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*Total number of authors:*  
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LUND UNIVERSITY

PO Box 117  
221 00 Lund  
+46 46-222 00 00

# CO-EXISTENCE OF TRISOMIES OF CHROMOSOMES 12 AND 19 IN CHRONIC LYMPHOCYTIC LEUKEMIA OCCURS EXCLUSIVELY IN THE RARE IgG-POSITIVE VARIANT

*To the Editor:*

Most cases of chronic lymphocytic leukemia (CLL) express surface IgM and surface IgD at levels lower than normal B cells. IgG-positive CLL is a relatively rare variant of CLL (frequency 6-10%), whose origin and ontogenetic relationship to the common IgM/IgD variant remains unknown.

Important biological and prognostic information in CLL is obtained by cytogenetic analysis. In particular, certain recurrent cytogenetic aberrations in CLL have been associated with different clinical outcomes; for instance, abnormalities of chromosome 17p are associated with poor prognosis, whereas deletions of chromosome 13q are associated with a favorable prognosis (1). Trisomy 12 is a recurrent chromosomal aberration occurring in 10-20% of CLL that has been associated with an intermediate risk (1-2). A small percentage, between 1.6% and 5%, of patients with trisomy 12 also have trisomy 19 and may constitute a distinct subgroup (3-5). This cytogenetic profile was recently shown to be associated with somatically mutated immunoglobulin heavy variable (IGHV) genes (4-5). We here significantly extend previous observations by reporting 20 additional cases with co-existing trisomies of chromosomes 12 and 19, with 18/20 cases expressing mutated IGHV genes and 15/20 cases also exhibiting trisomy 18. Importantly, we also demonstrate that the distinct karyotypic profile +12,+19 is significantly associated with very high CD38 expression and occurs exclusively in isotype-switched sIgG positive CLL.

The present study included 968 CLL cases from collaborating institutes in Greece, the UK and Sweden with available karyotypic data from: (i) classic G-banding cytogenetics (n=929); (ii) interphase FISH analysis (n=766)\_using probes for chromosome 12 (CEP12, Vysis) and chromosome 13 (LSI 13q14/D13S319 and LSI 13q34/13S25 Vysis); and, (iii) high-density 250K SNP-arrays (39 CLL cases from Sweden, all with trisomy 12). The group of UK cases without trisomy 12 includes only those cases that were confirmed to be non trisomic by both classic G-banding cytogenetics and FISH, therefore does not represent the entire CLL patient population without trisomy 12. Within this cohort, we identified a subgroup of 20 cases (2.1%) with co-

existence of trisomies of chromosomes 12 and 19 (“+12+19” subgroup); the +12+19 profile was detected by G-banding cytogenetics or SNP-arrays in 15 and 5 cases, respectively (Table 1). A further 313/968 cases (32.3%) were found to exhibit trisomy 12 yet without co-existing trisomy 19 (“+12 non +19”); and, finally, 635 cases did not show trisomy 12 (“no +12”). The sole criterion for assignment to the two latter subgroups was the presence or absence of trisomy 12, regardless of any other cytogenetic/genomic aberrations (Figure 1).

Demographic, clinical and biological data for the “+12+19” cases are summarized in Table 1. Interestingly, 15/20 cases exhibiting this cytogenetic profile were also found to carry trisomy 18. Prompted by these findings, we queried the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (<http://cgap.nci.nih.gov/Chromosomes/Mitelman>) for CLL cases with trisomy 19 and retrieved 43 hits (Supplemental Table 1). Among these, 36/43 (83.7%) and 22/43 cases (51.2%) displayed a coexisting trisomy 12 or trisomy 18, respectively; finally, 19/43 cases (44.2%) had a +12,+18,+19 karyotype. A similar query for CLL cases with trisomy 18 retrieved 62 hits (Supplemental Table 2): 22/62 +18 cases displayed a co-existing trisomy 12, and of these 18 were common hits in both queries, i.e. exhibited the +12+18+19 profile. On these grounds, we propose that the co-existence of trisomies 12 and 19 may define a distinct cytogenetic subgroup of CLL, while the emergence of trisomy 18 in such cases likely represents a clonal evolution event, whose high frequency indicates selection due to a clonal advantage.

