and *IGH*	45
4	ALL	8	f	BM	46,XX,t(12;21)(p12;q22)	Translocation, break centromeric of *TERT*, juxtaposition of *TERT* and *IGH*	73
5	SMZL	82	m	PB	46,XY,**t(5;7)(p15;p11)**, del(14)(q22q32)/46,XY	Translocation and deletion, break between *TERT* and *CLPTM1L,* FISH-pattern as in case 2	90
6	CLL	35	f	PB	46,XX,**t(5;10)(p15;q25)**/ 46,idem, del(13)(q14q21)	Translocation, break centromeric of *TERT*, FISH-pattern as in cases 3 and 4	86

7	MCL	62	m	spleen	46,XY,add(1)(q21), **add(5)(p?15)**,add(7)(p?21), del(10)(p?11),t(11;14)(q13;q32), der(?)t(17;?)(q21;?)/46,XY	Translocation, break centromeric of *TERT*, FISH-pattern as in cases 3 and 4	39
8	MCL	78	m	PB	46,XY,del(2)(p12p22), **t(5;9)(p15;q31~33)**, der(8)t(3;8)(q13;p21~22), t(11;14)(q13;q32), r(17)(?)	Translocation, break centromeric of *TERT*, FISH-pattern as in cases 3 and 4	67
9	MCL	70	m	LN	46,XY,**?t(5;19)(p14;p13)**, t(11;14)(q13;q32), ?t(11;17)(q23;q11~12)	Translocation, break centromeric of *TERT*, FISH-pattern as in cases 3 and 4	39
10	MCL	66	f	LN	46,XX,del(2)(p23),i(3)(q10),-9, del(10)(q24),t(11;14)(q13;q32), t(12;14)(q12;p12), +mar/46,XX	Translocation, break centromeric of *TERT*, FISH-pattern as in cases 3 and 4	48
11	MCL	85	f	LN	80-85,XXX,-X,-1,-2, der(2)t(2;5)(?;?)x2, +3,-4,-5, der(5)t(2;5)(?;?), der(6)t(6;22)(q13;?q11),-9, del(9)(?p11), der(9)t(5;9)(?;?), der(10)t(10;22)(?;?), der(11)t(11;14)(q13;q32)x2, del(13)(q21q22), der(13)t(13;16)(q22;?), +r(13)(p10q22),-14, der(14)t(11;14) (q13;q32), t(11;17)(q13.5;q23)x2,-15, -16,der(16)t(16;18)(?;?), der(16)t(16;22)(?;?), der(16)ins(16;18) (?q;?q),-17, der(17)t(11;17)(q13.5;q23)x2, -18,-21,-22,-22	HSR-amplification (> 5 copies)	88
12	MCL	73	m	LN	42,X,-Y,ins(1;?)(p22;?), **del(5)(p14)**,-7,-9, ?del(11)(q23-24), t(11;14)(q13;q32),-13,-15,+1mar	High-level amplification (> 7 copies)	71

[a] At date of diagnosis.

ALL, B-cell precursor acute lymphoblastic leukemia; BM, bone marrow; CLL, chronic lymphocytic leukemia; f, female; LN, lymph node; m, male; MCL, mantle cell lymphoma; PB, peripheral blood; SMZL, splenic marginal zone lymphoma.

The study was performed in the framework of the network projects “Molecular mechanisms in malignant lymphomas” and “European MCL Network”, for which central and local IRB approval was obtained (D425/03, B300/04 und B226/04). The material was pseudonymized to comply with the German law for correct usage of archival tissue for clinical research (Deutsches Ärzteblatt 2003; 100 A1632). The study was conducted according to the Declaration of Helsinki.

