Additional trisomies amongst patients with chronic lymphocytic leukemia carrying trisomy 12: the accompanying chromosome makes a difference

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Supplementary material

Additional trisomies amongst patients with chronic lymphocytic leukemia carrying trisomy 12: the accompanying chromosome makes a difference

The Supplementary material contains information regarding the patient series and the methodologies utilized.

Classic Cytogenetic and FISH analysis

For metaphase induction, 10^6 per mL peripheral blood mononuclear cells were cultured using two different protocols. More specifically, until 2008, cells were cultured for 72 and 96 hours in RPMI 1640 medium with 20% fetal calf serum and phorbol-12-myristate-13-acetate (TPA) at 50ng/mL; colcemid (0.01 μg/ml) was added 45 min before harvest. Since 2008, metaphases were obtained after culturing in RPMI 1640 medium with 20% fetal calf serum in the presence of the immunostimulatory CpG-oligonucleotide DSP30 and interleukin 2 (IL-2) (200 U/mL); after 48 hours, colcemid (0.015 μg/ml), was added for an additional 24 hours prior to the preparation of chromosomes. Hypotonic treatment was performed using 0.075 M KCl, and fixation of the chromosomes was accomplished using a methanol:glacial acetic acid (3:1) fixative solution. Chromosome preparation and staining was performed according to standard protocols. A minimum of 15 mitotic cells were examined and karyotypes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN) 2009. Karyotypes obtained before 2009, were re-classified following the ISCN from 2013.

Interphase *fluorescence in situ hybridization* (FISH) analysis was conducted using probes to detect the recurrent genomic aberrations in CLL. More specifically probes were used to detect (del)11q23 (LSI ATM and CEP11), del(13q14) (D13S319 and LSI13q34) for del(17p13) (LSI TP53 and CEP17). For trisomy 12 only CEP12 was used.

Microarray single nucleotide polymorphism analysis (250K Affymetrix arrays)

SNP-array experiments were performed according to the standard protocols for Affymetrix GeneChip® Mapping NspI-250K arrays and as previously described[1].

Analysis of gene mutations

Mutational screening was performed for the following genes: *NOTCH1*: entire exon 34 or targeted analysis for del7544-45/p.P2514Rfs*4; *TP53*: exons 4-8 as previously described[2].

CD38 expression

CD38 expression was assessed with flow-cytometry (threshold for positivity: 30%).

PCR amplification and sequence analysis of IGHV-IGHD-IGHJ rearrangements

PCR amplification and sequence analysis of IGHV-IGHD-IGHJ rearrangements were performed on either genomic DNA (gDNA) or complementary DNA (cDNA), as previously described or following the BIOMED-2 protocol[3]. PCR amplicons were subjected to direct sequencing on both strands. Sequence data were analyzed using the IMGT® databases and the IMGT/V-QUEST tool (http://www.imgt.org). Only productive rearrangements were evaluated. Output data from IMGT/V-QUEST for all productive IGHV-IGHD-IGHJ rearrangements were parsed, reorganized, and exported to a spreadsheet through the use of computer programming. Information was extracted regarding IG gene repertoires, VH CDR3 length and amino acid sequence and the % germline identity.

Statistical analysis

Descriptive analysis included frequency distributions for all the categorical variables. Associations regarding categorical variables were assessed using the Chi-square or Fisher's exact test for independence, in case the evaluated group included less than 20 cases. Overall survival (OS) was calculated using the date of diagnosis and the date of death or last follow-up. Kaplan Meier survival analysis and the log-rank test were used to assess differences in OS

between patient subgroups for different variables. All tests were two sided and significance was defined as a p value less than 0.05. Statistical analysis was performed using the Statistica Software 10·0 (StatSoftInc, Tulsa,OK) and R-3.2.1 programming language.

Supplemental Table 1. +12 CLL carrying additional trisomies.