IGHV usage across the “+12+19” subgroup was heterogeneous, although 4/20 cases (20%) were found to utilize the IGHV3-7 gene. No cases with stereotyped heavy complementarity-determining region 3 (VH CDR3) sequences were recognized. Following the 98% cut-off value of identity to the germline, 18/20 cases (90%) were considered as IGHV-mutated, whereas the remainder (2/20 cases, 10%) were considered as IGHV-unmutated, albeit with less than 100% identity to the germline. Statistically significant differences were identified between the three groups regarding mutational status, with a far lower proportion of IGHV-unmutated cases in the “+12+19” subgroup compared to either the “+12 non +19” or “no +12” subgroups (Table 2). These findings corroborate a previous report that trisomy 19 is associated with mutated IGHV genes (5). In addition, they strongly indicate that “+12+19” cases should be considered as distinct from the “+12 non +19” cases, since overall, they exhibit very different IGHV gene mutational profiles.

Ample evidence that the “+12+19” subgroup is distinctive was obtained by examining the immunophenotypic features of the leukemic clones. We found that the great majority of CLL cases of this subgroup exhibit very high CD38 expression, even when compared to “+12

isolated” cases, who have previously been associated with increased CD38 expression (2). In particular, with a 30% cut-off value for positivity, 16/19 cases of the present series with available data (84.2%) were considered as CD38-positive; this was significantly higher ( $p < 0.001$ ) than either the “+12 non +19” or the “no +12” cases (Table 2). Additionally, the “+12+19” subgroup was distinctive for a very high median percentage of CD38+ cells among positive cases (Table 2). Similar results were also obtained even when using other cut-off values for positivity regarding CD38 expression, i.e. 7% or 20%.

From an immunophenotypic perspective, however, perhaps even more distinctive was the fact that all 15 cases with available FACS data were found to express surface IgG without any evidence for a population of IgM/IgD expressing cells. The remaining five cases were shown to exclusively carry switched, tumor-derived IGHV-D-J-C  $\gamma$  transcripts (RT-PCR using appropriate IGHV sense and IGHC antisense primers). Therefore, the present study documents for the first time that the “+12+19” cytogenetic profile is exclusive to the rare IgG-switched variant of CLL. The fact that the co-existing trisomies are not detected in the entire neoplastic clone but only in a proportion of CLL cells, as evidenced by the consistent finding of a variable number of normal metaphases (Table 1), might be taken as indirect evidence for a secondary event occurring in a cell which has already undergone class switch recombination (although, in principle, normal metaphases could indicate the presence of residual healthy cells). Furthermore, given the low frequency both of trisomy 19 and sIgG expression in CLL, we argue that their simultaneous occurrence is unlikely to be fortuitous.

Recently, trisomy 12 was reported to be associated with a subset of cases expressing stereotypic unmutated IGHV4-39/IGKV1-39 (1D-39) B cell receptors (BCRs) of the rare IgG-positive variant (6). Notably, this subset was subsequently shown to be associated with the chromosomal translocation  $t(14;19)(q32;q13)$  co-existing with trisomy 12 (7-8). In such cases, the *BCL-3* proto-oncogene at 19q13 is juxtaposed to the immunoglobulin heavy chain gene locus at 14q32, leading to overexpression of the BCL-3 protein with resultant deregulation of the NF- $\kappa$ B pathway. Although the +12,+19 profile described here identifies a CLL subgroup distinct from subset 8, especially in terms of the IGHV repertoire and mutational status, the fact that aberrations of chromosome 19 are biased to sIgG-switched CLL might be considered as evidence of genetic evolution along distinctive yet related, likely antigen-driven and B cell receptor-mediated, pathways. In the “+12+19” subgroup, the ordered gain of trisomies, often also including trisomy 18, indeed points to a shared genetic “route” during leukemogenesis and further indicates a common cellular origin for this subset.