Table S2. Probes used for fluorescence *in situ* hybridization

Probe name	Company	Chromosomal band	Fluorescent dye
LSI IGH probe	Abbott, IL, USA	14q32	Spectrum green/spectrum orange
Clone name	Vector type	Genomic localization of insert [bp][a]	Fluorescent dye
RP11-316G9	BAC	chr2:89,561,552-89,772,752	Spectrum orange
RP11-525L16	BAC	chr2:88,570,647-88,680,773	Spectrum green
RP11-1021F11	BAC	chr2:88,767,510-88,939,350	Spectrum green
RP11-678B2	BAC	chr5:851,472-1,041,755	Spectrum green
RP11-117B23	BAC	chr5:1,260,045-1,422,139	Diethylaminocoumarin (DEAC)
WI2-1943L8	Fosmid	chr5:1,327,987-1,368,909	Spectrum green
WI2-841J3	Fosmid	chr5:1,349,298-1,386,902	Spectrum green
WI2-1054L12	Fosmid	chr5:1,363,945-1,399,036	Spectrum green
WI2-1945M12	Fosmid	chr5:1,373,051-1,408,036	Spectrum green
RP11-94J21	BAC	chr5:1,377471-1,540,909	Spectrum orange
WI2-2731B8	Fosmid	chr5:1,377,634-1,414,077	Spectrum green
WI2-1224D6	Fosmid	chr5:1,386,147-1,426,553	Spectrum green
RP11-1125A11	BAC	chr5:1,405,987-1,562,881	Spectrum orange
RP11-161F13	BAC	chr5:1,562,895-1,724,268	Spectrum green
RP11-356C5	BAC	chr5:2,224,377-2,406,663	Spectrum orange
CTA-526G4	BAC	chr22:20,634,858-20,740,796	Spectrum green
RP11-60B5	BAC	chr22:21,835,020-21,838,494	Spectrum orange
CTA-865E9	BAC	chr22:27,735,801-27,843,216	Spectrum orange

BAC-clones were obtained from Invitrogen (Karlsruhe, Germany) and Fosmid-clones were obtained from the Sanger Institute (Cambridge, USA).
[a]NCBI Build 36.1

Fluorescence *in situ* hybridization (FISH). For generation of FISH-probes vector containing *Escherichia coli* bacteria were cultured over night in medium containing appropriate antibiotics. Bacterial DNA was extracted with the Perfectprep® Plasmid Maxi Kit (Eppendorf, Hamburg, Deutschland) and labeled using the Bioprime® DNA-Labeling System (Invitrogen, Karlsruhe, Deutschland) which was modified by using dUTP spectrum green (Abbott), dUTP spectrum orange (Abbott) and dUTP-Diethylaminomethyl coumarin (DEAC, NEN, Zaventem, Belgium) instead of the dNTP-reagent provided by the Bioprime® DNA-Labeling System. The reactions were purified with Microcon® YM-30 columns (Millipore, Schwalbach, Deutschland) and precipitated together with Cot-1-DNA (Invitrogen). FISH was performed on fixed cells from bone marrow, peripheral blood or lymph node cell suspensions as described previously[1].

Array CGH to custom designed arrays. DNA from patient-samples (cells in DMSO-containing medium) and controls (peripheral blood) was extracted using the PUREGENE DNA Isolation Kit (Gentra systems, Minneapolis, MN, USA) according to manufacturers instruction. 1 µg of test DNA and 1 µg of reference DNA from a pool of 10 healthy donors matched for sex were hybridized. The microarrays were designed using the eArray-software from Agilent (Santa Clara, CA, USA) and the 4x44k format. High definition (HD) oligos from chromosomal region 5p15.3 (chr5: 1,320,000 bp - 1,400,000 bp; NCBI build 36.1) were selected for analysis and the design was filled with randomly selected oligos across the genome. The experimental procedures were performed according to the manufacturer's instructions with slight

modifications. We used the Bioprime Array-CGH Genomic Labeling System (Invitrogen) and the Oligo aCGH Hybridization Kit (Agilent). The arrays were scanned with the GenePix4000B Scanner (Axon Instruments, Inverury, Scotland) at a resolution of 5 µm/pixel. Signal intensities from the generated images were measured and evaluated with the Feature Extraction v9.5.3 software (Agilent). The log ratios obtained from the CGH Analytics 4.0.81 software (Agilent) were plotted.

