

Research paper

Complex karyotype as a predictor of high-risk chronic lymphocytic leukemia: A single center experience over 12 years



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ABSTRACT

Objectives: A complex karyotype (CK) is considered a poor prognostic marker in chronic lymphocytic leukemia (CLL).

Methods: The study analyzed 644 untreated CLL patients (pts) using conventional/molecular cytogenetics to reveal the presence of a CK and its composition and to assess its predictive value. The mutational status of *TP53* was detected by next generation sequencing.

Results: A CK was detected in 79 pts (12.3%). Patients with a CK showed shorter overall survival (OS) compared to those without a CK (77 months vs. 115 months, $p < 0.0001$). Chromosomes most frequently included in a CK were 13, 11, 17, 8, 2, and 6. The most common aberrations in a CK were translocations, numerical changes and dicentric chromosomes (with no effect on OS). Patients with aberrations of *TP53* and *ATM* were shown to have adverse prognosis comparable to patients with a CK without these abnormalities. A stronger impact of a CK on OS of female and older CLL patients was observed.

Conclusions: The determining of the presence of a CK is essential in modern clinical CLL practice. According to recent studies, the presence of a CK affects clinical and treatment decision-making.

1. Introduction

Chronic lymphocytic leukemia (CLL) is a biologically heterogeneous disease with a highly variable clinical course ranging from indolent to very aggressive. Chromosomal and genomic aberrations detectable by conventional and molecular cytogenetics occur in approximately 82% of patients at the time of diagnosis. Some of them are strongly related to prognosis and, apart from other important clinical and laboratory parameters, they are used to predict response to treatment and an outcome. The most frequent aberrations with a known prognostic impact are deletions of 13q, 11q, 17p and trisomy 12 detected by interphase fluorescence *in situ* hybridization (iFISH) [1]. Nevertheless, numerous changes of unknown significance are missed by iFISH targeted only at selected specific loci. Such abnormalities occurring across the

entire genome could be detected by karyotyping. In the past few years, the use of conventional cytogenetics in the detection of chromosomal aberrations in CLL was not very successful due to reluctance of B cells to grow in culture leading to a lack of metaphase chromosomes. A higher proliferation rate of CLL cultured cells was achieved by the use of mitogenic stimulation with CpG oligonucleotides and interleukin 2 more than ten years ago [2], making conventional cytogenetics a reliable and reproducible tool in CLL again. As a result, the number of detectable chromosomal changes in CLL patients increased and the term complex karyotype started to be discussed in CLL [3–7].

A complex karyotype (CK) occurs in 10–20% of CLL patients at the time of diagnosis and is defined as the presence of three or more independent chromosomal aberrations coexisting in one clone. It has recently been considered as a strong independent prognostic marker of

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rapid disease progression, short time to first treatment, progression-free survival and overall survival (OS) [4,8–10]. The incidence of changes included in a CK as well as their relationship to other clinical and laboratory parameters have been intensively studied. Dicker et al. [2] noted a correlation between the presence of a CK, unmutated *IGHV* and positivity of CD38, while Mayr et al. [11] observed that chromosomal translocations were often associated with the CK. Brugat et al. [12] have found that the presence of a CK may also be related to the presence of non-functional telomeres leading to defects in repairing damaged DNA and apoptosis induced by damaged DNA.

A complex karyotype may also occur at the time of relapse or progression of disease in clonal evolution. During clonal evolution, the importance of previous treatment is significant [13]. Ouillette et al. reported that changes that dominated a CK at the time of relapse had been found in patients even before therapy. The presence of TP53 mutation at the time of diagnosis results in complicated pathological clones dominating at relapse.

The present cytogenetic and molecular cytogenetic analysis aimed to determine the incidence of a CK in a group of untreated patients and to evaluate its impact on OS. Other objectives were to evaluate the frequency and types of chromosome changes in detected complex karyotypes, to identify the most frequently altered chromosomes and to evaluate the clinical significance of these findings, in order to contribute to a further understanding of the causes and consequences of genomic complexity in CLL.

