CYTOGENETIC COMPLEXITY IN CHRONIC LYMPHOCYTIC LEUKEMIA: DEFINITIONS, ASSOCIATIONS WITH OTHER BIOMARKERS AND CLINICAL IMPACT

Supplemental Material

Supplemental Material includes detailed information regarding methodology as well as Supplemental Figures 1-7 and Supplemental Tables 1-6.

Chromosome Banding Analysis (CBA)

For metaphase induction, 10^6/ml peripheral blood mononuclear cells were cultured using two different protocols: phorbol-12-myristate-13-acetate (TPA) (n=2631, 50%, A) or immunostimulatory CpG-oligonucleotide DSP30 plus interleukin 2 (IL-2) (n=2659, 50%, B) (A) cells were cultured for 72 and 96 hours in RPMI 1640 medium supplemented by 20% fetal calf serum (FCS) with phorbol-12-myristate-13-acetate (TPA) at 50ng/mL; colcemid (0.01 µg/ml) was added 45 min before harvest; (ii) metaphases were obtained after cell culture in RPMI 1640 medium supplemented by 20% FCS in the presence of the immunostimulatory CpG-oligonucleotide DSP30 and interleukin 2 (IL-2) (200 U/mL); after 48-72 hours, colcemid (0.015 µg/ml), was added for another 4-24 hours before chromosome preparation. Hypotonic treatment was performed with 0.075 M KCl, fixation of chromosomes was accomplished with 3v methanol: 1v glacial acetic acid solution. Chromosome preparation and staining was done according to standard protocols. Karyotypes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN) 2016. Karyotypes obtained before 2016 were re-classified following ISCN 2016 recommendations[1].

Supplemental Table 1. Main clinicobiological features of the entire cohort versus patients included in multivariable analysis.

Feature	Entire cohort (n=5290)	Cases included in the MVa (n=2376)	P-value
Male	3302/5290, 62%	1541/2376, 65%	0.04
Median age (diagnosis)	64.6 years	64.1	0.62
MBL	383/4454, 9%	197/2376, 8%	0.66
Binet A	3030/4454, 68%	1633/2376, 68%	0.55
Binet B/C	1041/4454, 23%	546/2376, 25%	0.71
U-CLL	1514/3453, 44%	1034/2376, 14%	0.81
CK	794/5290, 15%	320/2376, 13%	0.08
***TP53*abs**	657/4968, 13%	275/2376, 12%	0.05
del(11q)	487/4500, 11%	322/2376, 14%	0.008
Trisomy 12	685/4500, 15%	377/2376, 16%	0.48
idel(13q)	1734/4500, 38%	883/2376, 37%	<0.26

MVa: Multivariable analysis; CK: complex karyotype, ≥3 aberrations; MBL: Monoclonal B-cell lymphocytosis, U-CLL: Unmutated IGHV genes, *TP53*abs: deletion of chromosome 17p and/or *TP53* mutation, del(11q): deletion of chromosome 11q, idel(13q) isolated deletion of chromosome 13q. Statistical significant level was defined as 0.008 following the Bonferroni correction for multiple testing.

Supplemental Table 2. Main clinicobiological features of patients analyzed with different stimulation protocols. Enrichement for all recurrent cytogenetic abnormalities was seen in cases analyzed with the CpG/IL-2 protocol, except for trisomy 12 (+12) where concordant results were obtained with both protocols.

Feature	TPA n=2630	CpG/IL-2 N=2659	P-value
Male gender	1624/2630, 62%	1677/2659, 63%	0.32
Median age (diagnosis)	64.5 years	64.3 years	0.14
MBL	383/2543, 15%*[1]	-	-
Binet A	1575/2543, 62%	1455/1911, 76%	<0.0001
Binet B/C	585/2543, 23%	456/1911, 24%	0.5
U-CLL	523/1178, 44%	990/2274, 44%	0.62
CK	286/2630, 11%	508/2659, 19%	<0.0001
Tested before treatment	2229/2436, 92%	2217/2431, 92%	0.7
*TP53*abs	229/2315, 10%	427/2652, 16%	<0.0001
del(11q)	190/2014, 9%	318/2485, 13%	0.0004
Trisomy 12	351/2014, 17%	356/2485, 14%	0.005
del(13q)	732/2014, 36%	1101/2485, 44%	<0.0001

