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Research paper

Improving risk-stratification of patients with chronic lymphocytic leukemia using multivariate patient similarity networks

Peter Turcsanyi[a], Eva Kriegova[b,*], Milos Kudelka[c], Martin Radvansky[c], Lenka Kruzova[a], Renata Urbanova[a], Petra Schneiderova[b], Helena Urbankova[a], Tomas Papajik[a]

[a] Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, Olomouc, Olomouc, Czech Republic
[b] Department of Immunology, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, Olomouc, Czech Republic, Olomouc, Czech Republic
[c] Department of Computer Science, Faculty of Electrical Engineering and Computer Science, VSB – Technical University of Ostrava, Ostrava, Czech Republic

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ABSTRACT

Background: Better risk-stratification of patients with chronic lymphocytic leukemia (CLL) and identification of subsets of ultra-high-risk (HR)-CLL patients are crucial in the contemporary era of an expanded therapeutic armamentarium for CLL.
Methods: A multivariate patient similarity network and clustering was applied to assess the prognostic values of routine genetic, laboratory, and clinical factors and to identify subsets of ultra-HR-CLL patients. The study cohort consisted of 116 HR-CLL patients (F/M 36/80, median age 63 yrs) carrying del(11q), del(17p)/*TP53* mutations and/or complex karyotype (CK) at the time of diagnosis.
Results: Three major subsets based on the presence of key prognostic variables as genetic aberrations, bulky lymphadenopathy, splenomegaly, and gender: profile (P)-I (n = 34, men/women with CK + no del(17p)/*TP53* mutations), P-II (n = 47, predominantly men with del(11q) + no CK + no del(17p)/*TP53* mutations), and P-III (n = 35, men/women with del(17p)/*TP53* mutations, with/without del(11q) and CK) were revealed. Subanalysis of major subsets identified three ultra-HR-CLL groups: men with *TP53* disruption with/without CK, women with *TP53* disruption with CK and men/women with CK + del(11q) with poor short-term outcomes (25% deaths/12 mo). Besides confirming the combinations of known risk-factors, the used patient similarity network added further refinement of subsets of HR-CLL patients who may profit from different targeted drugs.
Conclusions: This study showed for the first time in hemato-oncology the usefulness of the multivariate patient similarity networks for stratification of HR-CLL patients. This approach shows the potential for clinical implementation of precision medicine, which is especially important in view of an armamentarium of novel targeted drugs.

1. Introduction

Chronic lymphocytic leukaemia (CLL), the most common type of leukemia in adults in western counties, is associated with significant clinical heterogeneity, varying from an indolent disease course to a progressive one, reflecting its biological diversity. Nowadays, several different treatment options are available for CLL patients, including novel targeted drugs. Better prognostic stratification and more accurate prediction of treatment response are therefore crucial in the contemporary era of an expanded therapeutic armamentarium and precision medicine for patients with CLL, a disease displaying an extremely variable clinical behaviour. The availability of effective new first-line regimens with pathway inhibitors and their combinations makes prognostication and prediction of response to treatment an important

Abbreviations: BM, bone marrow; CLL, chronic lymphocytic leukemia; CLL-IPI, CLL-International Prognostic Index; CK, complex karyotype; del, deletion; HR-CLL, high-risk chronic lymphocytic leukemia; LN, lymph nodes; OS, overall survival; PB, peripheral blood; PFS, progression-free survival; PSN, patient similarity networks; TTFT, time to first treatment

* Corresponding author at: Department of Immunology, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, Hnevotinska 3, 775 15, Olomouc, Czech Republic.
E-mail addresses: Peter.Turcsanyi@fnol.cz (P. Turcsanyi), eva.kriegova@email.cz (E. Kriegova), Milos.Kudelka@vsb.cz (M. Kudelka), Martin.Radvansky@vsb.cz (M. Radvansky), Lenka.Kruzova@fnol.cz (L. Kruzova), Renata.Urbanova@fnol.cz (R. Urbanova), Petra.Schneiderova@fnol.cz (P. Schneiderova), Helena.Urbankova@fnol.cz (H. Urbankova), Tomas.Papajik@fnol.cz (T. Papajik).

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exercise particularly for high-risk (HR) patients, as they respond poorly to a standard immunochemotherapy regimen, relapse early, and have significantly shorter overall survival (OS).

Currently, there is a plethora of prognostic biomarkers in CLL. Of these, the most reliable in guiding risk stratification in daily clinical practice are the deletion of 17p (del17p), 11q (del11q), complex karyotype (CK) [1,2], *IGHV* mutational status [2], mutations in *TP53*, *ATM*, *BIRC3*, *NOTCH1*, and other genes [3–5]. The most prognostically relevant aberration is the inactivation of *TP53*, caused by the deletion of 17p and/or *TP53* mutations, occurring in 4–10% of patients at the time of first-line treatment, and in 20–40% in relapsed CLL [6–8]. Patients with this abnormality have rapidly progressive disease with a time to first treatment (TTFT) of 9 mo, inferior survival outcomes (OS 32 mo) and poor response to fludarabine- or bendamustine-based immunochemotherapy [4,9,10]. 11q23 deletion is the next poor prognostic aberration, occurring in 5–20% of CLL patients at the time of diagnosis [11,12]. It is often associated with progressive disease, large and multiple lymphadenopathies, and unmutated *IGHV* status [13]. When compared to non-deleted 11q and 17p cases, patients with del (11q) have a TTFT of 13 mo, remission of shorter duration, and shorter OS following fludarabine-based immunochemotherapy with median 79 mo [4,6,14]. FCR immunochemotherapy improved significantly progression-free survival (PFS) and OS in patients del(11q) resulting in long-term remissions in previously untreated CLL patients [9]. Complex karyotypes (CK) with three or more chromosomal abnormalities in one clone have also been associated with a poor prognosis in CLL [15–18]. They are detected in nearly 16% of patients before treatment initiation [15] and often occur in association with *IGHV* unmutated status [16] and 11q or 17p deletions [17]. Regarding prognostic significance, CK predicts a shortened TTFT and OS in CLL patients (OS 25 mo) [15,18].

