Chromosome banding analysis and genomic microarrays are both useful but not equivalent methods for genomic complexity risk stratification in chronic lymphocytic leukemia patients

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Ramos-Campoy et al.

SUPPLEMENTARY DATA

Supplementary Methods

Patient cohort

Patients were diagnosed between 1983 and 2018 according to current guidelines.[1-4] Clinical information collected at diagnosis included demographics (age and gender), Binet stage, genetic and molecular data. Regarding information on evolution, dates of treatment administration and last follow-up were collected. Of note, data from CBA or GM of some patients have been included in previous publications although they were not used with the same purpose as the present study.[5-13]

Cytogenetic analyses

Peripheral blood (PB) or bone marrow (BM) samples cultures using either phorbol-12-myristate-13-acetate (TPA) (n=228; 67%), immunostimulatory cytosine guanine dinucleotide (CpG)-oligonucleotide DSP30 plus interleukin 2 (IL-2) (n=19; 5.6%) or both (n=93; 27.4%) as mitogens were established following standard procedures.[14] At least 20 metaphases were analyzed in cases with normal karyotype while for abnormal karyotypes, the minimum were 10. Number and type of abnormalities were recorded. Balanced rearrangements included translocations and inversions, while chromosome additions, duplications, insertions, isochromosomes, as well as derivative, dicentric, ring and marker chromosomes were considered unbalanced rearrangements and were counted as one aberration.

Interphase fluorescence *in situ* hybridization (FISH) results were available in 320/340 (94.1%) cases using probes for the chromosomal regions 13q14, 11q22 (*ATM*) and 17p13 (*TP53*) and the centromere of chromosome 12 (CEP 12). In five cases, whole chromosome painting was performed in order to study the discrepancies observed between CBA and GM.

Genomic microarray analyses

Genomic microarrays data were already available or obtained from DNA extracted in a period of time less than one year from the date of CBA in order to avoid the emergence of additional abnormalities (median time from CBA to GM=0 months; range: 0-12). GM

were assessed on DNA from whole PB (n=113; 33%), PB mononuclear cells (n=63; 19%), PB CD19+ purified cells (n=110; 32%) or from BM samples (n=54; 16%). Only DNA that fulfilled quality controls required was amplified, labelled and hybridized to different genomic microarray platforms according to the manufacturer's protocols. Obtained data were visually revised and copy number variants found as benign polymorphisms in the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home) were excluded. For defining genome coordinates, annotations of genome version GRCh37/hg19 were used. Chromothripsis-like and chromothripsis patterns were defined by the presence of ≥7 and ≥10 oscillating switches, respectively, between two or three copy number states on an individual chromosome.[7,8,15]

Although the objectives of the study did not consider the analysis of copy-number neutral loss of heterozygosity (CN-LOH), in those cases in which the microarray platform included single nucleotide polymorphisms (SNP) probes, a global screening for CN-LOH was performed. CN-LOH were recorded when detected in a region larger than 10Mb and extending to chromosome telomeres. They were not included in the counting of CNAs.

TP53 mutation analysis

A total of 308 (90.6%) cases were screened for *TP53* mutations. For the assessment of *TP53* mutations exons 4-8 were sequenced (exons 9-10 were also included in some centers) following ERIC recommendations.[16] Sixty (19.5%) cases were screened by Sanger sequencing whereas the remaining (n=248; 80.5%) were analyzed by next-generation sequencing. Only mutations with a variant allele frequency >10% were considered.

IGHV mutational analysis

IGHV mutational status was analyzed in 307 (90.3%) patients following established international guidelines.[17] Sequences were examined and interpreted using the IMGT database and the IMGT/V-QUEST tool. Clonotypic IGHV gene sequences with <98% germline identity were defined as mutated (M-IGHV) whereas those with ≥98% identity were classified as unmutated (U-IGHV).

Statistical analyses

As different European centers were involved in the present study, before performing the survival analyses we evaluated the homogeneity of the results in terms of time to first treatment (TTFT). We found out that in three institutions, TTFT in the non-CK group was notably shorter than previously reported in other studies[11] because CBA in

these centers were mainly performed at recruitment for clinical trials. Therefore, in order to avoid biases in the results reported herein, 81 cases were not included in the survival analyses. As for the CK group, no differences were observed between the collaborating centers. Consequently, survival analyses were performed in 259 patients.

Supplementary Results

Risk stratification of the genomic complexity observed by CBA and GM

Regarding CBA, when results obtained with each mitogen were considered separately, those cases stimulated with IL-2+DSP30 exhibited a higher proportion of complex cases. Significant differences were observed in the percentage of patients classified into intermediate-risk categories (3-4 abnormalities; 20.6% with TPA vs. 32.1% with IL-2+DSP30) or those showing the highest risk (≥5 abnormalities; 14.9% and 27.7%, respectively) (p<0.001). However, when comparing with GM classification, both methods presented a similar moderate agreement (TPA: κ=0.464; IL-2+DSP30: κ=0.530).

Number and type of abnormalities detected by CBA and GM

Regions with CN-LOH were detected in 23 (7.5%) patients as the microarray platform used in 306 cases also contained SNP probes. Median size of CN-LOH was 50.1Mb (range: 11.9-159Mb) and they were found in several chromosomes. Notably, two of the three cases with CN-LOH affecting 17p arm and the only case with CN-LOH involving *ATM* gene had *TP53* and *ATM* genes mutated, respectively. Nevertheless, CN-LOH data were not included in the analyses.

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Supplementary Tables

Table S1. Genomic microarray platforms used in this study.

Genomic microarray platform	n (%)
Whole-Genome 2.7M (ThermoFisher)	62 (18.2)
CytoScan HD array (ThermoFisher)	87 (25.6)
Affymetrix SNP6.0 (ThermoFisher)	82 (24.1)
SurePrint G3 Human CGH 8x60K (Agilent)*	11 (3.2)
SurePrint G3 ISCA CGH+SNP Bundle, 4x180K (Agilent)	75 (22.1)
Illumina Human Omni1-Quad array (Illumina)	12 (3.5)
Illumina Human Omni2.5-Quad array (Illumina)	11 (3.2)

*Custom design described in Salaverria I, Martín-Garcia D, López C, et al. Detection of chromothripsis-like patterns with a custom array platform for chronic lymphocytic leukemia. Genes Chromosomes Cancer. 2015;54(11):668-80.