Rachel Ibbotson<sup>1</sup>, Anastasia Athanasiadou<sup>2</sup>, Lesley-Ann Sutton<sup>3</sup>, Zandie Davis<sup>1</sup>, Anne Gardiner<sup>1</sup>, Panagiotis Baliakas<sup>2</sup>, Rebeqa Gunnarsson<sup>3</sup>, Achilles Anagnostopoulos<sup>2</sup>, Gunnar Juliusson<sup>4</sup>, Richard Rosenquist<sup>3</sup>, David Oscier<sup>1</sup>, Kostas Stamatopoulos<sup>2,5</sup>

<sup>1</sup>Haematology Department, Royal Bournemouth Hospital, Bournemouth, UK

<sup>2</sup>Hematology Department and HCT Unit, G. Papanicolaou Hospital, Thessaloniki, Greece

<sup>3</sup>Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden

<sup>4</sup>Department of Laboratory Medicine, Stem Cell Center, Hematology and Transplantation, Lund University, Lund, Sweden

<sup>5</sup>Institute of Agrobiotechnology, Center for Research and Technology, Thessaloniki, Greece

**Running title:** Co-existence of trisomies 12 and 19 in slgG-switched CLL

**Corresponding author:**

Kostas Stamatopoulos

Hematology Department and HCT Unit

Exokhi, 57010 Thessaloniki, GREECE

Phone: +30.2313.307992

Fax: +30.2313.307579

E-mail: [kostas.stamatopoulos@gmail.com](mailto:kostas.stamatopoulos@gmail.com)

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## FIGURE LEGEND

**Figure 1.** Venn diagram highlighting the relationships of the following three groups of CLL patients of the present study: (i) +12: n=333 (including all cases with co-existing trisomy 12 and any other cytogenetic aberration); (ii) +12+19, regardless any additional aberrations: n=20; (iii) +12+18+19: n=15.



**Table 1.** Clinical and biological data for CLL patients with co-existing trisomies of chromosomes 12 and 19 expressing slgG.