Nowadays, there is a challenge to select more accurate prognostic factors and their combinations particularly for HR-CLL patients, as they may profit from novel targeted therapies and their combinations. Moreover, in the era of precision medicine and growing number of available drugs and their combinations, the risk-stratification of CLL patients is needed to obtain optimal treatment response generally. Therefore, we applied multivariate patient similarity networks (PSN) to stratify HR-CLL patients to subsets and to assess the prognostic relevance of routinely tested genetic, laboratory, and clinical factors. In identified patient subsets, the clinical course, treatment response and OS were evaluated. Our study shows that used patient similarity networks may provide precise identification of patient risk subsets for treatment targeting in the daily clinical practice, and not only in CLL.

2. Patients and methods

2.1. Patient cohort

The study cohort consisted of 116 HR-CLL patients with del(11q), del(17p)/*TP53* mutations, and/or CK (F/M 36/80, median age 63 yrs, range 34–87 yrs) diagnosed in accordance with the IWCLL diagnostic criteria between 2000 and 2015 at a single reference center. Evaluations were performed of cytogenetic aberrations, *TP53* mutations, age, gender, Binet stage, blood counts, serum levels of beta-2-microglobulin, thymidine kinase (TK), and lactate dehydrogenase (LDH), splenomegaly (palpable spleen and/or enlarged spleen as assessed by CT scan or ultrasound), and bulky lymphadenopathy (defined as lymph node diameter > 5 cm as assessed by CT scan or ultrasound) in all the patients at the time of diagnosis. Indication, response to treatment, and disease progression were guided according to the IWCLL criteria, except that bone marrow aspirate/biopsy was not performed in some patients. Fludarabine-refractoriness was defined in this study as stable/progressive disease after therapy, or early relapse/progression < 6 months after chemotherapy. Detailed patient characteristics are shown in Table 1.

The selection of the treatment regimen was dependent on the age and performance status of the patients and their comorbidities. Of the patients who were enrolled, 78 (67%) were treated by standard chemotherapy (regimens containing fludarabine or anthracycline, cyclophosphamide, and their combinations; 79% with rituximab) and 14 (12%) by palliative regimens (leukeran, COP) (Table 1). Of the 24 patients (21%) who received no treatment, 11 were able to get only symptomatic treatment and 13 did not meet the IWCLL criteria for treatment initiation.

Table 1
Clinical and demographical characteristics of enrolled HR-CLL patients.

Patient Characteristics	HR-CLL patients (n = 116)
Age, median (range)	63 (34–87)
Gender, Male/Female, n (%)	80 (69) / 36 (31)
Binet stage, n (%)	47 (41) /37 (32) / 31
A/B/C/n.a.	(27) / 1 (1)
Bulky adenopathy ≥5 cm, n (%)	18 (16) / 98 (84)
Yes/No	
Splenomegaly, n (%)	22 (19) / 94 (81)
Yes/No	
Genetic aberrations	
del(11q)	76
del(11q), solo aberration	47
del(17p), solo aberration	14
del(17p)/*TP53* mutations	36
TP53 mutations	23
CK (>3 changes)	54/62
Yes/No	
CK alone + (no del(11q), no del(17p)/*TP53* mutations)	11
CK + del(17p)/*TP53* mutations	15
CK + del(11q)	22
CK + del(11q) + del(17p)/*TP53* mutations	6
no CK + del(11q) + del(17p)/*TP53* mutations	1
del(11q) + del(17p)/*TP53* mutations	7
***IGHV* mutation status, n (%)**	93 (80) / 16 (14) / 7 (6)
Unmutated / Mutated / n.a.	
β2-microglobulin ≥ 3.5 (mg/dL), n (%)	47 (41) / 49 (42) / 20 (17)
Yes/No/n.a.	
LDH greater than 2× the upper limit of normal (normal = 243 IU/L), n (%)	45 (39) / 50 (43) / 21(18)
Yes/No/n.a.	
Serum thymidine kinase (TK1) ≥13.5 (mg/dL), n (%)	65 (56) / 32 (28) / 19 (16)
Yes/No/n.a.	
Standard/Palliative/No treatment, n (%)	78 (67) / 14 (12) / 24 (21)
Fludarabine-based regimens	61 (78) / 17 (22)
Yes/No	
Fludarabine-sensitive/refractory, n (%)	36 (59) / 25 (41)
Rituximab at first-line (standard) treatment, n (%)	62 (79) / 16 (21)
Yes/No	

Written informed consent was obtained from all the patients who were enrolled in accordance with the Helsinki Declaration, and the study was approved by the local ethical committee.

2.2. Genetic analysis

The samples of peripheral blood (PB) and/or bone marrow (BM) or lymph nodes (LN) were analyzed by conventional cytogenetics, fluorescence in situ hybridization (*FISH*), and the arrayCGH technique, as reported previously [19]. A complex karyotype was defined by the presence of more than or equal to 3 chromosomal abnormalities in a single clone [15,20].