Table S2. Detection of the four classical CLL abnormalities by chromosome banding analysis in those patients with FISH results available.

	FISH		CHROMOSOME BANDING ANALYSIS				
Locus affected	**Patients tested**	**Altered cases** *n* (%)	**Abnormalities in CLL *loci*** *n* (%)	**Detail of the abnormalities**	n (%)	**Confirmed by FISH**	**Overall concordance**
13q14	327	188 (57.5)	97 (29.7)	Monosomy 13	13 (13.4)	12/13 (92.3%)	50.0%
				Deletion 13q14	66 (68.0)	64/66 (97.0%)	
				Balanced translocation involving 13q14	9 (9.3)	9/9 (100%)	
				Unbalanced translocation in 13q14	9 (9.3)	9/9 (100%)	
Chromosome 12	327	56 (17.1)	54 (16.5)	Trisomy 12	54 (16.5)	54/54 (100%)	96.4%
11q22q23 (*ATM*)	329	71 (21.6)	67 (20.4)	Monosomy 11	1 (1.5)	1/1 (100%)	87.3%
				Deletion 11q22q23	58 (86.6)	55/58 (94.8%)	
				Additional material in 11q22q23	1 (1.5)	1/1 (100%)	
				Balanced translocation involving 11q22q23	2 (2.9)	1/2 (50.0%)	
				Unbalanced translocation in 11q22q23	5 (7.5)	4/5 (80.0%)	
17p13 (*TP53*)	329	69 (21.0)	67 (20.4)	Monosomy 17	14 (20.9)	13/14 (92.9%)	91.3%
				Deletion 17p13	10 (14.9)	10/10 (100%)	
				Additional material in 17p	16 (23.9)	14/16 (87.5%)	
				Isochromosome (17)(q10)	5 (7.5)	5/5 (100%)	
				Dicentric chromosomes affecting 17p	4 (5.9)	4/4 (100%)	
				Unbalanced translocation in 17p	18 (26.9)	17/18 (94.4%)	

Table S3. Detection of the four classical CLL abnormalities by genomic microarrays in those patients with FISH results available.

	FISH		GENOMIC MICROARRAYS				
Locus affected	**Patients tested**	**Altered cases** n (%)	**Altered cases** n (%)	**Median size** Mb (range)	**Minimal abnormal region** Cytobands (coordinates: GRCh37/hg19)	**Overall concordance**	**Detail of discordant cases**
13q14	327	188 (57.5)	171 (52.3)	1.94 (0.035-95.7)	q14.2-q14.2 (50,632,951 - 50,659,544)	88.8% (167/188)	- 21 only positive by FISH (20/21 <30% nuclei) - 4 only positive by GM
Chromosome 12	327	56 (17.1)	55 (16.8)	133.60 (132.3-133.8)	p13.33-q24.33 (192,539 - 132,349,534)	98.2% (55/56)	- 1 only positive by FISH (7% nuclei)
11q22q23 (*ATM*)	329	71 (21.6)	68 (20.7)	25.81 (0.151-54.5)	q22.3-q22.3 (108,125,328-108,276,581)	91.5% (65/71)	- 6 only positive by FISH (5/6 <30% nuclei) - 3 only positive by GM
17p13 (*TP53*)	329	69 (21.0)	58 (17.6)	21.47 (0.470-25.9)	p13.1-p13.1 (7,481,305-7,678,604)	82.6% (57/69)	- 12 only positive by FISH (11/12 <20% nuclei) - 1 only positive by GM

Table S4. Abnormalities detected by chromosome banding analysis and genomic microarrays in the ten patients classified in opposite risk categories depending on the technique employed for their study.

Case	CHROMOSOME BANDING ANALYSIS		GENOMIC MICROARRAYS					
	Karyotype	Number of aberrations	Type	Chr.	Start-end	Size (Mb)	Number of CNA	Number of CNA ≥5Mb
#37	47,XY,add(8)(p23),-9,add(12)(q24),-13,-18,+4mar[41]/46,XY[8]	9	GAIN	12	p13.33-q24.33 (173786-133777902)	133,604	2	1
			GAIN	18	p11.31-p11.23 (6929190-8087455)	1,158		
#38	47,XY,-4,del(7)(p?),+12,add(15)(q26),+mar[20]/47,XY,+12[20]/ 46,XY[10]	5	GAIN	12	p13.33-q24.33 (173786-133777902)	133,604	1	1
#100	46,XY,add(19)(q13.4)[25]/45,XY,der(2)t(2;6)(p23;p12),del(6)(q?), -20,del(21)(q22)[5]/46,XY[21]	5	GAIN	2	p25.3-p13.3 (0-70151030)	70,151	1	1
#119	46,XX,i(17)(q10)[7]/45,XX,-13,add(14)(q11),i(17)(q10)[4]/ 44,XX,del(6)(q14q24),add(7)(q36),-12,-15,i(17)(q10)[1]/ 43,XX,-5,del(6)(q14q24),add(7)(q36),add(10)(q22),-12, -15,i(17)(q10)[7]/46,XX[4]	9	GAIN	17	q11.1-q25.3 (25270397-81041938)	55,772	2	2
			LOSS	17	p13.3-p11.1 (525-22261792)	22,261		
#121	47,XY,+12[9]/47,XY,del(X)(q25),add(5)(q31),add(8)(q24),+12, del(14)(q22q32)[4]/46,XY[7]	5	GAIN	12	p13.33-q24.33 (173786-133777902)	133,604	2	2
			LOSS	14	q23.2-q32.12 (63953105-93505497)	29,552		
#152	46,XX,del(14)(q24q32)[1]/44,XX,der(4;21)(q10;q10),del(14)(q24q32), der(15;22)(q10;q10)[5]/43,XX,der(1)t(1;17)(p11;q11), der(4;21)(q10;q10),der(11)t(1;11)(p11;q25),del(14)(q24q32), der(15;22)(q10;q10),-17[5]/ XY 46,XX[6]	6	LOSS	4	p16.3-p14 (0-39309957)	39,310	4	2
			LOSS	4	p12-p11 (45427534-49174296)	3,747		
			LOSS	4	q11-q12 (52697788-55087565)	2,390		
			LOSS	14	q24.1-q32.33 (69262059-106067093)	36,805		
#180	46,Y,der(X)t(X;2)(q26;p15)[5]/46,XY,der(2)t(2;2)(p24;p15)[5]/ 46,XY,der(5)t(2;5)(p15;q35),r(8)(p11q24),der(11)t(5;11)(?;q24)t(2;5) (p15;?)[4]/46,XY,der(6)t(2;6)(p15;q27)[2]/46,XY[14]	6	GAIN	2	p25.3-p15 (0-62206329)	62,206	2	2
			LOSS	13	q14.2-q14.3 (49874813-51747327)	1,873		
#228	45,X,-Y,del(11)(q?14),del(13)(q14q22)[7]/ 44,idem,der(12)t(12;13)(p?13;q?12)[5]/45,idem,t(1;11)(p?36;q13)[3]/ 46,XY[2]	5	LOSS	11	q14.1-q23.3 (79261152-116172518)	36,911	3	2
			LOSS	13	q14.11-q21.33 (43292880-72523559)	29,231		
			LOSS	X	q27.3-q27.3 (145094655-145177733)	0,083		