CK: complex karyotype, ≥3 aberrations; MBL: Monoclonal B-cell lymphocytocis; U-CLL: Unmutated IGHV genes; *TP53*abs: deletion of chromosome 17p detected by FISH and/or *TP53* mutation; del(11q): deletion of chromosome 11q; del(13q): deletion of chromosome 13q detected by FISH; TPA: phorbol-12-myristate-13-acetate; CpG/IL-2: CpG-oligonucleotide DSP30 plus interleukin 2. Statistical significant level was defined as 0.008 following the Bonferroni correction for multiple testing. *[1]: All cases with MBL where analyzed with the TPA protocol.

Supplemental Table 3. Clinicobiological profiles of patients carrying complex karyotypes (≥3 aberrations) detected by different stimulation protocols.

Feature	CK with TPA n=286	CK with CpG/IL-2 n=508	P-value
Male gender	189/286, 66%	333/508, 66%	0.87
Median age (diagnosis)	63.9 years	65.6 years	0.28
MBL	30/259, 12%	-	-
Binet A	142/259, 55%	245/382, 64%	0.02
Binet B/C	87/259, 33%	137/382, 36%	0.55
U-CLL	87/142, 62%	240/372, 64%	0.49
*TP53*abs	93/257, 36%	227/507, 44%	0.02
del(11q)	55/211, 26%	95/411, 23%	0.41
Trisomy 12	65/211, 31%	69/411, 17%	<0.0001
del(13q)	58/211, 28%	86/411, 21%	0.07

CK: complex karyotype, ≥3 aberrations; MBL: Monoclonal B-cell lymphocytocis; U-CLL: Unmutated IGHV genes; *TP53*abs: deletion of chromosome 17p detected by FISH and/or *TP53* mutation; del(11q): deletion of chromosome 11q determined by FISH; del(13q): deletion of chromosome 13q detected by FISH; TPA: phorbol-12-myristate-13-acetate; CpG/IL-2: CpG-oligonucleotide DSP30 and interleukin 2. Statistical significant level was defined as 0.008 following the Bonferroni correction for multiple testing.

Supplemental Table 4. Univariable and multivariable analysis for Overall Survival (OS).

Parameter	Univariable analysis (n=5095)			Multivariable analysis (n=2376)		
	HR	95% CI	p-value	HR	95% CI	p-value
Male	1.202	1.070-1.350	0.001	1.123	0.946-1.338	0.18
CK (≥3abs)	2.059	1.789-2.370	<0.001	1.578	1.267-1.966	<0.0001
idel(13q)	0.894	0.792-1.010	0.07	-	-	-
Trisomy 12	1.310	1.125-1.525	<0.001	1.139	0.926-1.401	0.21
del(11q)	1.942	1.659-2.273	<0.001	1.109	0.883-1.393	0.37
***TP53*abs**	2.904	2.517-3.350	<0.001	2.183	1.761-2.707	<0.001
U-CLL	2.851	2.467-3.295	<0.001	2.371	1.973-2.850	<0.001
Binet B/C	2.036	1.793-2.312	<0.001	1.585	1.324-1.897	<0.001

CK: complex karyotype; U-CLL: Unmutated IGHV genes; *TP53*abs: deletion of chromosome 17p detected by FISH and/or *TP53* mutation; del(11q): deletion of chromosome 11q determined by FISH; idel(13q): isolated deletion of chromosome 13q detected by FISH.

Supplemental Table 5. Main clinicobiological features of patients with complex karyotype (CK) carrying +12+19 plus another trisomy or structural abnormality.

	+12+19+other trisomy (n=43)	+12+19+structural (n=38)	P-value
Age at diagnosis (median)	64.6	56.6	0.7
Male	33/43, 77%	30/38, 79%	0.81
Binet B/C	8/39, 21%	6/24, 25%	0.68
M-CLL	19/21, 90%	32/34, 94%	0.61
del(13q)	39/43, 91%	30/35, 86%	0.49
del(11q)	0/39, 0%	1/36, 0.3%	0.29
*TP53*abs	2/41, 0.5%	1/37, 0.3%	0.61