Briefly, the banding of chromosomes was performed using standard G-banding procedures after cell culturing for 24 h (BM, LN) or 72 h (PB) in an RPMI 1640 medium or BM medium (Gibco, USA; Biological Industries, USA) after stimulation with 12-O-tetradecanoylphorbol-13-acetate (Sigma Aldrich, Germany). *FISH* was used to detect the del (11q), del(17p), del(13q), and *IGH* rearrangements (Abbott Molecular, IL, USA; Kreatech Diagnostics, Netherlands; MetaSystems, Germany).

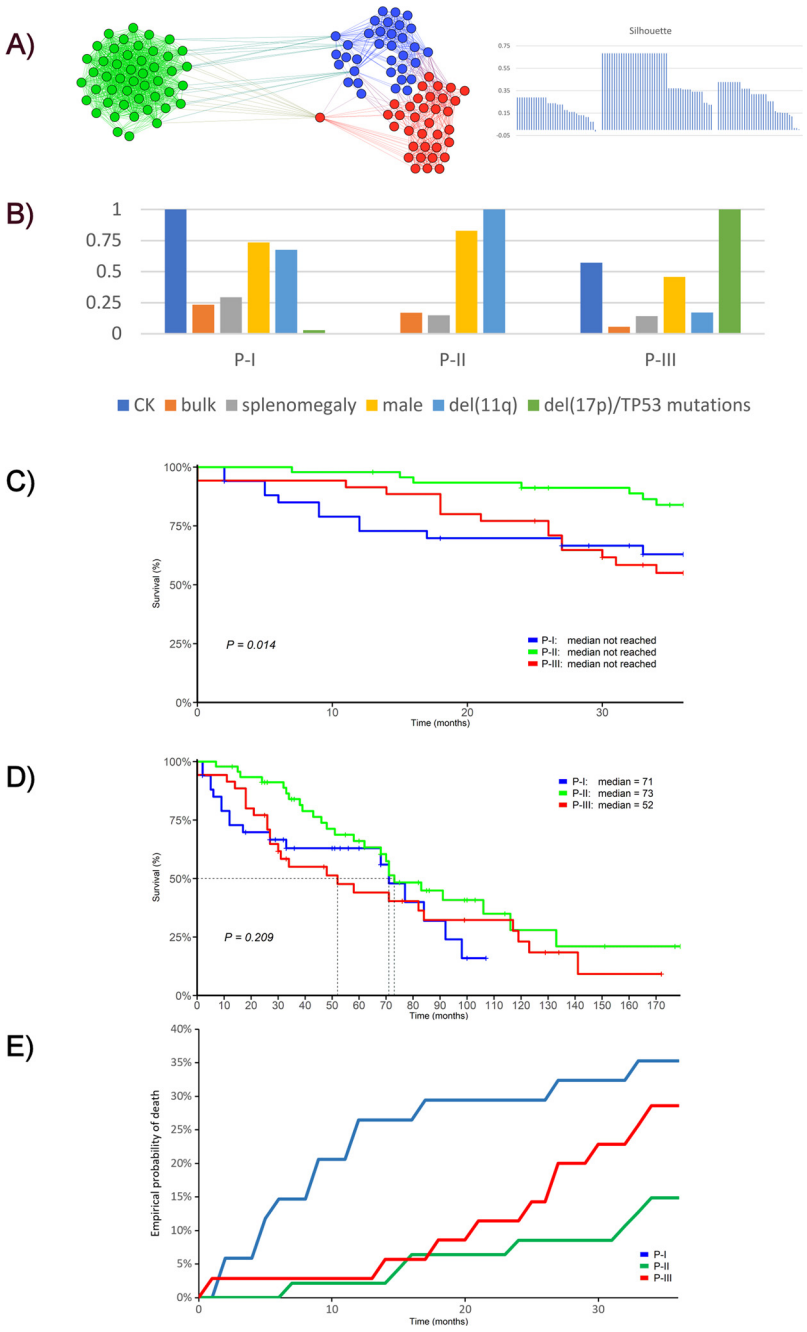

Fig. 1. Network analysis for the identification of major HR-CLL subgroups.
The network **(A)** and the characteristics of obtained subgroups based on the combinations of del(11q), del(17p)/*TP53* mutations, CK, bulk, splenomegaly, and gender **(B)**. The quality of the clusters that were obtained was checked by silhouette analysis. The Kaplan–Meier curves show the overall survival in subgroups of patients at short **(C)** and long follow-up **(D)**. The empirical probability of death in subgroups **(E)**.

The arrayCGH technique was applied using the following platforms: 1Mb 3 K platforms (LUMC, Leiden, the Netherlands, Leuven, Belgium), CytoChip ISCA 4 × 44 K (BlueGnome, UK), Human Genome CGH Microarray Kit, 4 × 44 K (Agilent, USA), and SurePrint G3 Hmn CGH + SNP 4 × 180 K Microarray Kit (Agilent, USA). All procedures were performed according to the manufacturer's recommendations.

2.3. Sequencing of TP53 gene and IGHV mutational status

The analysis of the full coding sequence of the *TP53* gene (exons 2–11, NM_000546) was performed using targeted sequencing on an MiSeq instrument (Illumina, San Diego, USA) as reported previously [21]. The detection limit of low variant allelic frequency was 5%. Mutational *IGHV* status was examined by direct Sanger sequencing.