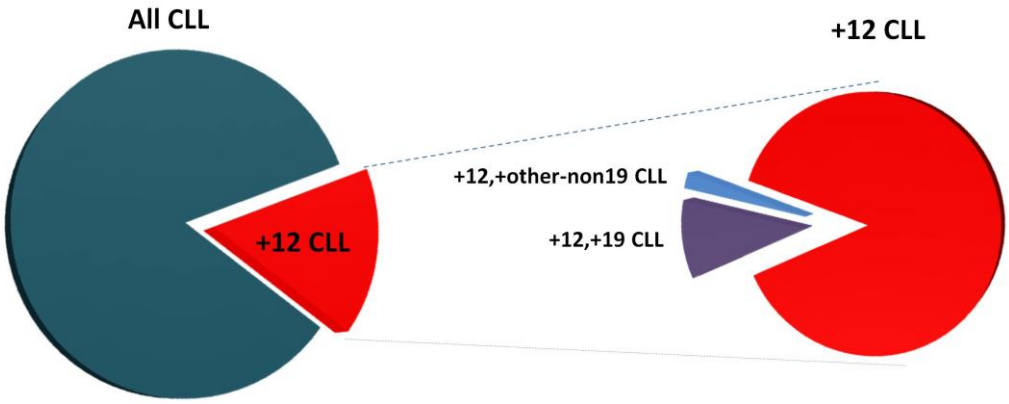
Case	Classic cytogenetic analysis
+12+19 CLL	
1	48,XY,+12,+19[16]
2	49,XY,+12,+18,+19[4]/49,XY,idem,del(13)(q14)[2]/46,XY[10]
3	49,XX,+12,+18,+19[18]
4	48,XY,+12,+19[18]
5	48,XY,+12,+19[5]/46,XY[10]
6	50,XY,+12,+18,+19,+22[2]/46,XY[18]
7	50,XY,+12,+18,+19,+22[2]/46,XY[38]
8	49,XY,+12,+18,+19[4]/46,XY[16]
9	49,XY,+12,+18,+19[6]/46,XY[14]
10	50,XY,+8,+12,+18,+19 [13]/50,idem,?del(3)(p2?2)[2]
11	49,XY,+12,+18,+19[18]/46,XY[2]
12	47,X,-Y,+12,+19[6]/46,XY[14]
13	48,XX,+12,+19[20]
14	49,XX,+12,+19,+22[20]
15	48,XY,+12,+18[3]/49,idem,+19[11]/46,XY[7]
16	49,+12,+18,+19[15]/46,XY[4]
17	48,XY,+12,+19[6]/46,XY[13]
18	48,XY,+12,+19[4]/48,idem,t(6;14)(p2?3;q2?3)[6]/46,XY[14]
19	50,XY,+12,+18,+19,+22[6]/46,XY[14]
20	49,XY,+12,+18,+19[8]/46,XY[12]
21	49,XY,+12,+18,+19[15]/46,XY[5]
22	49,XY,+12,+18,+19[6]/46,XY[14]
23	49,XY,+12,+18,+19[10]/46,XY[9]
24	48,XY,+12,+19[8]/46,XY[12]
25	47,XY,+12[2]/48,idem,+19[14]/46,XY[4]
26	+12,+19*[1]
27	+12,+18,+19*[1]
28	+12,+18,+19*[1]
29	+12,+19*[1]
30	+12,+19*[1]
31	46,XY,del(13q14)[6]/49,XY,+12,+18,+19[4]/46,XY[3]
32	48,XY,+12,+19[9]/46,XY[11]
33	49,XY,+12,+18,+19[7]/46,XY[13]
34	49,XY,+12,+19,+mar[2]/46,XY[18]
35	49,XY,+12,+18,+19[6]/46,XY[9]
36	48,XX,+12,+19[17]/46,XX[20]

37	49,XX,+12,+18,+19[3]/46,XX[17]
38	49,XX,+12,+18,+19[5]/46,XX[15]
39	47,X,-X,+12,+19[7]/46,XX[13]
40	48,XY,+12,+19[15]/46,XY[5]
41	49,XY,+12,+18,+19[10]/46,XY[11]
42	48,XX,+12,+19[1]/46,XX[22]*[2]
43	49,XY,+12,+18,+19[6]/46,XY[9]
44	50,XY,+Y,t(10;10)(q25;q23),+12,+18, +19[4]/46,XY[12]
45	48,XY,+12,+19[3]/46,XY[17]
46	48,XX,+12,+19[3]/46,XY[17]
47	48,XY,+12,+19[3]/46,XY[17]
48	50,XY,+8,+12,del(13)(q11q14),t(13;14)(q14;q24),+18,+19[20]
49	49,XY,+12,+18,+19[14]/46,XY[6]
50	49,XY,+12,+18,+19[3]/46,XY[17]
51	49,XY,+12,+18,+19[8]46,XY[22]
52	49,XY,t(9;13)(q?32;q?14),+12,+18,+19 [15]/46,XY[3]
53	49,XX,+12,+18,+19[9]/46,XX[11]
54	49,XY,+12,+18,+19[17]/46,XY[3]
55	49,XY,+12,+18,+19,?del(9)(q?),?add(13)(q?)[7]/46,XY[13]
56	49,XX,+12,+18,+19[17]/46,XX[3]
57	49,XY,+12,+18,+19[20]
58	49,XY,+12,?del(13)(q14q22),+18,+19[8]/46,XY[12]
59	49,XY,+12,+18,+19 [9]49,idem,t(13;14)(q14;q24)[2]/46,XY[9]
60	48,XY,?11,t(12;13)(p11;q?12),+12+19[10]/46,XY[10]
61	49,XY,+12,+18,+19[8]/46,XY[12]
62	49,XX,+12,+18,+19[10]/46,XX[10]
63	48,XX,+12,+19[7]/46,XX[13]
64	48,XY,+12,+19[9]/46,XY[11]
65	48,XY,+12,+19[3]/46,XY[16]
66	49,XY,+12,+18,+19[20]
67	49,XY,+12,+13,+18,+19[17]/49,idem,del(17)(p11)[2]
68	48,XY,+12,+19[20]
+12+other-non19 CLL	
69	48,XX,+12,+21[7]/46,XX [13]
70	48,XY,+12,+22[6]/46,XY[14]
71	48,XY,+3,+12[9]/46,XY[11]
72	+12,+18*[1]
73	48,XY,+8,+12[10]/46,XY[10]
74	49,XY,+3,+12,+18[7]/46,XY[15]
75	47,X,-Y,+3,+12[3]/46,XY[17]