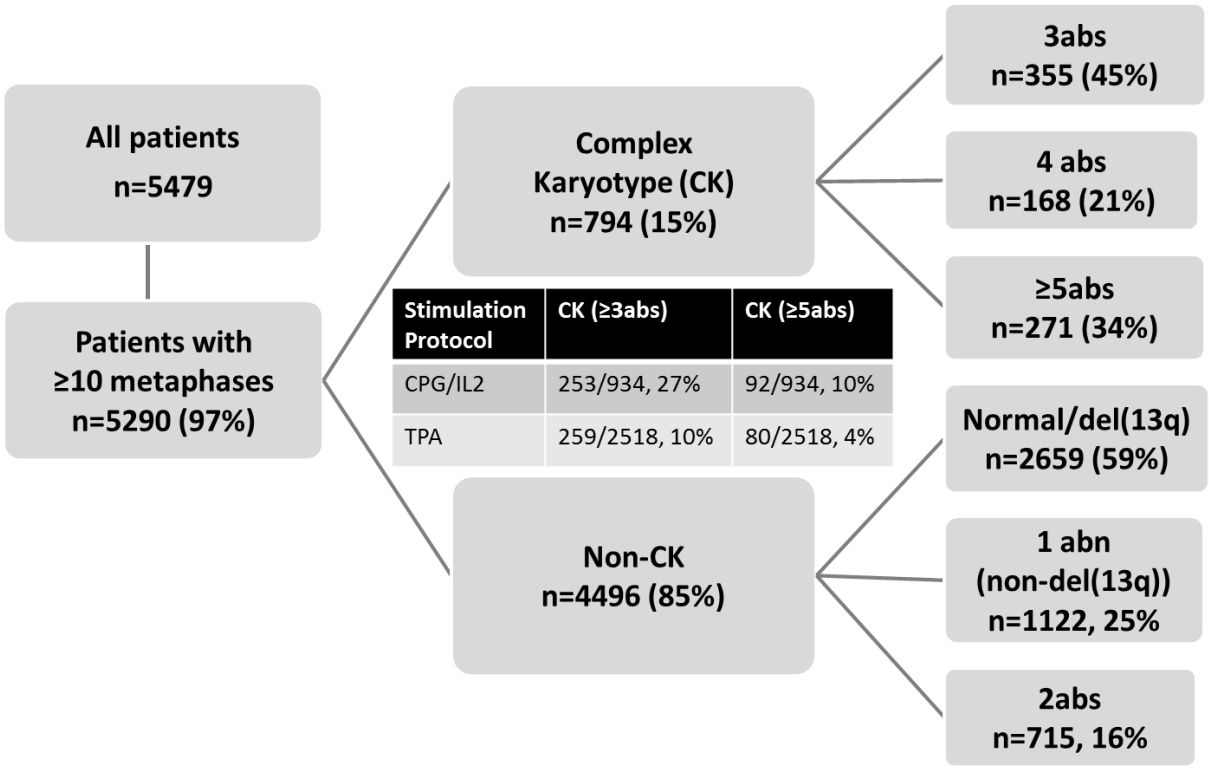
CK: complex karyotype; M-CLL: Mutated IGHV genes; *TP53*abs: deletion of chromosome 17p detected by FISH and/or *TP53* mutation; del(11q): deletion of chromosome 11q detected by FISH; del(13q): deletion of chromosome 13q detected by FISH. Statistical significant level was defined as 0.008 following the Bonferroni correction for multiple testing.

Supplemental Table 6. Main clinicobiological features of patients with complex karyotype (CK) carrying 3 and 4 aberrations (low-CK and intermediate-CK, respectively).

Feature	Low-CK N=365	intremediate-CK N=178	Pvalue
Male gender	238/365, 65%	113/178, 63%	0.69
Median age (diagnosis)	63.9 years	65.6 years	0.75
MBL	23/285, 8%	4/127, 3%	0.06
Binet A	176/285, 62%	87/127, 66%	0.18
Binet B/C	86/285, 30%	36/127, 31%	0.7
U-CLL	132/240, 55%	69/111, 62%	0.2
*TP53*abs	89/341, 26%	62/160, 39%	0.004
del(11q)	78/289, 27%	38/124, 30%	0.44
Trisomy 12	91/289, 31%	26/124, 21%	0.03
del(13q)	88/289, 30%	19/124, 15%	0.001