#c362	44,XY,der(5;17)t(5;17)ins(5;17)(q1?3;p11q25)[2]/46,XY[12]	1	LOSS	5	q12.3-q13.1 (64558088-66809076)	2,251	10	7
			LOSS	5	q13.2-q23.1 (70908308-119198005)	48,290		
			LOSS	5	q31.3-q32 (144329951-149326255)	4,996		
			LOSS	5	q33.1-q33.2 (149834719-155509902)	5,675		
			LOSS	11	q22.3-q23.3 (105315158-115742438)	10,427		
			LOSS	13	q14.2-q14.3 (48675135-51631607)	2,956		
			LOSS	17	p13.3-p11.2 (1-19149275)	19,149		
			LOSS	17	q11.1-q11.2 (25270425-26179601)	0,909		
			LOSS	18	p11.32-p11.21 (454728-14318059)	13,863		
			LOSS	20	p13-p11.1 (60001-26107860)	26,048		
#c377	46,XY[29]	0	LOSS	1	q42.12-q42.12 (225692953-225845385)	0,152	7	5
			LOSS	1	q42.12-q44 (225961441-249224401)	23,263		
			LOSS	4	p16.3-p15.2 (10001-27127332)	27,117		
			LOSS	4	p14-q21.21 (39184089-81142337)	41,958		
			LOSS	4	q31.3-q31.3 (153332112-154672325)	1,340		
			LOSS	13	q14.13-q14.3 (46725019-52636989)	5,912		
			LOSS	13	q14.2-q14.3 (50339821-51605362)	1,266		

Those CNA highlighted in grey were non-classical CLL abnormalities smaller than 5Mb.

Table S5. Initial and rewritten karyotypes from six patients in which the formula was modified after genomic microarrays analysis.

Case	Initial karyotype	N abn.	Abnormalities by genomic microarrays				Rewritten karyotype	N abn.
			Type	Chr.	Start-end	Size (Mb)		
#16	45,XY,-13,add(15)(p11),der(17)t(13;17)(q11;p11)[10]/ 46,XY[10]	3	GAIN	3	q11.1-q29 (93626178-197851986)	104,226	45,XY,-13,der(15)t(3;15)(q11;p11), der(17)t(13;17)(q11;p11)[10]/46,XY[10]	3
			LOSS	3	p26.3-p26.1 (311066-6061949)	5,751		
			LOSS	13	q14.2-q14.3 (50595391-51485770)	0,890		
			GAIN	15	q22.2-q26.3 (60417426-102345371)	41,928		
			LOSS	17	p13.3-p11.1 (9474-22227062)	22,218		
#43	47,XX,del(11)(q23),-14,+2mar[10]/46,XX[20]	4	GAIN	2	p25.3-p11.2 (12770-89129064)	89,116	47,XX,+i(2)(p10),del(11)(q23),del(13)(q14q22)[10]/ 46,XX[20]	3
			LOSS	11	q14.1-q23.3 (77108160-117201998)	40,094		
			LOSS	13	q14.2-q22.3 (49894796-77764277)	27,869		
			LOSS	14	q23.2-q24.1 (64199833-69665479)	5,466		
#48	47,XY,der(12)(q?),+der(12)(q?),del(13)(q?)[30]	3	GAIN	12	p13.33-q23.2 (173786-102013163)	101,839	47,XY,der(12)(q?),+der(12)t(12;13)(q23;q21), del(13)(q14q21)[30]	3
			GAIN	13	q21.32-q34 (67265752-115107733)	47,842		
			LOSS	13	q14.13-q21.31 (46950690-63774667)	16,824		
			LOSS	17	p12-p12 (14000097-14622477)	0,622		
#58	46,XY,-5,-6,-14,+3mar[9]/46,XY[11]	6	LOSS	5	q14.2-q23.1 (82410385-115495863)	33,085	46,XY,del(5)(q14q23),der(6)inv(6)(?),del(13)(q12q14)[9]/ 46,XY[11]	3
			LOSS	13	q14.2-q14.3 (50140480-51426156)	1,286		
			LOSS	13	q12.3-q14.3 (32145265-52311881)	20,167		
#61	43-45,X,-X,del(2)(p15),+4,-7,add(11)(q21),-12,-13, add(14)(q32),add(17)(p11)[cp6]/46,XX[9]	9	GAIN	2	p25.3-p13.1 (12770-73803026)	73,790	45,X,-X,del(2)(p15),+4,der(7)t(2;7)(p13;q36), del(11)(q21q23),-12,del(13)(q14),der(14)t(2;14)(p13;q32), dic(17;18)(p10;p10),+3mar[6]/46,XX[9]	12
			LOSS	11	q21-q23.2 (95086750-112810693)	17,724		
			LOSS	13	q14.2-q14.3 (50691182-51659251)	0,968		
			LOSS	17	p13.3-p11.2 (525-21565553)	21,565		
			LOSS	18	p11.32-p11.21 (136226-13719291)	13,583		
#62	46,XX,t(2;5)(p16;p15),del(4)(q31),add(15)(q26)[6]/ 46,XX[18]	3	No aberrations				46,XX,t(2;15)(p16;q26),t(4;5)(q31;p15)[3]/46,XX[18]	2