2. Materials and methods

2.1. Biological material

A total of 644 untreated CLL patients (diagnosed according to the 1996 and 2008 iwCLL criteria [14,15]) were examined in our center (between 1996 and 2017). Biological material (peripheral blood, bone marrow or lymph nodes) was investigated at the time of diagnosis or during the course of the disease before treatment. All patients gave informed consent approved by the institutional ethics board in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2. Conventional cytogenetics

Peripheral blood cells were cultured in RPMI 1640 medium or BM medium (Gibco, Life technologies, Carlsbad, California, USA; Biological Industries, Cromwell, Connecticut, USA) without stimulation or in the presence of DSP30 (TIBMolbiol, Berlin, Germany) or ECAMPO10 (EuroClone, Pero, Italy) and interleukin 2 (Sigma Aldrich, Darmstadt, Germany) for 72 h. Bone marrow or lymph node cells were cultured in RPMI 1640 medium or BM medium for 24 h. Banding of chromosomes was performed using standard G-banding procedures [3,5]. Chromosome analysis was performed in all patients on metaphases of biological material. At least 10 metaphases were evaluated for each patient, less than 10 evaluable metaphases were considered as an unsuccessful cytogenetics. The karyotypes were compiled according to the current ISCN 2016 [16] using software Ikaros (MetaSystems, Altlußheim, Germany).

2.3. Fluorescence in situ hybridization (FISH)

All patients were examined by FISH with commercially available probes for detection of deletions of *ATM*, *TP53*, 13q14 (D13S319/*DLEU1*, *DLEU2*), 13q34 (*LAMP1*), 6q21, trisomy of chromosome 12, duplication of *MYC*, *IGH* rearrangements (Abbott Molecular, Des Plaines, Illinois, USA; Kreatech Diagnostics, Vlierweg, Netherlands; MetaSystems). Other locus-specific, centromeric, painting, BAC probes (Cambio, Cambridge, UK; Abbott Molecular; Dako, Glostrup, Denmark; MetaSystems; BlueGnome, Cambridge, UK), or mFISH (24Xcyte DNA

probe Kit, MetaSystems) were also used in accordance with the manufacturers' instructions to determine and specify complex karyotypes. The hybridization results were evaluated under the Olympus BX60 fluorescence microscope and scanned using the ISIS software (MetaSystems).

2.4. Array comparative genomic hybridization (arrayCGH)

Sixty-six patients with available DNA extracted from peripheral blood, bone marrow or lymph nodes were examined by arrayCGH. The following platforms were used: 1Mb 3 K platforms (Leiden University Medical Center, Leiden, the Netherlands; Department of Human Genetics, Catholic University Leuven, Leuven, Belgium), CytoChip ISCA 4 x 44 K (BlueGnome), Human Genome CGH Microarray Kit, 4 x 44 K (Agilent, Santa Clara, California, USA) and SurePrint G3 Hmn CGH + SNP 4 x 180 K Microarray Kit (Agilent). In all cases, the manufacturers' recommendations were followed. The results were evaluated using the BlueFuse Multi software (BlueGnome), Agilent Genomic Workbench or Agilent CytoGenomics (Agilent).

2.5. Mutational status of *IGHV*

The mutational status of *IGHV* genes was assessed in the peripheral blood mononuclear fraction or bone marrow cells of patients. RNA was extracted using the TRIzol solution (Invitrogen, Carlsbad, California, USA) and reverse transcribed into cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California, USA). The cDNA was amplified in 6 individual reactions for the detection of clonal proliferation (Ampli-Taq Gold polymerase; Applied Biosystems), followed by direct Sanger sequencing (Big Dye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems) using ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The nucleotide sequences were then aligned to NCBI IgBlast (www.ncbi.nlm.nih.gov/igblast). A mutated status was assigned when there was $\geq 2\%$ deviation from the germline *IGHV* sequence.

2.6. *TP53* mutations

Analysis of the full coding sequence of the *TP53* gene (exons 2–11, NM_000546) was performed by next-generation sequencing on the MiSeq instrument (Illumina, San Diego, California, USA). Amplicon-based libraries with dual indexing were paired-end sequenced (2×151 bp). The detection limit for low variant allele frequency was 5%, with a coverage depth of $\geq 2000 \times$. After mapping to the human reference genome (GRCh38) using the Burrows-Wheeler Aligner-MEM algorithm (version 0.7.12), variant calling was performed using Genome Analysis Toolkit (version 3.8): Unified Genotyper, Haplotype Caller and MuTect (version 1.1.7). All variants detected were annotated using Variant Effect Predictor (version 83).

2.7. Statistical and data mining methods

Kaplan-Meier survival plots were constructed using the R statistical software package, a free software environment for statistical computing and graphics (www.r-project.org/). Overall survival was defined as the time from diagnosis to the date of the last follow-up or the date of death (event) from any cause. A p-value < 0.05 was considered significant. A co-occurrence network [17] was constructed and visualized for the most common combinations of chromosomes involved in the CK rearrangements.