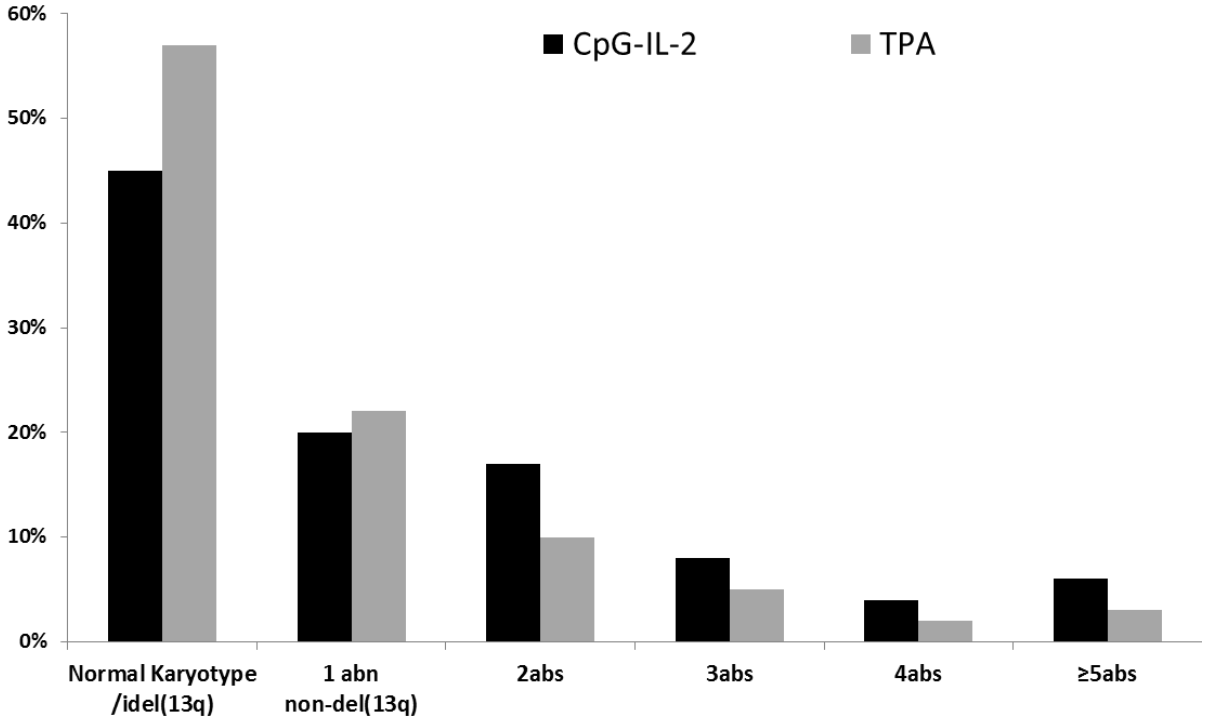
low-CK: 3 aberrations; intremediate-CK; 4aberrations; U-CLL: Unmutated IGHV genes; *TP53*abs: deletion of chromosome 17p detected by FISH and/or *TP53* mutation; del(11q): deletion of chromosome 11q detected by FISH; del(13q): deletion of chromosome 13q detected by FISH. Statistical significant level was defined as 0.008 following the Bonferroni correction for multiple testing.

Supplemental Figure 1. Overview of the cohort and the detected cytogenetic aberrations