Abbreviations: Abn.= Abnormalities, Chr.= Chromosome

Table S6. Frequency of different genetic features in the three subgroups defined by chromosome banding analysis and genomic microarrays (n=259)

	CHROMOSOME BANDING ANALYSIS					GENOMIC MICROARRAYS				
	Non-CK	**Low/ intermediate-CK**	**High-CK**	***p*-value**	***p*-value** low/int vs high-CK	**Low-GC**	**Intermediate-GC**	**High-GC**	***p*-value**	***p*-value** intermediate vs high-GC
Known CLL genetic prognostic factors										
Del(13)(q14)	63 (62.4%)	47 (58.0%)	49 (63.6%)	0.744	0.470	68 (54.8%)	50 (71.4%)	41 (63.1%)	0.071	0.301
Trisomy 12	18 (17.8%)	19 (23.5%)	8 (10.4%)	0.095	0.029	28 (22.6%)	15 (21.4%)	2 (3.1%)	0.002	0.001
Del(11)(q22)	12 (11.9%)	26 (32.1%)	23 (29.9%)	0.002	0.762	13 (10.5%)	28 (40.0%)	20 (30.8%)	<0.001	0.263
Del(17)(p13)/mutation *TP53* (n=239)	7 (8.4%)	23 (28.7%)	47 (61.8%)	<0.001	<0.001	11 (10.3%)	21 (30.9%)	45 (70.3%)	<0.001	<0.001
U-IGHV (n=227)	25 (28.1%)	42 (61.8%)	50 (71.4%)	<0.001	0.229	40 (36.7%)	35 (60.3%)	42 (70.0%)	<0.001	0.271
Type of abnormality by CBA										
Unbalanced rearrangements	3 (3.0%)	58 (71.6%)	76 (98.7%)	<0.001	<0.001	26 (21.0%)	49 (70.0%)	62 (95.4%)	<0.001	<0.001
Presence of material from unknown origin	1 (1.0%)	32 (39.5%)	45 (58.4%)	<0.001	0.017	15 (12.1%)	26 (37.1%)	37 (56.9%)	<0.001	0.021
Clonal evolution	1 (1.0%)	40 (49.4%)	41 (53.2%)	<0.001	0.627	20 (16.1%)	29 (41.4%)	33 (50.8%)	<0.001	0.277
Type of abnormality by GM										
Common CNA										
Gain 2p	2 (2.0%)	12 (14.8%)	27 (35.1%)	<0.001	0.003	3 (2.4%)	19 (27.1%)	19 (29.2%)	<0.001	0.788
Loss 3p	1 (1.0%)	5 (6.2%)	8 (10.4%)	0.021	0.335	3 (2.4%)	3 (4.3%)	8 (12.3%)	0.015	0.089
Gain 3q	0	6 (7.4%)	5 (6.5%)	0.024	0.822	0	4 (5.7%)	7 (10.8%)	0.002	0.283
Loss 4p	1 (1.0%)	7 (8.6%)	8 (10.4%)	0.019	0.708	1 (0.8%)	2 (2.9%)	13 (20.0%)	<0.001	0.002
Loss 6q	1 (1.0%)	5 (6.2%)	10 (13.0%)	0.004	0.144	1 (0.8%)	5 (7.1%)	10 (15.4%)	<0.001	0.128
Loss 8p	0	6 (7.4%)	10 (13.0%)	0.001	0.245	0	5 (7.1%)	11 (16.9%)	<0.001	0.079
Gain 8q	1 (1.0%)	8 (9.9%)	9 (11.7%)	0.010	0.713	0	4 (5.7%)	14 (21.5%)	<0.001	0.007
Loss 14q	2 (2.0%)	4 (4.9%)	9 (11.7%)	0.021	0.123	5 (4.0%)	4 (5.7%)	6 (9.2%)	0.348	0.521
Loss 15q	0	5 (6.2%)	11 (14.3%)	<0.001	0.091	1 (0.8%)	3 (4.3%)	12 (18.5%)	<0.001	0.009
Gain 17q	0	3 (3.7%)	9 (11.7%)	0.001	0.058	1 (0.8%)	3 (4.3%)	8 (12.3%)	0.002	0.089
Loss 18p	3 (3.0%)	6 (7.4%)	18 (23.4%)	<0.001	0.005	2 (1.6%)	8 (11.4%)	17 (26.2%)	<0.001	0.028
Gain 19q	1 (1.0%)	6 (7.4%)	6 (7.8%)	0.059	0.927	1 (0.8%)	8 (11.4%)	4 (6.2%)	0.004	0.282
Chromothripsis	1 (1.0%)	7 (8.6%)	22 (28.6%)	<0.001	0.001	1 (0.8%)	6 (8.6%)	23 (35.4%)	<0.001	<0.001

Abbreviations: CK = complex karyotype, non-CK = 0-2 abnormalities detected by chromosome banding analysis, low/intermediate-CK = 3-4 abnormalities, high-CK = ≥5 abnormalities, GC = genomic complexity, low-GC = 0-2 copy number abnormalities (CNA) detected by genomic microarrays, intermediate-GC = 3-4 CNA, high-GC = ≥5 CNA, U-IGHV = CLL with unmutated IGHV

Table S7. Univariate and multivariate analysis for time to first treatment (TTFT)

Variable	Univariate analysis		Multivariate analysis for CBA		Multivariate analysis for GM	
	Median TTFT in months (95% CI)	***p*-value**	**Hazard ratio (95% CI)**	***p*-value**	**Hazard ratio (95% CI)**	***p*-value**
CBA						
low/intermediate-CK vs. non-CK	18 (11-25) vs. NR	<0.001	2.85 (1.53-5.31)	<0.001	-	-
high-CK vs. non-CK	5 (1-9) vs. NR	<0.001	4.54 (2.18-9.44)	<0.001	-	-
GM						
intermediate-GC vs. low-GC	35 (0-74) vs. NR	0.022	-	-	1.60 (1.05-2.43)	0.029
high-GC vs. low-GC	3 (0-6) vs. NR	<0.001	-	-	3.52 (2.27-5.46)	<0.001
Unbalanced rearrangements	11 (5-17)	<0.001	0.97 (0.53-1.77)	0.916	-	-
Chromothripsis	2 (0-6)	<0.001	-	-	1.35 (0.83-2.20)	0.228

Abbreviations: CBA = chromosome banding analysis, CK = complex karyotype, non-CK = 0-2 abnormalities detected by CBA, low/intermediate-CK = 3-4 abnormalities, high-CK = ≥5 abnormalities, GM = genomic microarrays, GC = genomic complexity, low-GC = 0-2 copy number abnormalities (CNA) detected by genomic microarrays, intermediate-GC = 3-4 CNA, high-GC = ≥5 CNA, CI = confidence interval, NR = not reached.