2.4. Statistical methods and data mining methods

All the statistical analyses (Mann-Whitney-Wilcoxon test, receiver operating characteristic curves, Kaplan-Meier survival plot, Factor analysis of mixed data) were performed using the R statistical software package (http://www.r-project.org/). Overall survival (OS) was defined as the time from diagnosis (=time of genetic analysis) 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.

For the visualization of internal relationships (similarities) among clinical, demographic, and laboratory data in individual patients, the

method of network (graph) construction based on the nearest neighbor analysis was applied; for more details about used approach see [22]. Briefly explained, data with a selected group of markers was converted to a network [22]. The natural outcome of this procedure is in our case the multivariate network [23], which contains the values of the markers representing individual patients. Links between vertices of the network represent the similarity between patients. Different combinations of markers generate networks with different internal structure. The network construction phase was followed by the automatic detection of clusters in the network. For more details on patient similarity networks see the Supplementary file.

To assess the quality of the partitioning of the individual patient´s data into clusters in constructed multivariate networks, two parameters were used: weighted modularity and silhouette. Regarding weighted modularity [24], networks with high modularity have dense connections between vertices within clusters but sparse connections between vertices in different clusters. Silhouette, a traditional graphical representation of consistency within clusters, was used to study the separation distance between the resulting clusters [25]. The silhouette represents how well each patient belongs to its own cluster compared to other clusters.

3. Results

3.1. Stratification of HR-CLL patients according to the prognostic factors

The differences and similarities between individual HR-CLL patients on the basis of the clinical, demographic, and laboratory data were visualized using a patient network. The internal structure of the network represents profiles (=groups) of similar patients; links connecting pairs of network vertices (=patients) represent the nearest neighbors with the greatest similarity in terms of the parameters used. To obtain a set of characteristic parameters (most informative markers), we constructed multivariate networks based on different combinations of parameters and measured modularity and silhouette values. The goal was to select, for further processing, a combination with a small number of clusters and a combination with high values of both measured values.

Our data showed that the best separation of the HR-CLL patients used the following parameters: genetic aberrations (del(11q), del(17p)/*TP53* mutations, CK), bulk, splenomegaly, and gender, and their combination. The other available clinical and demographic data, such as age, Binet stage, blood counts, beta-2-microglobulin, thymidine kinase, and LDH were not discriminant for the subgrouping of HR-CLL patients. Similarly, the mutational status of the *IGHV* gene did not improve the discriminatory power of the network, as HR-CLL patients with mutated/unmutated status were distributed through all the subgroups (P-I, n = 5/29; P-II, n = 5/42; P-III, n = 6/29). Moreover, ROC curves were constructed to identify the cut-off for analytical parameters within the HR-CLL patient subsets, but none of the serum analytes were found differently expressed in the patient subgroups (data not shown).

A multivariate network based on the most informative markers was constructed, with the position of each HR-CLL patient in the network reflecting the combination of the most informative markers (del(11q), del(17p)/*TP53* mutations, CK, splenomegaly, bulky lymphadenopathy, gender). Colors distinguish the particular diagnostic subgroups. The network that was constructed clearly demonstrates that at least three subgroups (P-I, P-II, P-III) based on the similarity and diversity of occurrence of the most informative markers in individual HR-CLL patients can be suggested (Fig. 1). The quality of the clusters that were formed was checked by silhouette analysis (Fig. 1B). The absence of negative values indicates that none of the HR-CLL patients who were investigated was assigned to the wrong cluster and high values indicate that the patients are far from the decision boundary between two neighboring clusters.

To compared patient similarity networks with traditional multivariate methods, we applied on the data from our patient cohort a Factor analysis of mixed data (FAMD), a principal component method dedicated to analyzing a data set containing both quantitative and qualitative variables [26]. Our results showed that FAMD is less suitable to identify the most relevant features (=clinical and laboratory data) (Fig. S1, Tables S1, S2) with more attributes needed to reach the sufficient discrimination power (Fig. S2, Table S2) and to discriminate the particular patient subsets (Fig. S3) as shown by the silhouette analysis (Figs. S2, S4A) comparing to the patient similarity networks (Figs. 1, S4B).

3.2. Analysis of observed major subsets of HR-CLL patients

In order to study the characteristics of the patient subgroups identified by network analysis, we characterized the subsets in terms of the occurrence of prognostic markers, OS, age at diagnosis, relationship to survival, and its association with other clinical and laboratory parameters.

A multivariate patient similarity network revealed three major subgroups of HR-CLL patients, P-I (n = 34, men/women with CK + del (11q) + no del(17p)/*TP53* mutations), P-II (n = 47, predominantly men with del(11q) + no CK + no del(17p)/*TP53* mutations), and P-III (n = 35, men/women with del(17p)/*TP53* mutations, where about 60% of the patients had the co-presence of CK and 20% del(11q)) (Fig. 1A,B). The OS for P-I was 71 mo, for P-II 73 mo, and for P-III 52 mo (Fig. 1C). The biggest differences between the OS in the subsets that were studied were observed within the first 36 mo (Fig. 1D) ($P = 0.017$), with the shortest survival in P-I (Fig. 1E). As evident from the empirical probability of death in these profiles, the death rate in P-I reached 25% in first 12 mo after diagnosis. Importantly, the particular HR-CLL profiles did not differ in the distribution of any other demographic, laboratory, and clinical parameters, mainly because of the high variability of these markers across all subtypes.