3. Results

3.1. Basic characteristics of the patients

Complex karyotypes (≥ 3 chromosomal abnormalities in one clone)

Table 1
Basic characteristics of patients with complex changes.

Sex	N (%)
Male	57 (72)
Female	22 (28)
Age at diagnosis	Median (range) 64 (34–87)
Binet stage	N (%)
A	24 (30.4)
B	28 (35.4)
C	23 (29.1)
Not applicable	4 (5.1)
IGHV status	N (%)
Mutated	10 (12.7)
Mutated (VH3-21)	6 (7.6)
Unmutated	60 (75.9)
Not analyzed	3 (3.8)
Presence of a CK	N (%)
At diagnosis	59 (75)
Before treatment	20 (25)
Survival	N (%)
Alive	38 (48)
Died	41 (52)

N = number of patients.

were detected in 79/644 pts (12.3%). Although 11 out of 79 pts failed to fulfil the criteria of a CK precisely due to unsuccessful or uninformative conventional cytogenetics, they were still included in the CK cohort, because they carried three or more independent chromosomal aberrations coexisting in one clone without any doubt. Four of them met the criterion of a 70% positivity threshold (≥ 3 abnormalities detected by iFISH, each of them in at least 70% of interphase cells; thus, co-occurrence in one clone was certain in at least 10% of cells). In seven of them, the proportion of the changes was lower, but their co-occurrence in one clone was verified by another combination of FISH probes.

The CK cohort consisted of 57 men and 22 women with a median age at diagnosis of 64 years (range, 34–87 years). A CK was detected in 59 pts (75%) at the time of diagnosis and 20 pts (25%) before treatment (range, 6–128 months). The mutational status of *IGHV* was examined in 76/79 pts; only 16 pts (20.3%) had a mutated *IGHV* status, out of them 6 pts (7.6%) mutated *IGHV3-21* and 60 pts (75.9%) an unmutated status. For basic clinical characteristics of patients see Table 1.

Among these 79 pts with CK were 69 pts treated, the median time to first treatment (TTFT) was 197 days (6.5 months). In the cohort of 69 treated pts, 51 pts received immunochemotherapy with curable potential (fludarabine, bendamustine based). Five pts were treated by chemotherapy alone (the era without routine using of immunotherapy), 4 pts by BCR inhibitors in the first line (3x R-idelalisib, 1x ibrutinib) and 9 pts by palliative therapy regimens (5x leukeran monotherapy, 3x corticosteroids, 1x alemtuzumab monotherapy). Ten pts were not treated, 6 of them because of very bad performance status and 4 of them were not indicated to treatment.

3.2. Cytogenetics

The most frequently detected recurrent changes in patients with a CK were deletions of 13q14 (68%), 11q22 (43%), 17p13 (35%) and duplication of 8q24 (27%). Other cytogenetic abnormalities were duplication of the short arm of chromosome 2 (33%), deletion of 6q21 (14%), trisomy of chromosome 12 (10%) and rearrangement of the *IGH* gene (2.5%). Results of conventional cytogenetics combined with FISH and results of arrayCGH in 79 pts with a CK are summarized in Supplementary data (Table 1, Table 2). Chromosomes most often included in a CK and the links between them are shown in Fig. 1.

3.3. Survival analysis

3.3.1. Influence of a CK and its composition

Statistical analysis showed lower OS rates in patients with a CK compared to those without it (79 pts with a CK vs. 565 pts without a CK; 77 months vs. 115 months; $p < 0.0001$; Fig. 2). The effect of the presence of translocations, numerical changes and dicentric chromosomes as part of a CK on patient survival was not demonstrated ($p = 0.5$, $p = 0.35$ and $p = 0.62$, respectively). Furthermore, the number of aberrations (less than 5 aberrations vs. 5 or more aberrations) did not influence the OS in the cohort ($p = 0.35$).

3.3.2. Influence of *TP53* and *ATM* abnormalities

A total of 32 pts with a CK had aberrations (deletion and/or mutation) of the *TP53* gene. *TP53* was deleted in 28 pts with a CK (35%) compared to 18 pts (3%) in the cohort without a CK (565 pts). *TP53* gene mutations were detected in 24 out of 69 examined patients with a CK (35%); 19 out of 69 pts (28%) had both *TP53* deletion and mutation. The *ATM* gene was deleted in 34 pts with a CK (43%) compared to 79 pts (14%) in the cohort without a CK (565 pts). Five patients had both *TP53* and *ATM* deletions.