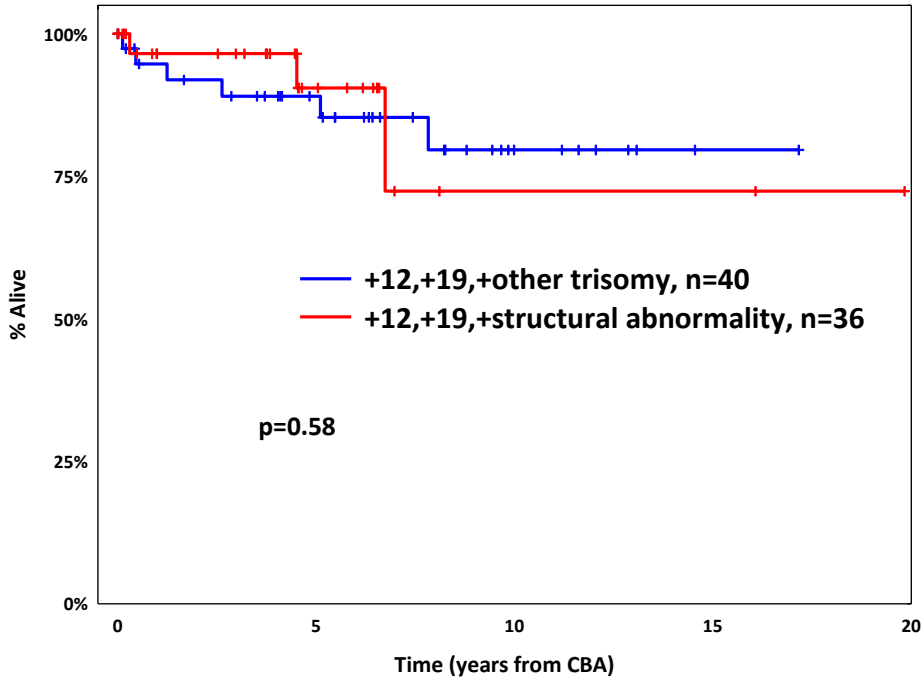
CPG/IL2: CpG-oligonucleotide DSP30 and interleukin 2, TPA: phorbol-12-myristate-13-acetate, abs: aberrations, abn: abnormality, del(13q): deletion of chromosome 13q, Normal: 46,XX or 46,XY.

Supplemental Figure 2. Number of aberrations detected depending on the used stimulation protocol.