| Case | Gender | Age at Diagnosis | Binet Stage at Diagnosis | Chromosomal profile   | Karyotype ascertainment | IgG+ detection method | IGHV gene | IGHV identity | CD38 % | Need for treatment |
|------|--------|------------------|--------------------------|---|-------------------------|-----------------------|-----------|---------------|--------|--------------------|
| 1    | F      | 56               | A                        | 49,XX,+12,+18,+19[17]/46,XX[17]   | G-banding               | FLOW                  | 1-69      | 95,0          | 95     | NO                 |
| 2    | M      | 65               | A                        | 49,XY,+12,+18,+19[8]/46,XY[22]  | G-banding               | FLOW                  | 2-26      | 92,3          | 98     | NO                 |
| 3    | M      | 45               | A                        | 49,XY,+12,+18,+19[17]/46,XY[11]   | G-banding               | FLOW                  | 2-5       | 98,9          | 99     | NO                 |
| 4    | F      | 73               | A                        | 49,XX,+12,+18,+19[13]/46,XX[17]   | G-banding               | FLOW                  | 3-15      | 95,2          | 98     | YES                |
| 5    | F      | 70               | B                        | +12,del(17)(p13),+19  | SNP array               | RT-PCR                | 3-30      | 89,2          | 9      | YES                |
| 6    | M      | 46               | A                        | 48,XY,+12,+19[8]/46,XY[12]  | G-banding               | FLOW                  | 3-33      | 98,6          | 2,4    | NO                 |
| 7    | M      | 66               | A                        | 50,XY,+12,+13,del(17)(p11),+18,+19[10]/46,XY[12]                              | G-banding               | FLOW                  | 3-48      | 92,7          | NA     | YES                |
| 8    | M      | 71               | A                        | 49,XY,+12,+18,+19[8]/46,XY[12]  | G-banding               | FLOW                  | 3-48      | 95,1          | 42,9   | YES                |
| 9    | M      | 59               | C                        | 49,XY,+12,+18,+19[6]/46,XY[14]  | G-banding               | FLOW                  | 3-49      | 95,8          | 54,1   | YES                |
| 10   | F      | 50               | A                        | 49,XX,+12,+18,+19[1]/46,XX[10]  | G-banding               | FLOW                  | 3-7       | 94,4          | <1     | YES                |
| 11   | M      | 70               | A                        | 49,XY,+12,del(13)(q14q22),+18,+19[10]/46,XY[22]                               | G-banding               | FLOW                  | 3-7       | 90,0          | 100    | NO                 |
| 12   | M      | 64               | A                        | 49,XY,t(9;13)(q?32;q?14),+12,+18,+19[15]/46,XY[1]                             | G-banding               | FLOW                  | 3-7       | 93,9          | 65     | NO                 |
| 13   | M      | 50               | B                        | +12,del(13)(q14),+18,+19  | SNP array               | RT-PCR                | 3-7       | 89,6          | 39     | YES                |
| 14   | M      | 64               | A                        | 48,XY,+12,+19[3]/46,XY[32]  | G-banding               | FLOW                  | 3-74      | 92,0          | 59     | NO                 |
| 15   | M      | 48               | A                        | +12,del(13)(q14),+19  | SNP array               | RT-PCR                | 4-30-2    | 96,9          | 40     | YES                |
| 16   | M      | 61               | A                        | 49,XY,+12,?dup(13)(q12q14),?13,+18,+19[5]/49,XY,idem,del(9)(p13)[2]/46,XY[18] | G-banding               | FLOW                  | 4-39      | 90,9          | 99     | YES                |
| 17   | M      | 57               | A                        | +12,+18,+19   | SNP array               | RT-PCR                | 4-59      | 93,7          | 96     | YES                |
| 18   | M      | 67               | A                        | +12,+19   | SNP array               | RT-PCR                | 4-59      | 93,3          | 85     | YES                |
| 19   | F      | 72               | A                        | 49,XX,t(4;8)(p16;q11),+12,+18,+19[6]/46,XX[14]                                | G-banding               | FLOW                  | 4-59      | 93,0          | 32,8   | YES                |
| 20   | M      | 43               | A                        | 49,XY,+12,+18,+19[15]/46,XY[5]  | G-banding               | FLOW                  | 7-4-1     | 97,2          | 95,7   | NO                 |

NA: not available

**Table 2.** CD38 expression and IGHV gene mutational status in cases assigned to the +12,+19 subgroup (“+12+19”) to cases exhibiting trisomy 12 yet without co-existing trisomy 19 (“+12 non +19”) and cases negative for trisomy 12 (“no +12”).

|                                     | <b>“+12+19”</b> | <b>“+12 non +19”</b> | <b>“No +12”</b> | <b>p value</b>     |
|-------------------------------------|-----------------|----------------------|-----------------|--------------------|
| CD38+ cells>30%                     | 16/19, 84.2%    | 136/241, 56.4%       | 111/561,19.8%   | <0.001             |
| Median % of CD38+ cells             | 95              | 72.5                 | 55              | <0.05 <sup>a</sup> |
| IGHV gene identity to germline ≥98% | 2/20, 10%       | 115/218, 52.7%       | 203/593, 34.2%  | <0.001             |
| IGHV gene identity to germline 100% | 0/20, 0%        | 94/218, 43.1%        | 145/593, 24.5%  | <0.001             |

<sup>a</sup>t-test: p=0.37 for comparison of “+12+19” versus “12 isolated”; p=0.04 for comparison of subgroups “+12+19” versus “no 12”; p=0.001 for comparison of subgroups “12 isolated versus “no 12”.