Quantitative reverse transcription PCR. RNA was extracted from tumor cell containing samples and control samples stored either in DMSO as cell suspension or as kryoblocks. RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and transcribed into cDNA with the QuantiTect Rev. Transcription Kit (Qiagen). *TERT* transcripts were amplified and detected as described elsewhere[2]. The *TERT* mRNA expression was calculated by comparative Ct method, in which the amount of target (*TERT* mRNA, respectively), normalized to an endogenous reference (housekeeping gene beta-glucuronidase, betaGUS) and relative to a calibrator (*TERT* mRNA in peripheral blood leukocytes) is given as 2-ΔΔCT .
The method is described in detail in the User´s Bulletin #2 (page 11, Applied Biosystems, Darmstadt, Germany). Accordingly, standardization of *TERT* values was carried out by subtraction of corresponding Ct-values of the housekeeping gene *beta-glucuronidase (betaGUS)*. Further on, standardized *TERT* mRNA expression in translocation containing cases was calibrated to the standardized *TERT* mRNA expression in peripheral blood lymphocytes from healthy individuals (ΔΔCt). Cases with translocations affecting the *TERT* locus were compared to appropriate disease controls as well as not infiltrated tissue controls without aberration in 5p15.33 by FISH where applicable. Samples with undetectable *TERT* mRNA expression but detectable house-keeping quantification transcript *betaGUS* were excluded from statistical analysis using the Mann-Whitney-Test.

TRAP-assay. The PCR-based telomeric repeat amplification protocol (TRAP) assay was performed as described previously[3,4]. Protein from tumor cell containing samples and control tissue was isolated from cells in DMSO-containing medium or fresh material according to Kim et al., 1994[5]. Protein extracts were diluted to 500 ng/µl protein content and the TRAP assays were performed in triplicates using 1 µg protein in each sample. The TAMRA-TS primer elongation was carried out for 30 min at 30°C followed by PCR under the following conditions: 94°C for 2 min and 35 cycles at 94°C for 30 s, 50°C for 30 s and 72°C for 40 s. The amplified telomerase products were analyzed by ABI Prism 310 capillary electrophoresis as described[6]. The relative telomerase activity was calculated by the ratio of integral values of the telomerase product peaks 2-6 and the integral value of the internal amplification standard (ITAS) product peak. Statistical analysis was performed using the GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Cases with

translocations affecting the *TERT* locus were compared to appropriate disease controls without aberration in this region by FISH.

Figure S1. Schematic diagram of the translocation t(5;14)(p15;q32) with regard to the *TERT* and *CLPTM1L* genes and the *IGH* segments. In the case of illegitimate VDJ-rearrangement as typical for ALL both *IGH*-enhancers, Eα (3'-enhancer) and Eμ (5'-enhancer), remain on the derivative chromosome 14. Through illegitimate class switch recombination frequently observed in mature B-cell neoplasms one of the enhancers moves to the derivative chromosome 5. Chromosome 5 is displayed in blue, chromosome 14 is displayed in orange.