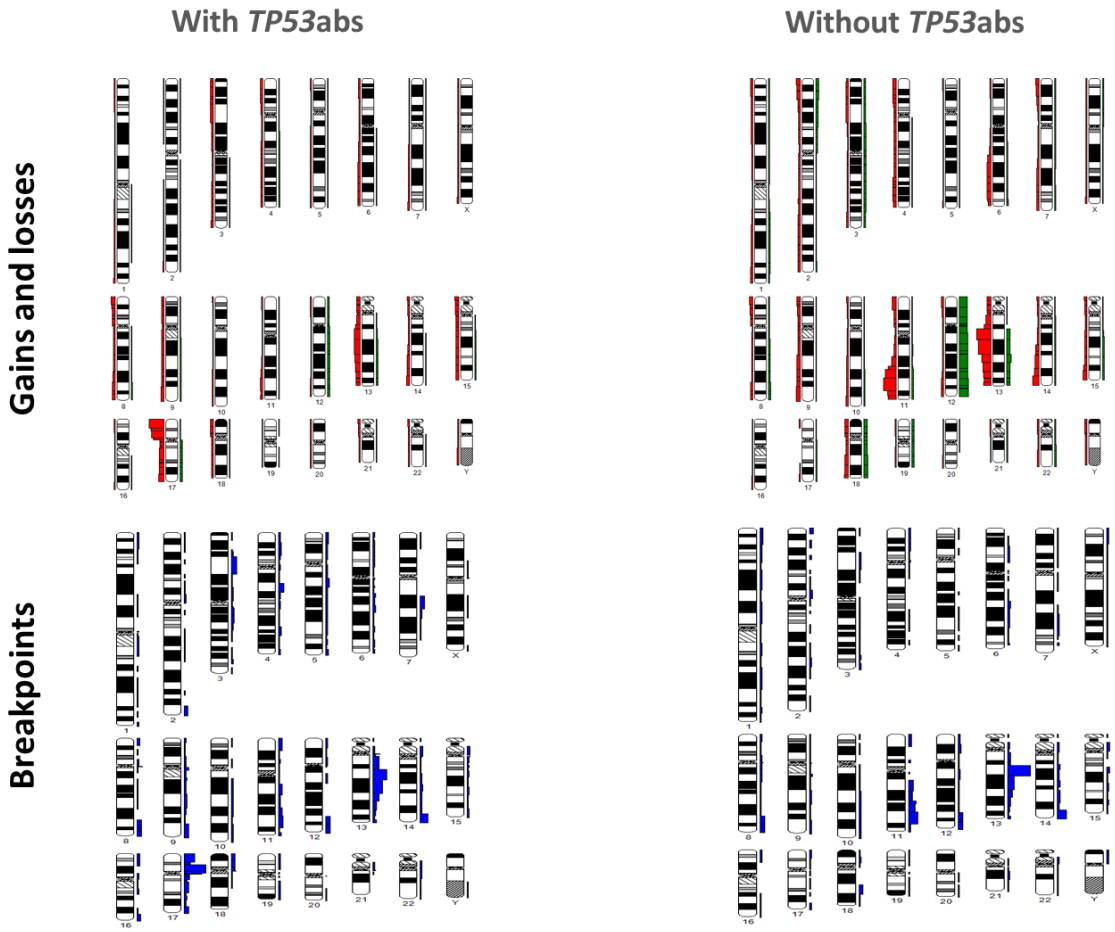


CPG/IL2: CpG-oligonucleotide DSP30 and interleukin 2, TPA: phorbol-12-myristate-13-acetate, abs: aberrations, abn: abnormality, del(13q): deletion of chromosome 13q,

Supplemental Figure 3. Kaplan Meier curves for Overall Survival (OS) for cases with complex karyotype (CK) carrying +12+19. No difference between cases carrying extra trisomies versus those carrying extra structural abnormalities.

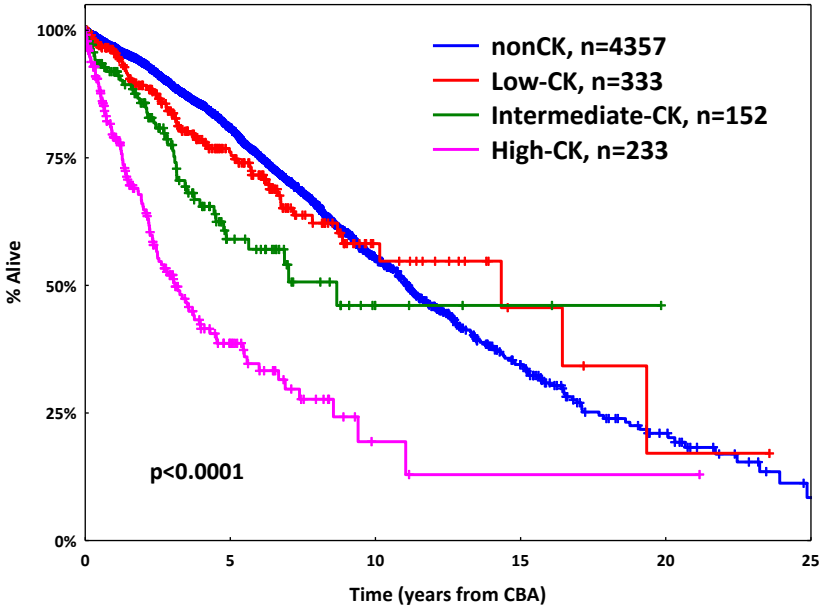


Supplemental Figure 4. Distribution of chromosome gains and losses as well as chromosomal breakpoints in the karyotypes of cases with ≥5 aberrations (High-CK). Right high-CK cases with *TP53*abs (deletion of chromosome 17p and/or *TP53* mutation), left: high-CK cases without *TP53*abs (gains: right green bars, losses: left red bars, translocation breakpoints: blue right bars adjacent to chromosomes). Ideograms were prepared with the CYDAS software package, freely available at www.cydas.org.