The survival of patients with a CK and *TP53/ATM* aberrations was not worse compared to that in patients with a CK but without *TP53/ATM* aberrations (48 months/ 68 months vs. 87 months/ 84 months; $p = 0.076/p = 0.49$; Fig. 3A, B). However, patients without a CK but with *ATM/TP53* aberrations (79 pts/ 33 pts) had a comparable negative prognostic impact to those with a CK ($p = 0.37/p = 0.78$). Moreover, patients with a CK and *TP53* mutation/deletion had worse OS compared to those with *TP53* aberrations without a CK (48 months vs. 137 months; $p = 0.0084$).

3.3.3. Influence of sex and age

We also observed an influence of sex and age on the OS of patients with a CK. A more adverse outcome was seen in women with a CK compared to those without a CK (37 months vs. 154 months; $p < 0.0001$) in comparison with men with a CK vs. men without a CK (84 months vs. 106 months; $p = 0.0049$). However, a significant difference in OS between men and women with a CK was not found (37 months vs. 84 months; $p = 0.14$) (Fig. 3C). Moreover, worse OS was seen in patients with a CK aged over 60 years (pts with a CK over 60 vs. pts with a CK under 60: 68 months vs. 123 months; $p = 0.0075$; Fig. 3D).

4. Discussion

A complex karyotype was detected in 79 untreated patients (12.3%), confirming the association with poor prognosis and shorter OS; this is consistent with the previously published data [2,4,6–11,18]. Our definition of a CK was based on precise cytogenetic (metaphase) analysis; cases were included in the CK cohort if they had three unrelated chromosomal abnormalities present in one clone. Cases with unsuccessful or uninformative cytogenetics were included in the CK cohort only if they had three or more abnormalities detected by interphase FISH, each of them in at least 70% or co-occurrence of these aberrations in one clone was detected. Patients with more abnormal noncomplex clones (carrying less than three changes in each clone) were not included in the CK cohort. Nevertheless, further studies comparing outcomes of these patients with those of patients with a CK could be interesting.

The patients displayed a variety of abnormalities across the entire genome, but the most frequent aberrations were deletions of 13q14 (68%), 11q22 (43%), 17p13 (35%), duplication of 2p (33%), 8q24 (27%) and deletion of 6q21 (14%). Once again, these results are in agreement with average proportions of these changes reported by other authors [1,9,19–22]. We analyzed the composition of a CK; however, neither the specific chromosome nor the type of aberration such as

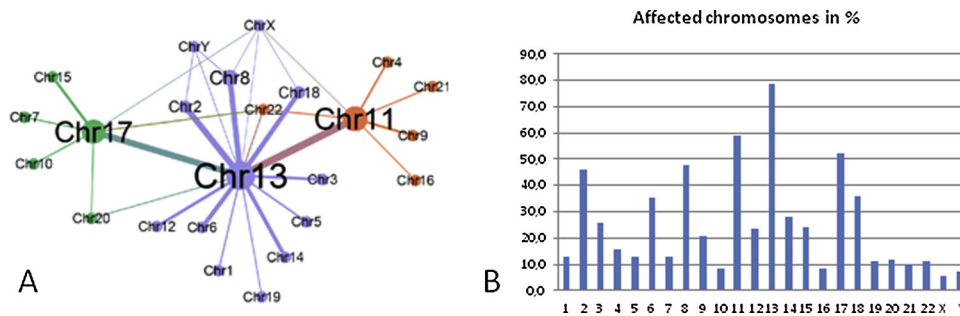


Fig. 1. (A) Co-occurrence network of the most commonly affected chromosomes in a CK and the links between them (chromosomes 13, 17 and 11 are most frequently included in changes; abnormalities of chromosome 13 are often accompanied by either chromosome 11 or 17, whereas aberrations of chromosomes 11 and 17 are very rarely seen together); (B) Graphical display of the most commonly affected chromosomes.