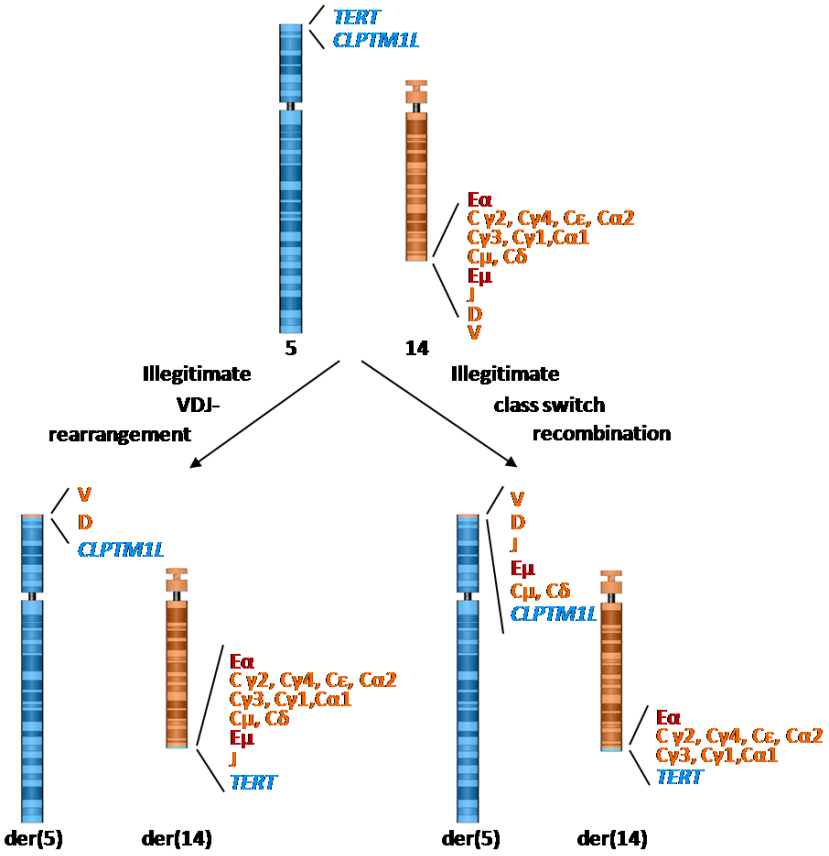


Figure S2. Custom designed array CGH of the cases 2 **(a)** and 5 **(b)**. A value of zero indicates a balanced status, shift of the dots below the line indicate the presence