Next, we assessed the distribution of the widely used CLL-International Prognostic Index (CLL-IPI) [27], which includes the major prognostic parameters as *TP53* status, *IGHV* mutational status, serum β2-microglobulin, clinical stage, and age, within our subsets. The P-I included predominantly patients with intermediate (54%) and high (38%) risk according CLL-IPI, and small number of patients with low (4%) and very high (4%) risk. The P-II were patients with low (10%), intermediate (53%), and high (37%) risk. The P-III included only patients with high (14%) and very high (86%) risk.

3.3. Subanalysis of subsets in major profiles of HR-CLL patients

To subanalyze the major profiles of HR-CLL patients, we further investigate whether it is also possible to subdivide subgroups P-I and P-III on the basis of the combination of del(11q), del(17p)/*TP53* mutations, CK), bulk, splenomegaly, and gender.

Further subdivision of the P-I profile, with the highest number of deaths within 36 mo after diagnosis, revealed three clearly separated subgroups (Fig. 2A,B); however, no differences in OS and other parameters were observed (Fig. 2C). The calculation of empirical probability of death showed that this profile has the highest death rate of 25% in the first 12 mo after diagnosis increasing to 40% after 36 mo (Fig. 2D).

Within the P-III risk profile, three clearly distinguished profiles were detected: A) predominantly men with *TP53* only or *TP53* + CK (P-III-A, n = 14), B) women with only *TP53* disruption (P-III-B, n = 11), and C) predominantly women with *TP53* + CK (P-III-C, n = 10). Regarding OS, women with only *TP53* disruption (P-III-B) had better OS (119 mo) compared to women with *TP53* + CK (P-III-C; 71 mo) and men with *TP53*, regardless of the presence of CK (P-III-A; 31 mo) ($P = 0.04$) (Fig. 3).

Moreover, the analysis of survival revealed two ultra-HR profiles (P-III-A and P-III-C) with the majority of patients dying within 3 yrs after

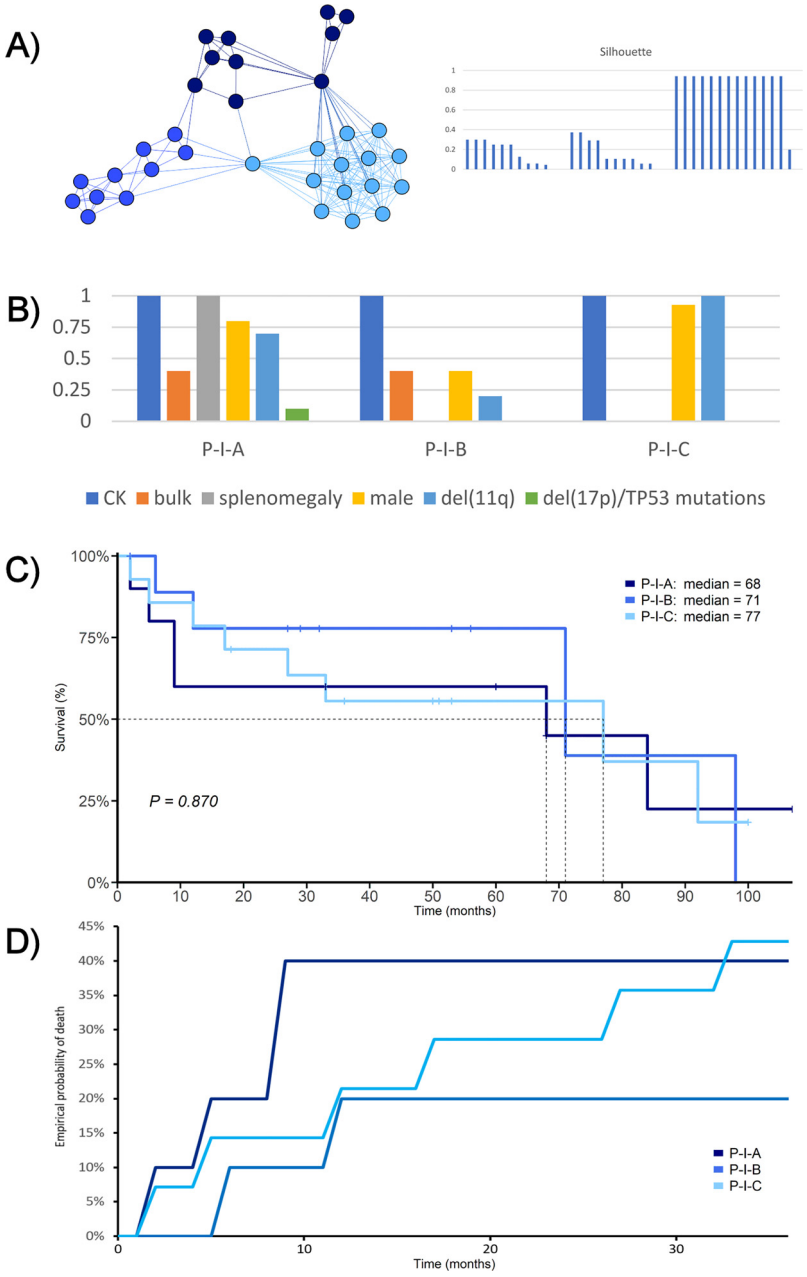

Fig. 2. Network subanalysis for short-term risk profile P-I.
The silhouette analysis of the clusters that were obtained **(A).** The characteristics of patient subgroups based on the combination of del(11q), del(17p)/*TP53* mutations, CK, bulk, splenomegaly, and gender **(B).** Overall survival among patient subgroups **(C).** The empirical probability of death in subgroups **(D).**

diagnosis. As is already evident from the OS curves, the best prognosis in the P-III risk group was observed in women with only *TP53* disruption (P-III-B), where two-thirds of the patients lived longer than 3 yrs (Fig. 3C). The empirical probability of death in particular subsets showed the highest death rate of 58% in P-III-A (Fig. 3D).