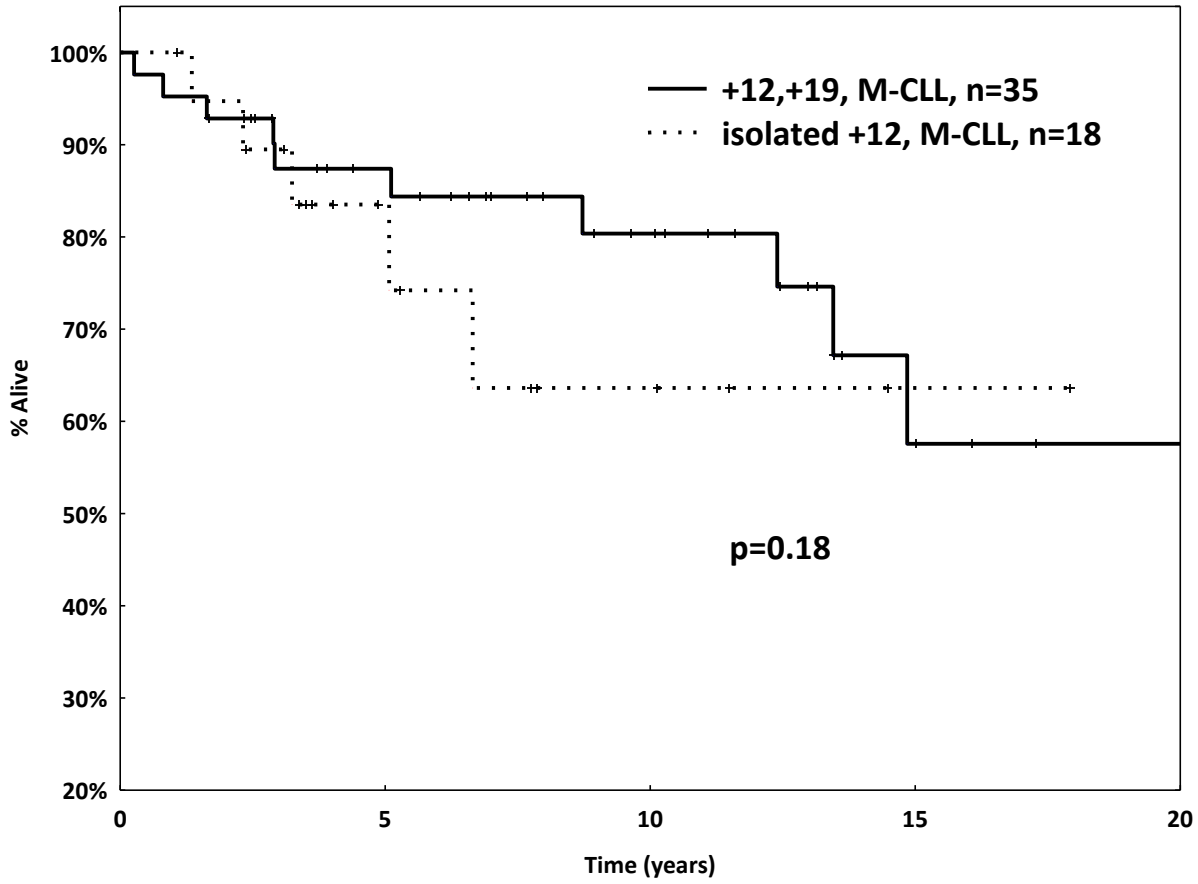
76	49,XY,+12,+18,+22[6]/46,XY[14]
77	48,XY,+3,+12[8]/46,XY[9]
78	48,XX,+3,+12[3]/46,XX[17]
79	48,XY,+3,+12[2]/46,XY[18]
80	48,XX,+12,+18 [4]46,XX[1]
81	49,XX,+12,+16,+18[7]/46,XX[22]
82	48,XY,+12,+21[22]/46,XY[7]
83	48,XY,+8,+12[16]/46,XY[6]
84	48,XY,+12,+22[6]/46,XY[15]
85	48,XY,+3,+12[9]/46,XY[11]
86	49,XY,+12,+18,+22[6]/46,XY[14]

*1: according to high-density 250K single nucleotide polymorphism analysis
*[2]: verified by FISH analysis

Supplemental Figure 1. Left: proportion of +12 CLL within the whole CLL cohort. Right: Proportion of +12,+19 and +12,+othet-non19 CLL within +12 CLL.



Supplemental Figure 2. Kaplan-Meier curves for overall survival (OS) within cases carrying mutated IGHV genes (M-CLL). No significant difference between cases with +12,+19 and those with isolated +12 is observed, however this could be due to low numbers.



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