of losses of genomic material. Below the Repeat Masker of the Human Genome Browser database (http://genome.ucsc.edu/) is indicated.

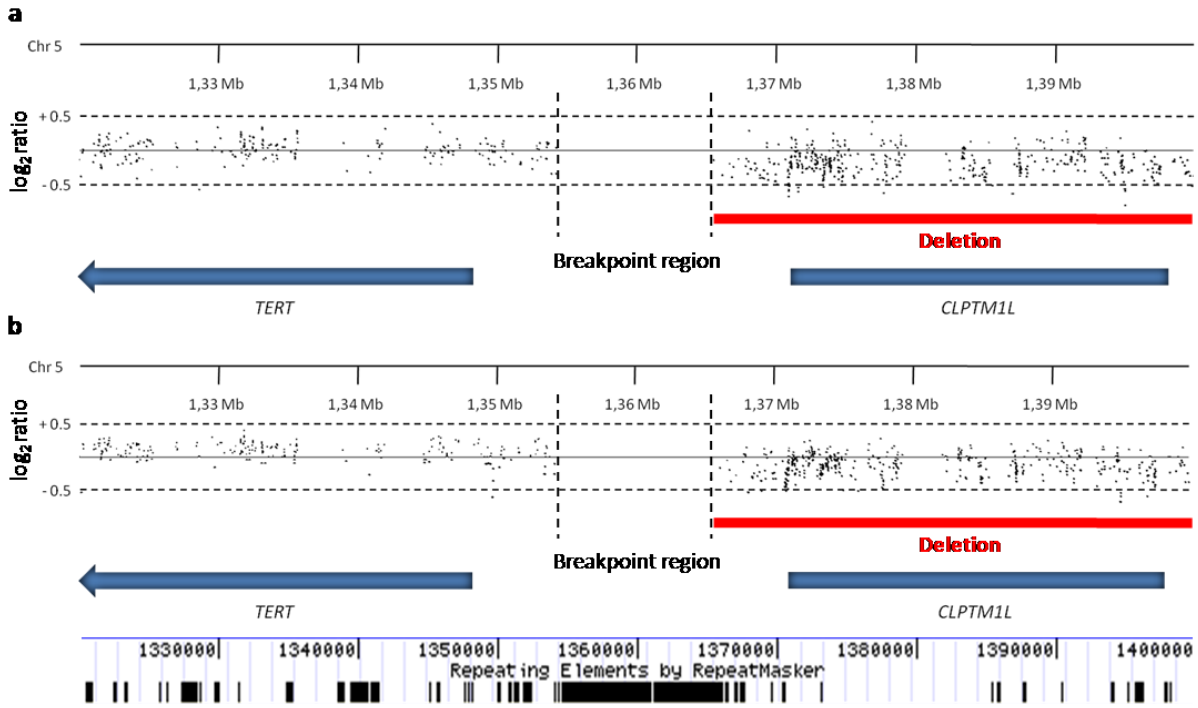
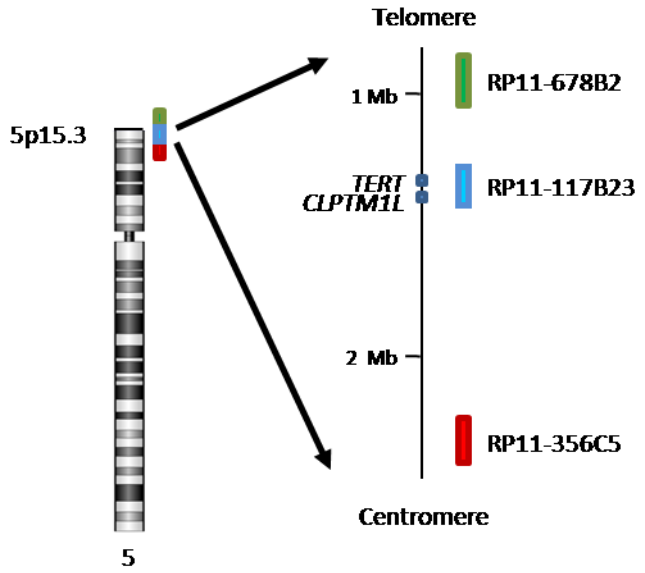