3.4. Treatment impact in particular profiles

In order to investigate the influence of the treatment initiation on the OS, we compared those who needed the treatment three or six months after diagnosis with those patients who were treated later. Those patients who required treatment shortly after diagnosis (< 3 mo) had the shortest OS (48 mo) versus those starting treatment later (OS 84 mo, $P = 0.034$), which is evident in all the profiles. The differences in OS between those patients who required treatment < 6 mo (68 mo) versus those treated later (OS 84 mo, $P = 0.14$) did not reach significance (Fig. 4A).

Next, we investigated the impact of adding rituximab to the therapy in the whole cohort of HR-CLL patients, as well as in particular profiles. No difference in OS was observed between patients treated in combination with rituximab (n = 62, OS 83 mo) and those without rituximab (n = 16, OS 70 mo) ($P = 0.73$), showing a positive effect only in long-lived HR-CLL patients after 70 mo of follow-up (Fig. 4B). The addition of rituximab did not improve OS in any prognostic profiles ($P = 0.98$).

Further, we were interested in whether particular profiles are associated with fludarabine refractoriness. Of 61 patients treated with a fludarabine-based regimen, 25 (41%) were refractory. Of the fludarabine-refractory patients, 29.4% belonged to P-I, 40.0% to P-II, and 83.3% to P-III. The fludarabine-refractory patients had a poor prognosis with OS 58 mo, in contrast to fludarabine-sensitive patients, with OS

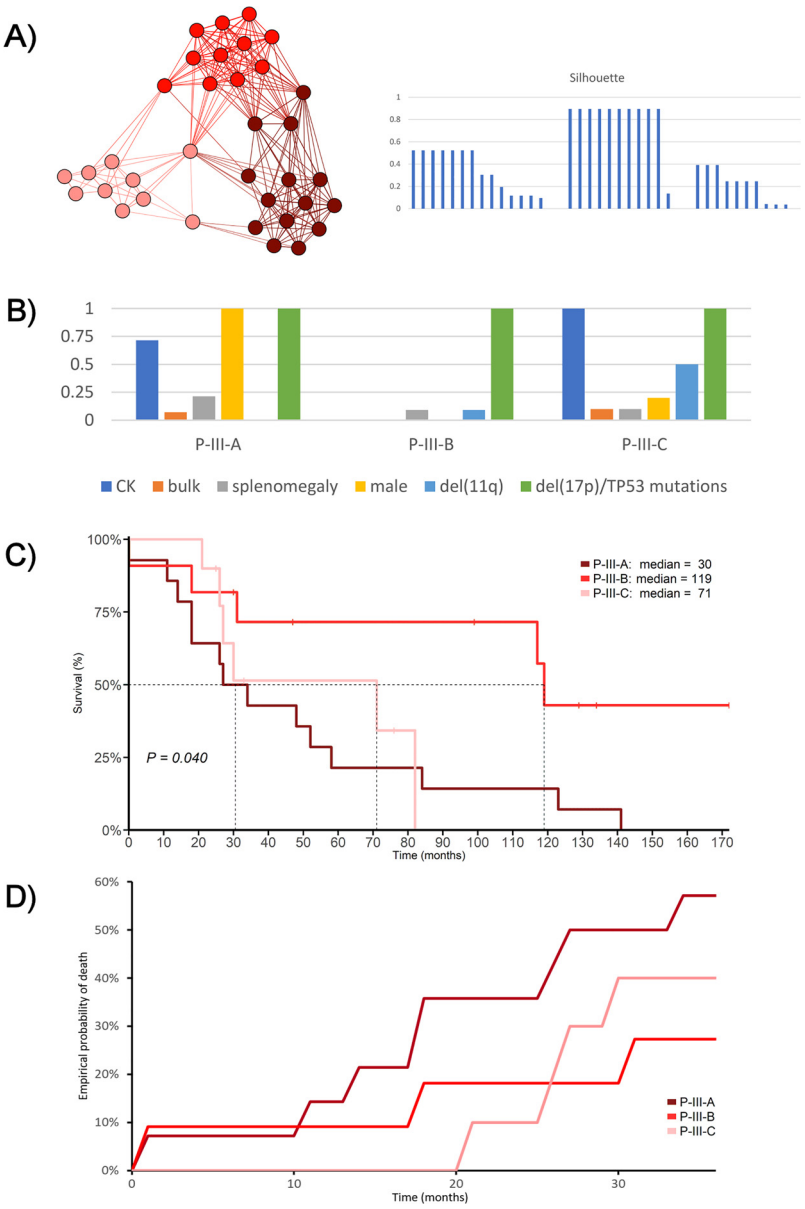

Fig. 3. Network subanalysis for risk profile P-III.
The silhouette analysis of the clusters that were obtained **(A)**. The characteristics of patient subgroups based on the combination of del(11q), del(17p)/*TP53* mutations, CK, bulk, splenomegaly, and gender **(B)**. Overall survival among patient subgroups **(C)**. The empirical probability of death in subgroups **(D)**.