Supplementary Figures

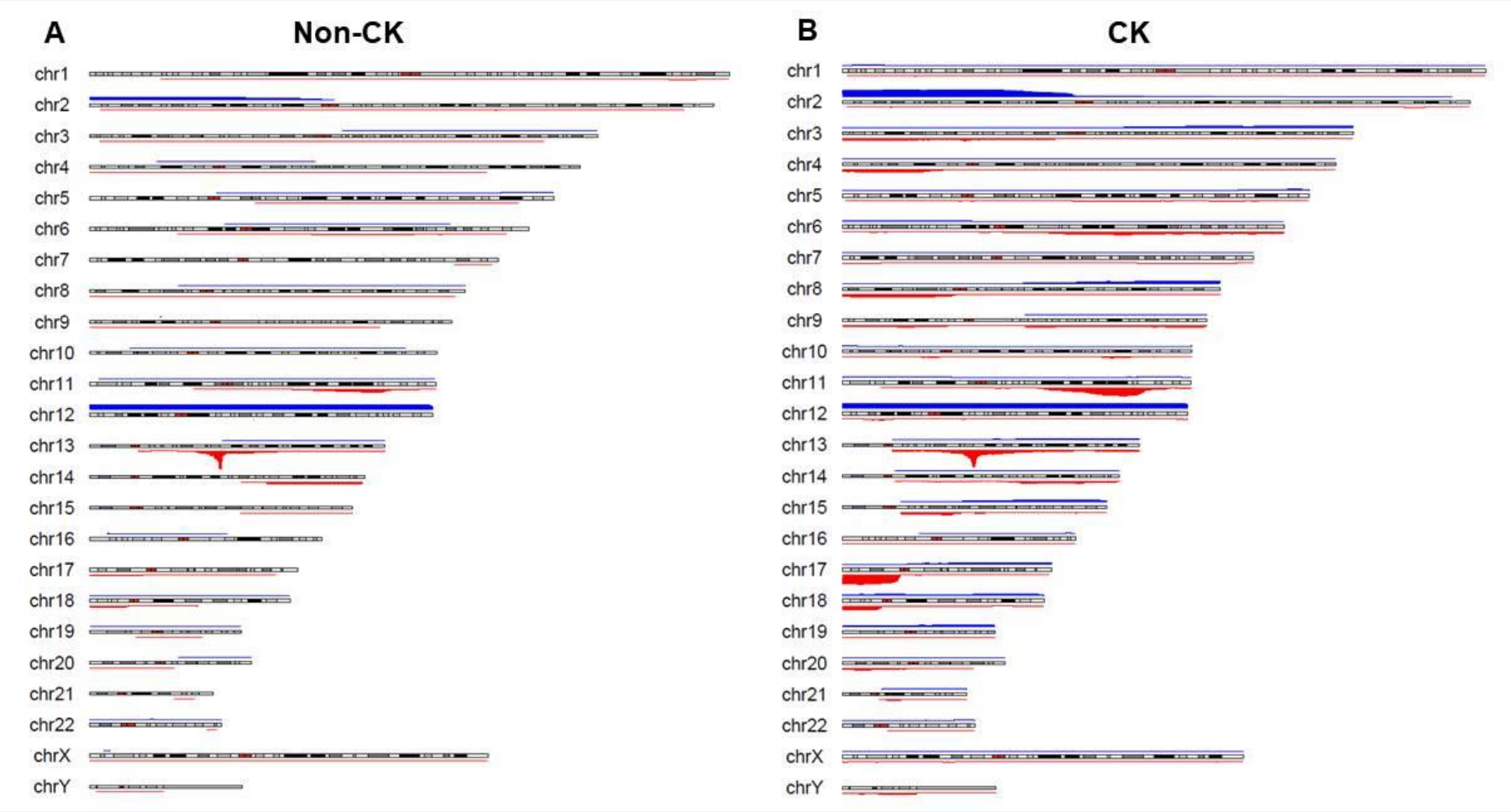

Figure S1. **Distribution of the copy number aberrations detected by genomic microarrays in non-CK and CK groups.** (A) Non-CK subgroup (0-2 abnormalities), (B) CK subgroup (≥3 abnormalities). Gains are represented in blue above and losses in red below the affected chromosomal regions. The thickness of the bars represents the number of cases showing the respective gain or loss. Figures were created by using KaryoploteR package of R.