Figure S3. FISH-probe design of the *TERT-CLPTM1L*-three-color break-apart assay. The BAC clones are indicated by bars in the color they were labeled for hybridization (spectrum green, diethylaminocoumarin (DEAC), spectrum orange).



normal

translocation with break in RP11-117B23

Figure S4. Relative *TERT* mRNA expression determined by quantitative RT-PCR. Three mantle cell lymphomas (MCLs, cases 7-9) with break in the *TERT-CLPTM1L* locus were compared to MCLs 1-5 without 5p15.33-break by FISH. The relevant cytogenetic aberrations and the tumor cell content are given in brackets. P-value was calculated using the Mann-Whitney-Test. Samples with undetectable *TERT* mRNA expression were excluded from statistical analysis.

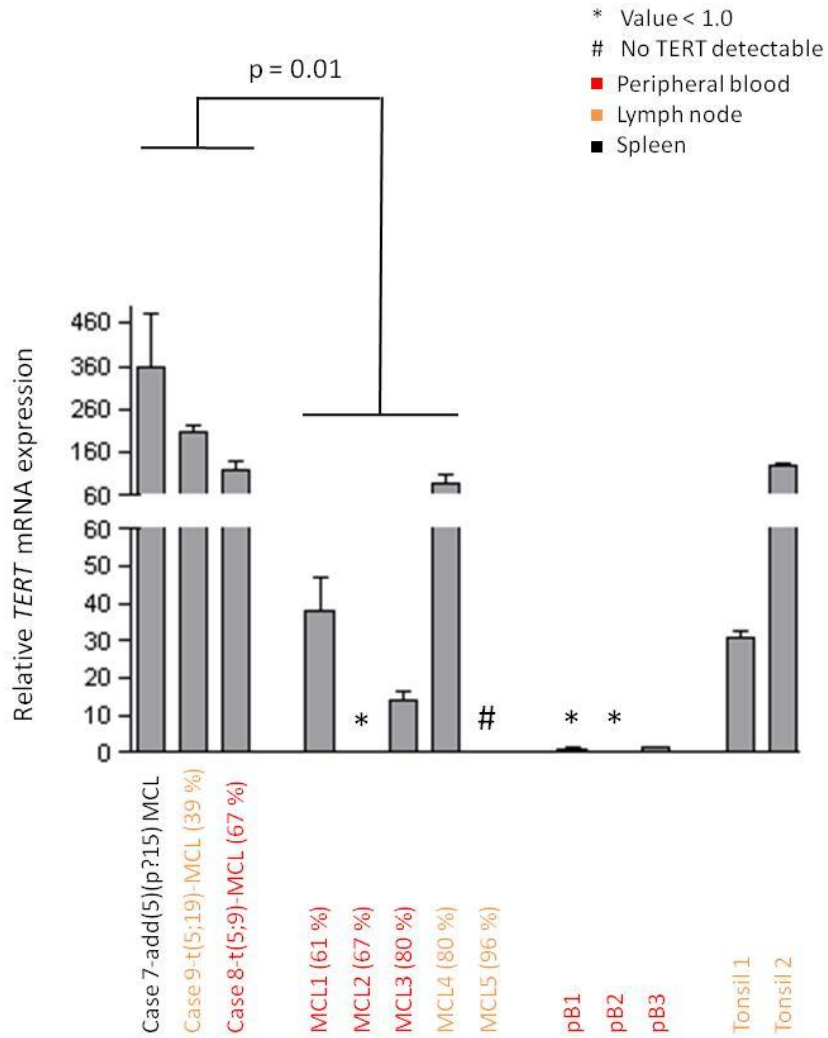


Figure S5. Relative *TERT* mRNA expression determined by quantitative RT-PCR. One acute lymphoblastic leukemia (ALL, case 4) with 5p15.33-breakby FISH were compared to ALLs 1-6 showing no 5p15.33-aberration by FISH. The relevant cytogenetic aberrations and the tumor cell contents are given in brackets.