101 mo ($P = 0.003$). Moreover, those patients treated with a fludarabine-based regimen had better outcomes than the others treated with regimens without fludarabine (R-CHOP), 82 vs 48 mo respectively ($P = 0.42$).

4. Discussion

Our study further refined subsets of HR-CLL patients using multivariate patient similarity network on spectrum of genetic, laboratory, and clinical parameters assessed at the time of diagnosis. Besides confirmation of known prognostic factors in which responses to chemoimmunotherapy are traditionally dismal, we identified further subsets of patients for precision medicine armed with an armamentarium of novel targeted drugs.

Nowadays, there is a challenge to further stratify HR-CLL patients, as different patient subgroups may profit from different novel molecules or their combinations as well as different first-line regimens. Taking into account that multivariate data analysis may stratify patients more precisely than univariate analyses [28], we applied a multivariate patient similarity network in order to assess the prognostic values of genetic, laboratory, and clinical parameters (=features) for the stratification of HR-CLL patients. As already shown by recent paper [29] and our previous studies [30–32], this approach is able to select the most informative parameters and their combinations, form subgroups of patients (clusters) based on the similarity of a spectrum of diverse features that are analyzed, and visualize the relationships between the parameters and patients. Moreover, the quality of the clusters (=subgroups) that are formed may be checked by the silhouettes and modularity values, thus ensuring the robustness and quality of the subgroups. When comparing to traditional multivariate methods (e.g. PCA - principal component analysis), the big advantage of used patient similarity network and its clustering is the independence on a pre-set number and size of the clusters, visualization of relationships between patients, selection of the most relevant features, possibility to re-analyse each cluster (= patient subsets) and excellent model interpretability [29,31,32].

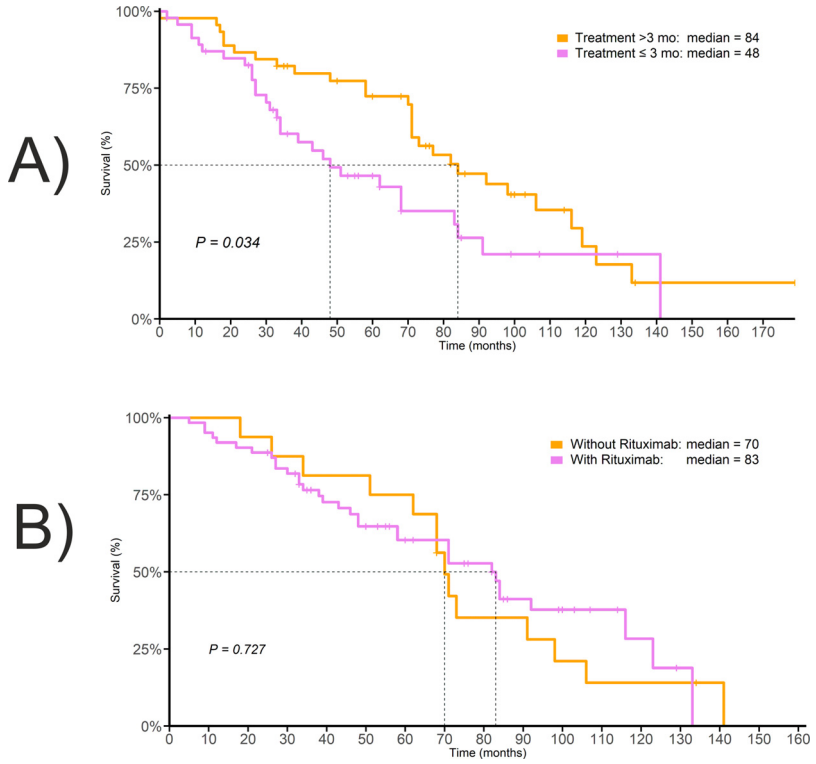

Fig. 4. The treatment impact in HR-CLL patients.
Comparison of overall survival between HR-CLL patients treated < 3 mo and those treated later after the time of the diagnosis **(A)** and those receiving first-line treatment with/without rituximab **(B)**.

Of the demographic, laboratory, and clinical parameters collected at the time of diagnosis, the combination of genetic aberrations (del(11q), del(17p)/*TP53* disruption and CK), bulk, splenomegaly, and gender showed the highest prognostic values for further stratification of HR-CLL. Based on these key prognostic variables, the multivariate patient similarity network revealed three major prognostically different subgroups of HR-CLL patients. The best prognosis was achieved for the profile P-II, consisting predominantly of men with only del(11q). This group had OS 73 mo and the longest average survival of all the major subgroups. Our results are in agreement with a recent study on 1044 CLL patients from a population-based cohort in British Columbia, Canada, which showed that patients with del(11q) have long OS [33]. Compared to other subgroups, the death rate in the first 3 yrs was low, accounting for less than one-quarter, which was mainly evident in older patients.