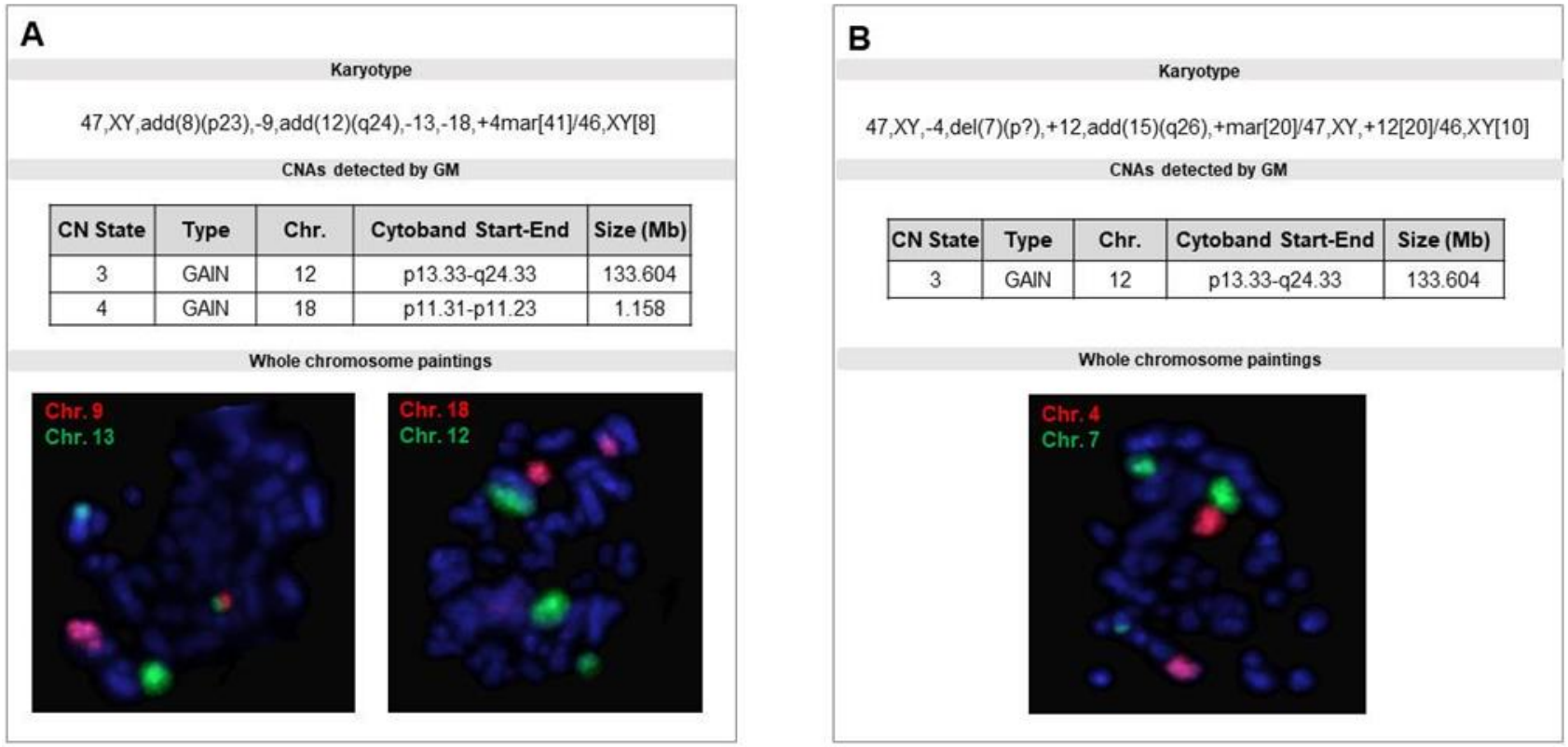

Figure S2. Whole chromosome painting FISH images of two high-CK cases classified as low-GC by genomic microarrays. (A) Nine aberrations were detected by CBA while only two were observed by GM. FISH was performed using chromosome painting probes for chromosomes 9 (red) and 13 (green), on the left image, and for chromosomes 12 (green) and 18 (red), on the right image. FISH revealed that chromosomes apparently lost in the karyotype appeared to be fragmented, either constituting the additional material of other chromosomes or being part of marker chromosomes. (B) Five aberrations were detected by CBA while only gain of chromosome 12 was detected by GM. FISH was performed using chromosome painting probes for chromosomes 4 (red) and 7 (green). According to FISH images, both chromosomes were present in the analyzed metaphases but were fragmented (chr.7) or considered as marker chromosomes (chr.4). Chromosomes were stained with DAPI.