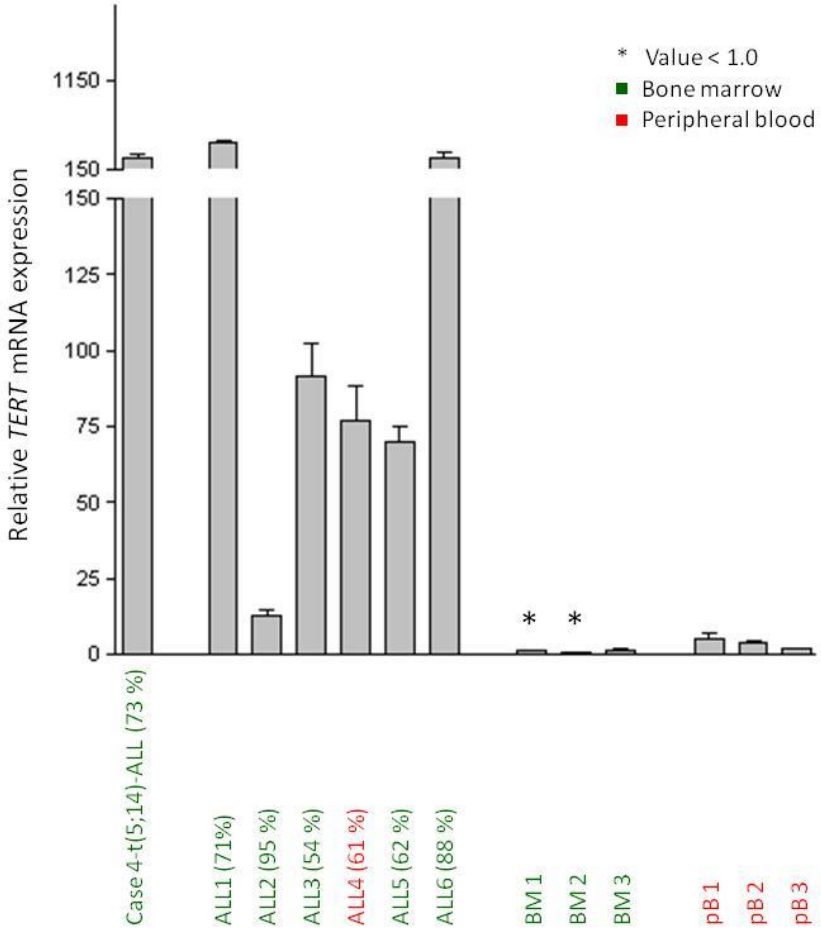


Table S3. TRAP assay raw data

Case	Telomerase-elongated peak area					total peak area	ITAS peak area	ratio: total peak area / ITAS peak area
	1	2	3	4	5			
B-CLL								
Case 1	14380	7851	5885	7379	5056	40551	17991	2,254
Case 1	15512	8434	5754	8742	5914	44356	17423	2,546
Case 1	14500	8063	5836	7525	5193	41117	18948	2,170
Case 6	12238	5751	3763	3245	1528	26525	3110	8,529
Case 6	11436	5269	3823	2803	1528	24859	3294	7,547
Case 6	15923	6395	3869	2626	1172	29985	2925	10,251
CLL 1	5823	2349	1734	1465	2047	13418	66588	0,202
CLL 1	1593	795	1035	879	1176	5478	70908	0,077
CLL 1	7868	3723	2834	2611	1914	18950	58093	0,326
CLL 3	3655	1464	2031	1603	901	9654	15339	0,629
CLL 3	5297	3288	1804	1875	1026	13290	31843	0,417
CLL 3	6762	1615	1345	2491	914	13127	26519	0,495
CLL 4	6039	2419	2504	2218	1419	14599	27166	0,537
CLL 4	5810	3142	3333	2714	1233	16232	53970	0,301
CLL 4	4839	2396	2579	2136	1490	13440	62192	0,216
CLL 6	6003	1061	1684	1191	1135	11074	54455	0,203
CLL 6	4812	1169	1180	2472	2715	12348	41984	0,294
CLL 6	7444	692	1288	1560	814	11798	32264	0,366
CLL 10	2597	796	488	0	0	3881	57035	0,068
CLL 10	2485	883	677	583	0	4628	59501	0,078
CLL 10	2900	0	0	0	0	2900	52841	0,055
CLL 11	10755	5718	3044	4434	2178	26129	29605	0,883
CLL 11	10648	6573	3100	4025	1897	26243	17237	1,522
CLL 11	12360	6257	3735	3775	2682	28809	14827	1,943
MCL								
Case 9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Case 9	11323	6417	4837	6051	4045	32673	4538	7,200
Case 9	13347	7615	5346	5635	3851	35794	6829	5,241
Case 8	8076	3466	3470	3601	2010	20623	2128	9,691
Case 8	10245	4220	2551	2774	1729	21519	4831	4,454
Case 8	9314	3932	2265	3090	1721	20322	4342	4,680
MCL 4	12409	5528	4491	5126	4244	31798	26563	1,197
MCL 4	12448	5927	4397	5141	3832	31745	12240	2,594
MCL 4	12242	6375	4665	5020	3691	31993	23533	1,359
MCL 5	14473	6496	5044	5509	4022	35544	9822	3,619
MCL 5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MCL 5	14492	6648	5172	6160	3951	36423	7656	4,757
ALL								
Case 4	16310	6920	5050	4416	3236	35932	20632	1,742
Case 4	15740	6641	4765	4734	2866	34746	25541	1,360
Case 4	16629	6973	5288	4637	3119	36646	16170	2,266

[a] internal amplification standard

Data interpretation was performed according to Klapper et al., 2003: the first five telomerase elongated primer product area values were summed and divided by the corresponding ITAS (internal amplification standard) peak area value. The ratio comprises the relative telomerase activity given in arbitrary units in correspondence to telomerase activity values in peripheral blood leukocytes obtained from healthy volunteers.

In accordance, each vertical line contains raw data (integrated peak area values) for one TRAP assay performed. Evaluable TRAP assay values are listed in columns as follows: (1) case label, (2) first, (3) second, (4) third, (5) forth and (6) fifth telomerase product peak area value, (7) sum of the telomerase product peak area values 1-5, (8) ITAS peak area value, and (9) ratios of corresponding (7) and (8) values as indicated above for relative telomerase activity.

B-CLL, chronic B-lymphocytic leukemia; MCL, mantle cell lymphoma, ALL, acute lymphocytic leukemia, n.a. data not available

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