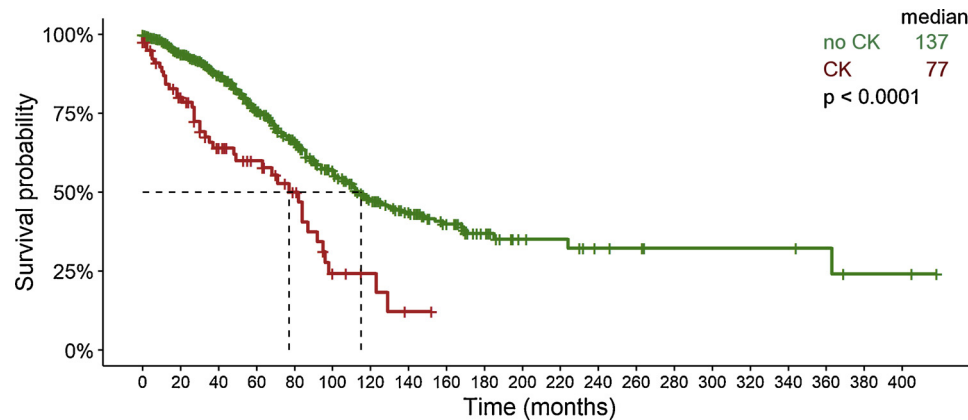


Fig. 2. Kaplan-Meier curves for OS in patients with/without a CK.

translocations, numeric changes or the presence of dicentric chromosomes showed any effects on patient survival. In conclusion, the composition of a CK, however complicated, did not influence OS in the present study, contrary to the previously published data [23,24]. According to Baliakas et al., there are several subgroups in a CK. One is a group of CKs with coexisting trisomies of chromosomes 12 and 19 which exhibits a more favorable profile. Nevertheless, there was only 1 patient in our cohort with trisomy of chromosome 12 in one clone and trisomy of chromosome 19 in other clone, therefore we were not able to assess their conclusion. Another subgroup defined by Baliakas et al., called high-CK, consists of pts with five or more cytogenetic abnormalities and shows adverse prognosis. According to them, there is no significant difference in OS between high-CK with *TP53* aberrations and high-CK without *TP53* aberrations. Although we observed a higher incidence of *TP53* aberrations in our subgroup with more than five abnormalities (48% vs. 27% in a group with 3 or 4 aberrations; Supplementary Table 1), the present study failed to show any impact of *TP53* aberrations on OS. Moreover we observed no impact of higher number of cytogenetic changes on OS as well. This could be possibly caused by much lower number of pts in our center.

The prognostic value of a CK was compared with other well-known cytogenetic risk factors, namely aberrations of *TP53* and *ATM*. Patients with aberrations of *TP53* and *ATM*, either included in a CK or not, were found to have an adverse prognosis comparable to that of patients with a CK but without these abnormalities. We also analyzed the influence of deletions of the *TP53* and *ATM* genes in a CK. Despite a higher incidence of the deletions, the negative prognostic impact in the CK cohort was not confirmed, consistently with findings in a study by Thompson et al. [25]. However, patients with a CK and *TP53* abnormalities (mutation/deletion) displayed a trend towards worse survival ($p = 0.076$). Patients without a CK with *TP53* or *ATM* deletions had a comparable negative prognostic impact, similar to patients with a CK ($p = 0.37/p = 0.75$). Moreover, patients with a CK and *TP53* mutation/deletion had worse OS compared to those with *TP53* deletions without a CK ($p = 0.0084$).

Men are more affected by CLL than women (almost 2:1) and they also have a worse prognosis than women [26]. Our CK cohort comprised 57 men and 22 women (2.6:1). Interestingly, OS was more significantly shortened in women with a CK compared to those without a CK ($p < 0.0001$) but no difference between men and women with a CK was demonstrated ($p = 0.14$), despite median OS rates of 84 months in men vs. 37 months in women. This could be explained by a higher incidence of *TP53* aberrations (55% in women vs. 35% in men). However, it is necessary to confirm this interesting phenomenon in international studies with a larger cohort of patients with a CK as these are results of a single-center study with a limited number of patients. To our best knowledge, these findings have not been reported by other authors so far.

The median age at diagnosis in CK patients was 64 years (range, 34–87 years). Age is another prognostic factor influencing the outcome of CLL patients [27]. In our study, a more adverse outcome was seen in patients with a CK who were older than 60 years ($p = 0.0075$).

In conclusion, the use of standard cytogenetic methods combined with modern molecular genetic analyses remains essential for risk stratification of CLL patients. Consistently with other authors, our results suggest that a complex karyotype is a strong negative prognostic factor and none of the other independent prognostic factors improve the OS of patients with a CK. Only results of ongoing clinical studies could reveal effective treatment strategy for patients with a CK. Finally, together with *TP53* aberrations, the CK has become a key prognostic factor in CLL regardless of its composition.