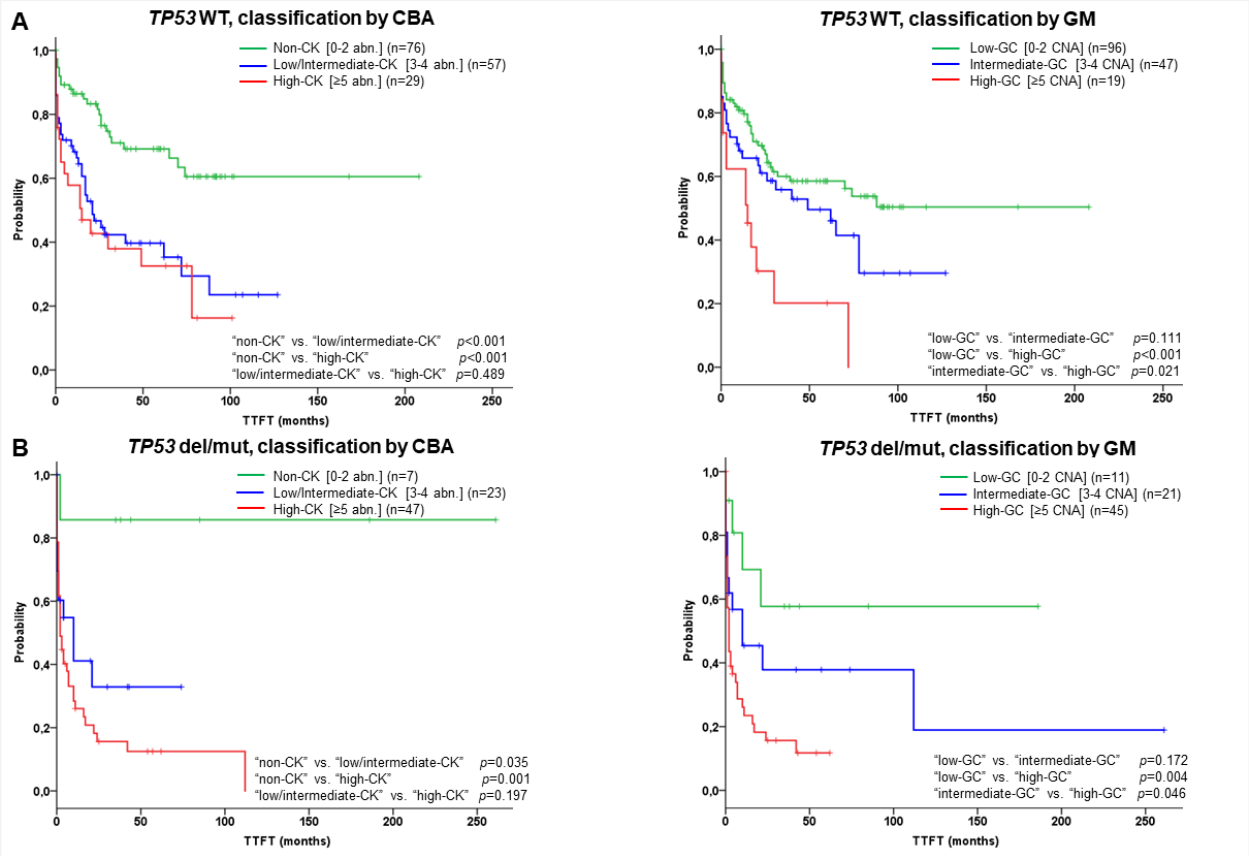

Figure S3. Effect on TTFT of risk categories defined by chromosome banding analysis and genomic microarrays in patients with abnormal *TP53* (deleted and/or mutated). Kaplan-Meier estimation for TTFT in risk categories defined by CBA (plots on the left) or GM (plots on the right) in patients with normal *TP53* (A) and in patients with deleted and/or mutated *TP53* (B).

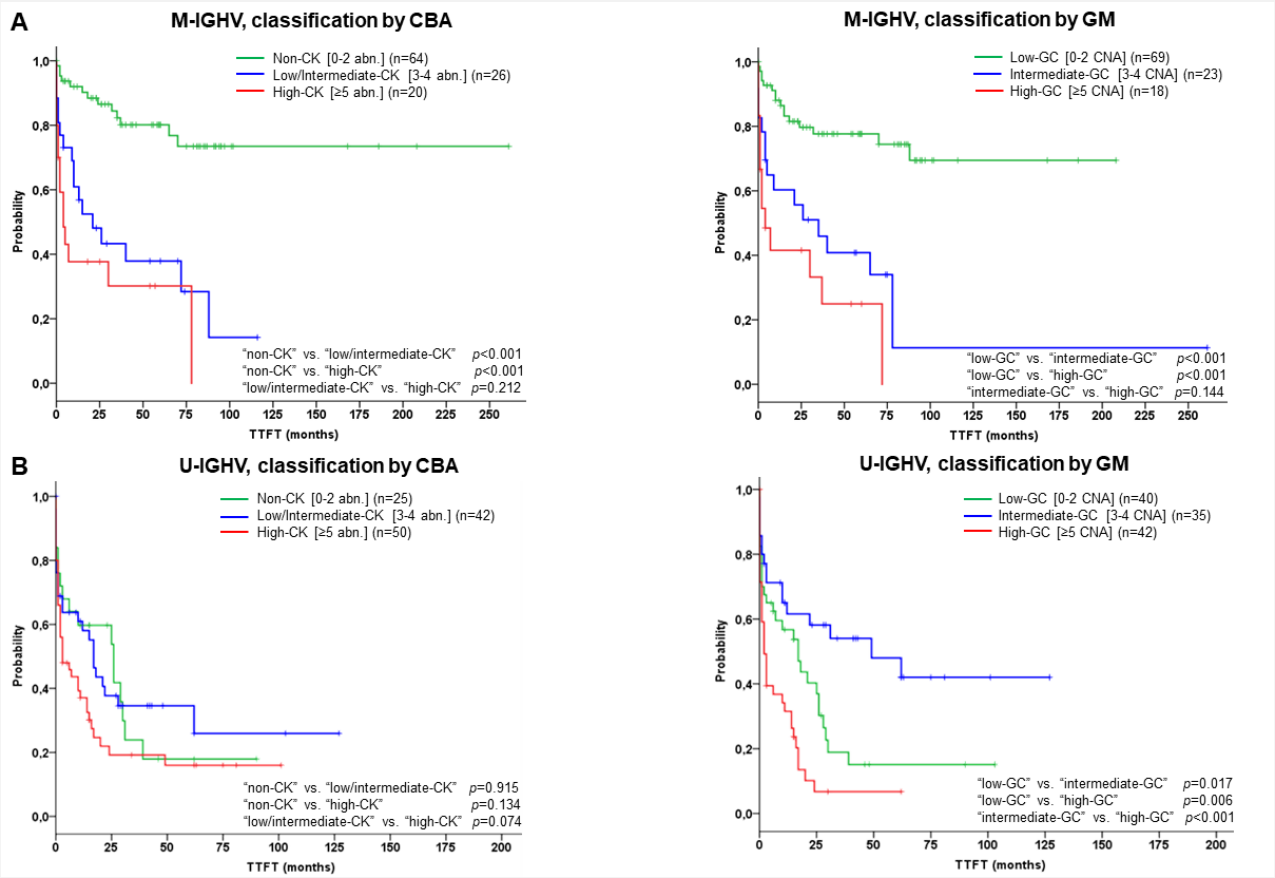

Figure S4. Effect on TTFT of risk categories defined by chromosome banding analysis and genomic microarrays in patients with M-IGHV or U-IGHV. Kaplan-Meier estimation for TTFT in risk categories defined by CBA (plots on the left) or GM (plots on the right) in patients with M-IGHV (A) and in patients with U-IGHV (B).