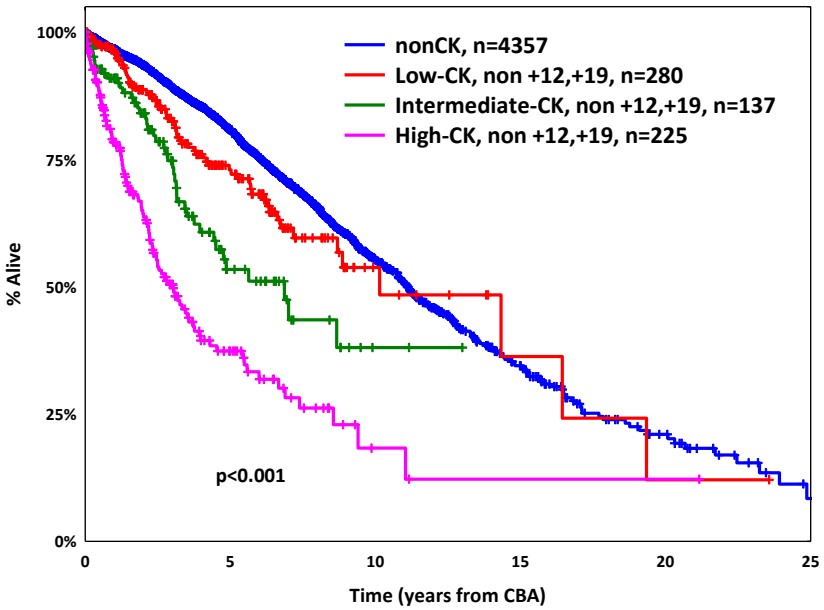


Supplemental Figure 5. Kaplan Meier curves for Overall Survival (OS). A) Patients with complex karyotype (CK) and 3, 4 and ≥5 aberrations (low-CK, intermediate-CK and high-CK respectively) as well as all the remaining nonCK CLL; B) patients with low-CK, intermediate-CK and high-CK well as all the remaining nonCK CLL after the exclusion of patients with CK carrying +12,+19.

A

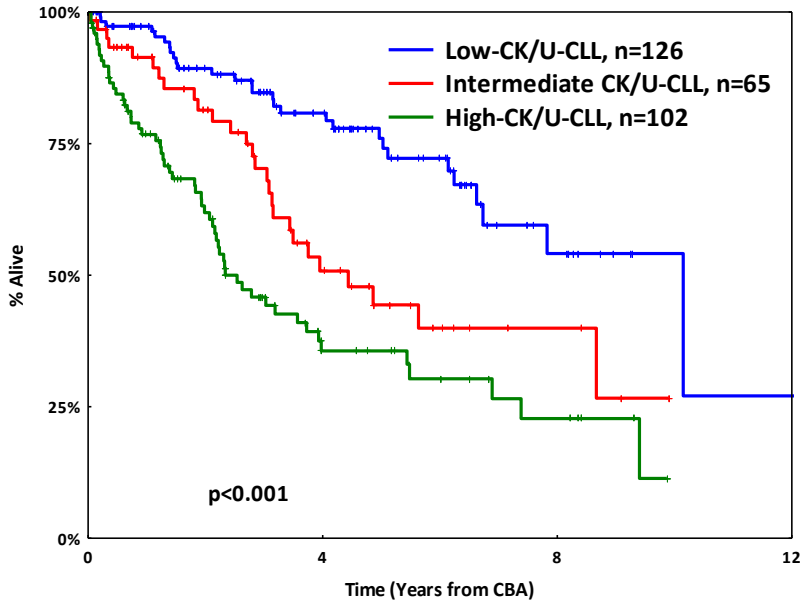


B



Supplemental Figure 6. Kaplan Meier curves for Overall Survival (OS) for patients with complex karyotype (CK) and 3, 4 and ≥5 aberrations (low-CK, intermediate-CK and high-CK respectively). (A) CLL with unmutated IGHV genes (U-CLL); (B) CLL with mutated IGHV genes (M-CLL).