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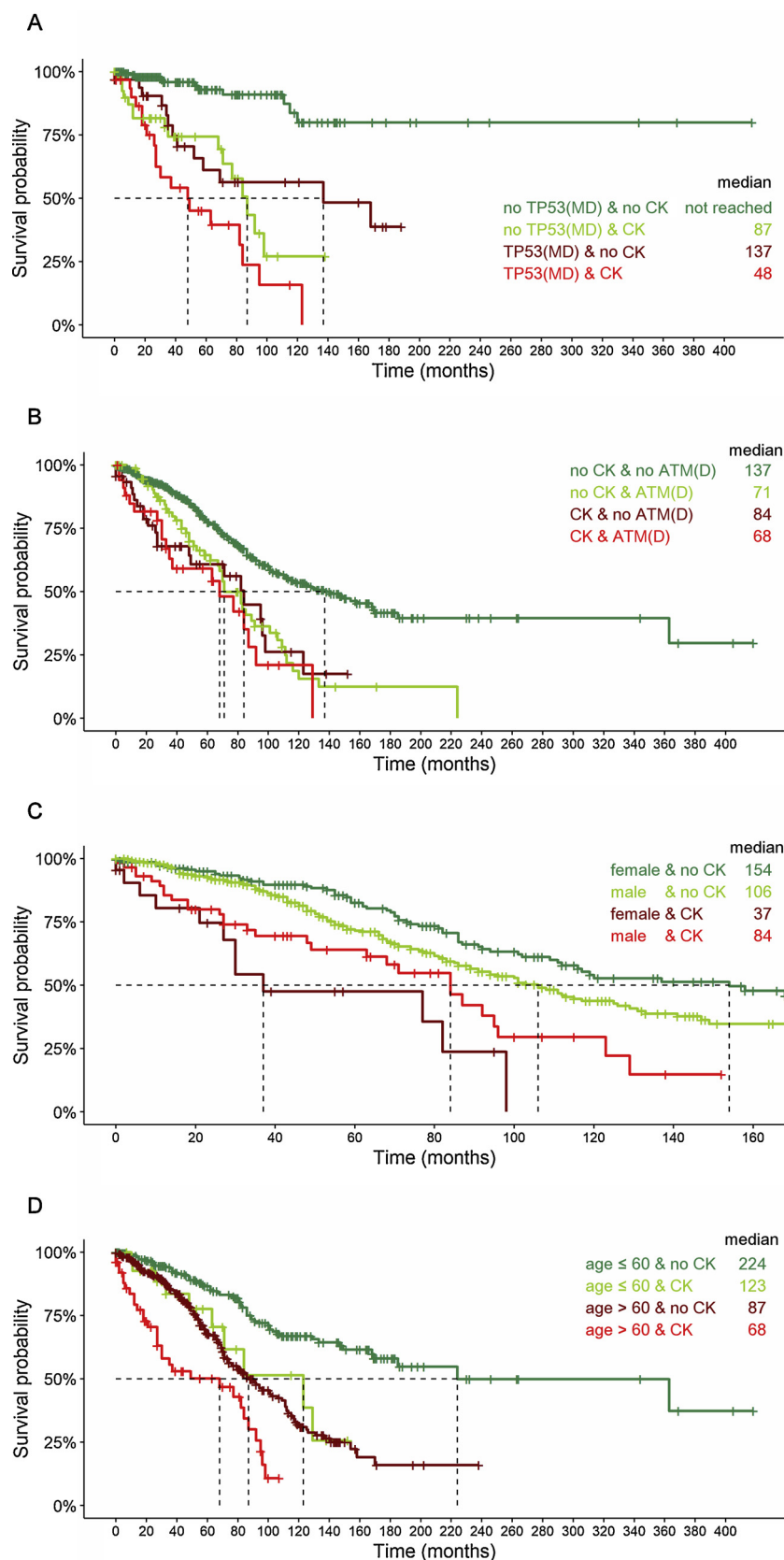


Fig. 3. Kaplan-Meier curves for OS in patients with/without a CK subdivided according to: (A) presence of *TP53* mutation/deletion: (with/without)*; (B) presence of *ATM* deletions: (with/without)**; (C) sex: (men/women); (D) age: (≤60 years/ > 60 years).

*Cohort of patients without *TP53* aberrations was selected with respect to *TP53* deletion and mutation assessment (362 pts with unknown *TP53* status in the cohort without a CK were discarded and so were 7 pts in the cohort with a CK);

**Cohort of patients without a CK and without *ATM* deletion was selected with respect to *ATM* deletion assessment (4 pts were discarded).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.leukres.2019.106218>.

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