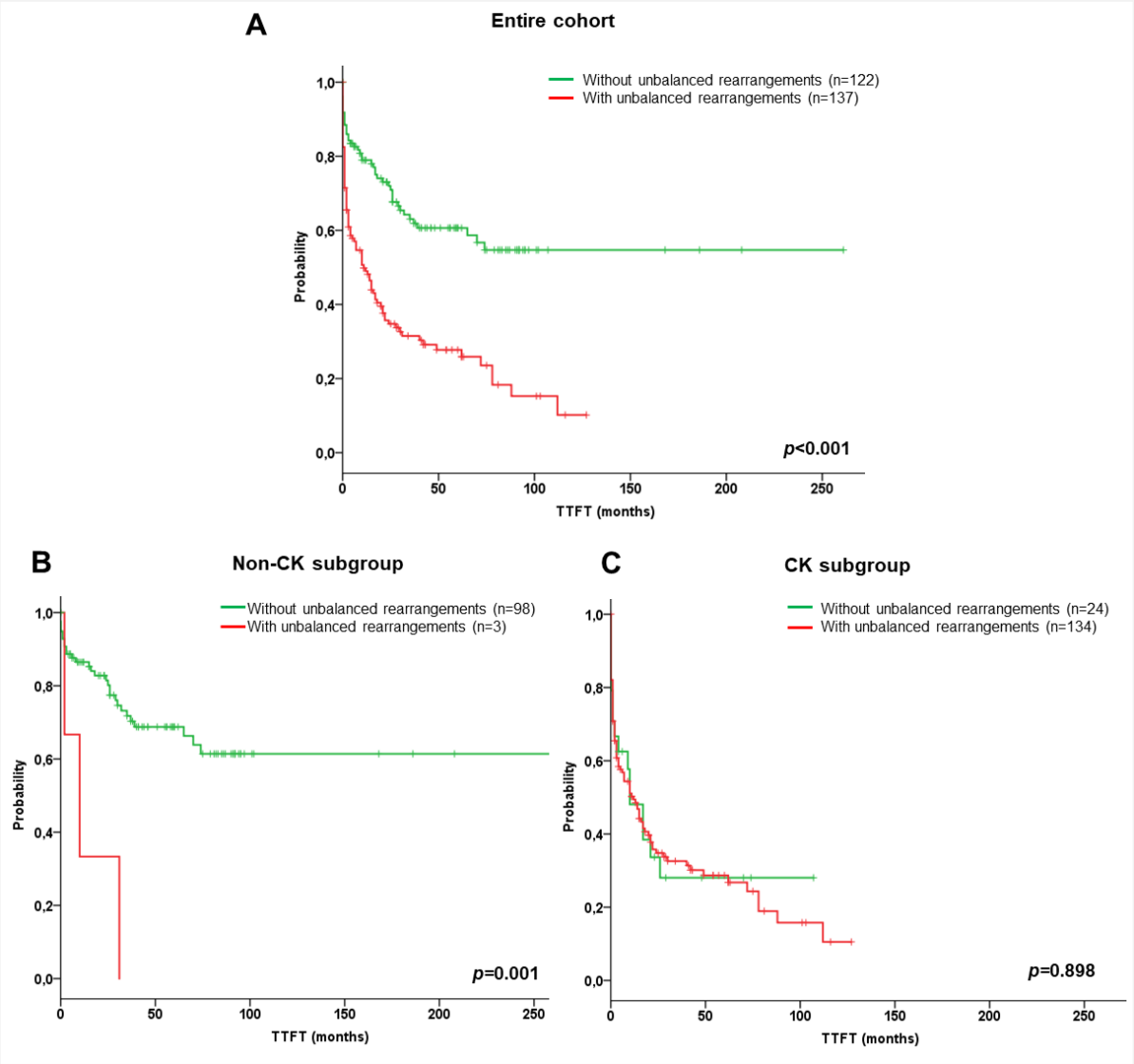

Figure S5. Effect on TTFT of unbalanced rearrangements detected by chromosome banding analysis in the entire cohort and within the non-CK and CK subgroups. Kaplan-Meier estimation for TTFT in patients with and without unbalanced rearrangements in the entire cohort (A) and in non-CK (B) and CK subgroups (C).

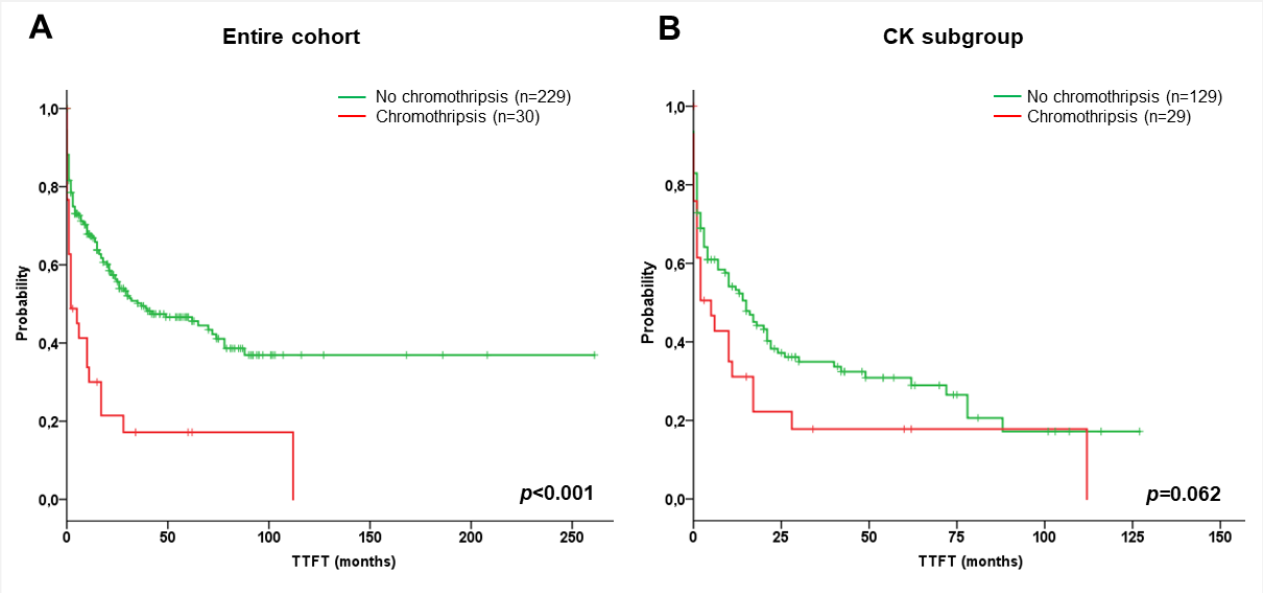

Figure S6. Effect on TTFT of chromothripsis in the entire cohort and within the CK subgroup. Kaplan-Meier estimation for TTFT in patients with and without chromothripsis in the entire cohort (A) and in CK subgroup (B). Survival plot for non-CK subgroup is not shown as only one case displayed chromothripsis.