A



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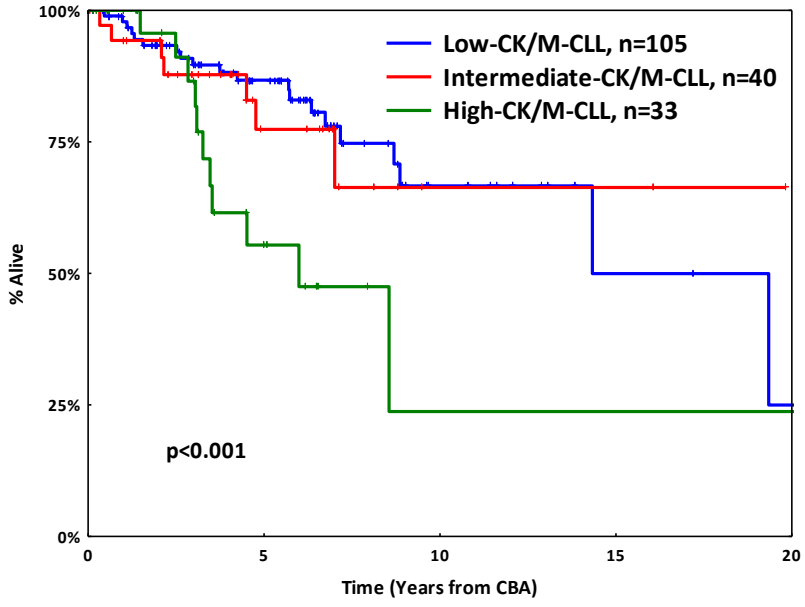
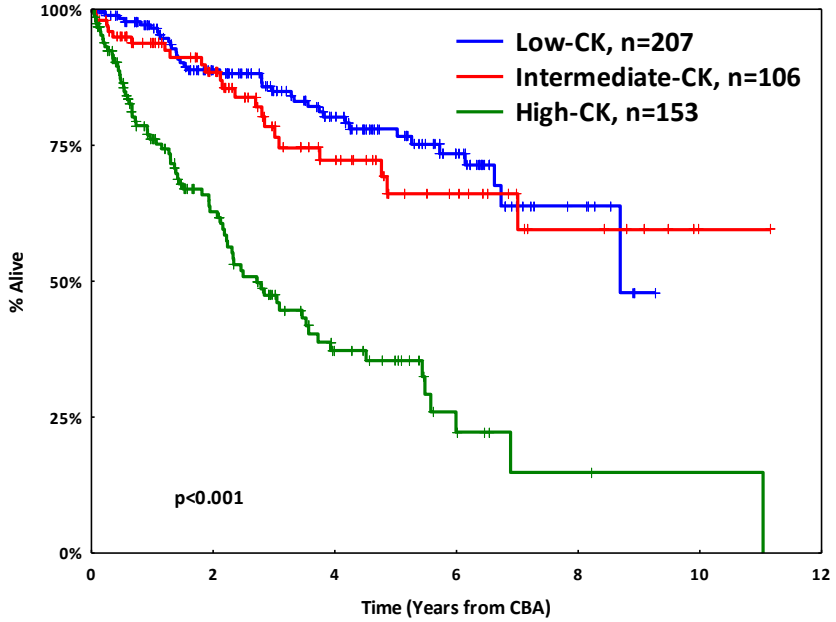


Supplemental Figure 7. Kaplan Meier curves for Overall Survival (OS) for patients with complex karyotype (CK) and 3, 4 and ≥5 aberrations (low-CK, intermediate-CK and high-CK respectively). (A) Patients analyzed with CpG-oligonucleotide DSP30 and interleukin 2 (CpG/IL-2); Patients analyzed with phorbol-12-myristate-13-acetate (TPA).

A



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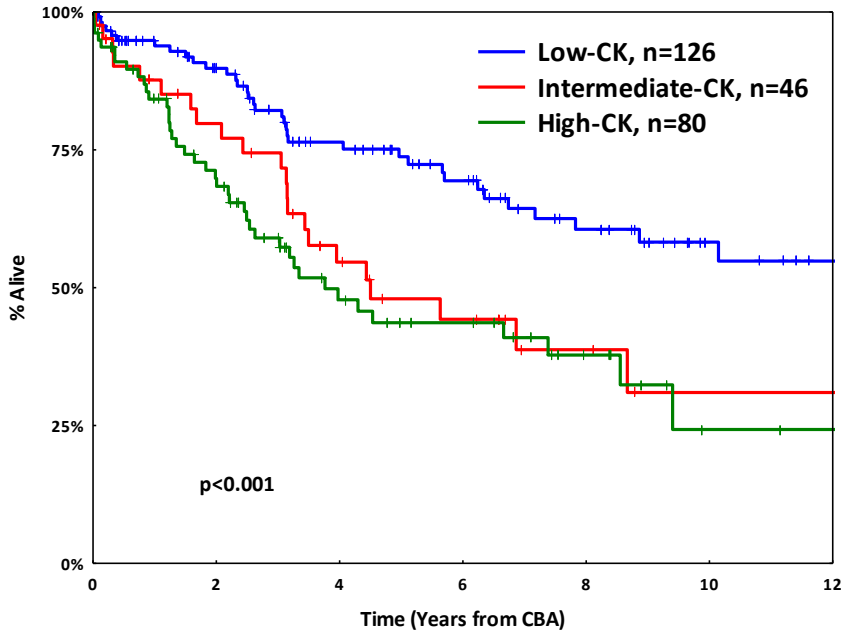

1. Nomenclature ISCoHC. ISCN : an international system for human cytogenomic nomenclature (2016): Basel ; New York : Karger; 2016.