The next subgroup (P-I) included patients with CK, with two-thirds being men, often with the co-occurrence of del(11q) and bulk disease. Recently, the CK at diagnosis or disease progression emerged as an independent predictor of inferior TTFT and shorter OS [16,34]. The mechanism responsible for the unfavorable prognosis in a heterogeneous group of CKs in CLL remains to be clarified, though it was shown that genetic complexity is associated with telomere shortening [35]. The OS of our patients with CK was 71 mo, comparable with P-II patients with only del(11q). However, a short-term follow-up of 3 yrs showed the shortest OS in this group compared to other subgroups, with the highest death rate being 25% in the first 12 mo after diagnosis. This observation highlights the selection of first-line therapy for those patients. Subgrouping using a network clustering approach revealed three subsets within P-I group, with no difference in OS. This observation further supports the opinion that CK represents a strong independent prognostic factor in CLL [36,37]. It has even been suggested that a genetic instability or dysregulation in CKs may overcome the prognostic impact of mutations in CLL-associated genes, except *TP53* mutations [15,38].

The prognostically worst profile (P-III) consisted of patients with del (17p)/*TP53* mutations, with OS 52 mo. This observation is in line with numerous studies reporting that disruption of the *TP53* pathway is associated with poor treatment response, short PFS and OS on immunochemotherapy and a generally bad prognosis [16,39–41]. In this group there were twice as many women as men, and about 60% of the patients had the co-presence of CK and 20% del(11q). One-quarter of our HR patients in the P-III profile died within 3 yrs after the diagnosis. In order to investigate whether the marked inter-individual differences in survival may be linked to a particular pattern, we further subdivided these patients using a patient similarity network and detected three subsets with marked differences in OS. and detected three subsets with marked differences in OS. The first subset (P-III-A) consisted predominantly of men with *TP53* disruption, with 75% of them having CK. The other two subsets included predominantly women with only *TP53* disruption (P-III-B) and those with *TP53* disruption + CK (P-III-C). The best prognosis (OS 116 mo) was observed in women with only *TP53* disruption, where two-thirds of the patients lived longer than 3 yrs and their OS far exceeded the OS in other major groups. The two ultra-HR-groups included men with *TP53* disruption with/without CK (OS 31) and women with *TP53* disruption and CK (OS 71), with about half of the patients dying within 3 yrs after diagnosis. Those ultra-HR-CLL patients do not profit from immunochemotherapy regimens, and thus novel targeted therapies and their combinations should be suggested in first-line treatment.

Next, we investigated whether the particular patient subgroups differ in terms of treatment response. Our analysis revealed that those patients who required treatment shortly after diagnosis (< 3 mo) had the shortest OS (48 mo) versus those treated later (84 mo). In our cohort of HR-CLL patients, improved outcomes in terms of OS were achieved in patients treated with a fludarabine-based regimen in comparison to other regimens, irrespective of the profile. Moreover, the adding of rituximab to the fludarabine-based regimen did not improve OS in our patients. Similarly, the data on a large population-based

cohort did not observe any differences in OS between CLL patients receiving fludarabine with or without rituximab [33]. On the other hand, our data suggests a positive effect of rituximab in long-lived HR-CLL patients (> 70 mo), which thus deserves further investigation. In line with other studies [9,42,43], we observed the highest rate of fludarabine-refractory patients in cases with *TP53* disruption (> 80%) comparing to patients with del(11q) and those with CK. Numerous studies already reported that patients with *TP53* deletions or mutations are typically resistant to immunochemotherapy, while fludarabine-based chemotherapy with rituximab may be efficient in previously untreated CLL patients with del(11q) [9,42–44].

We are aware that this study has several limitations. Firstly, our real-life cohort was modest, thus further studies on larger patient cohorts are needed. In addition, the prognosis for revealed subsets of patients was evaluated only for the treatment of immunochemotherapy, as new pathway inhibitors were only available in clinical trials during the enrollment of our patients. Therefore, further studies should evaluate the prognosis of nominated subsets of HR-CLL patients in the context of new targeted drugs and combinations thereof.

5. Conclusion

Our data revealed three major subsets within HR-CLL patients on subgroups based on their similarity in clinical/laboratory characteristics. Subanalysis identified two ultra-HR-CLL subsets of i) men with *TP53* disruption with/without CK and ii) women with *TP53* disruption and CK when treated with immunochemotherapy. Moreover, very poor short-term outcomes were achieved in patients with CK + del(11q). In addition to the known risk-factors associated with a poor response to immunochemotherapy, this study further refined subgroups of HR-CLL patients. Finally, we showed for the first time in hemato-oncology the usefulness of the multivariate patient similarity networks for patient stratification, not only in CLL. This approach shows the potential for clinical implementation of precision medicine, which is especially important in view of an armamentarium of novel targeted drugs.

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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.2019.02.005.

References

[1] C. Vetro, T. Haferlach, M. Meggendorfer, S. Jeromin, C.R. Baer, N. Nadarajah, W. Kern, C. Haferlach, In CLL with Normal Karyotype By Conventional and Genomic Array Karyotyping the Prognostic Impact of an Unmutated IGHV Status Is Stronger Than the Impact of Gene Mutations, Blood 128 (2016) 4357–4357.

[2] P.A. Thompson, C.S. Tam, S.M. O'Brien, W.G. Wierda, F. Stingo, W. Plunkett, S.C. Smith, H.M. Kantarjian, E.J. Freireich, M.J. Keating, Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in IGHV-mutated chronic lymphocytic leukemia, Blood 127 (2016) 303–309, https://doi.org/10.1182/blood-2015-09-667675.

[3] D.A. Landau, E. Tausch, A.N. Taylor-Weiner, C. Stewart, J.G. Reiter, J. Bahlo, S. Kluth, I. Bozic, M. Lawrence, S. Böttcher, S.L. Carter, K. Cibulskis, D. Mertens, C.L. Sougnez, M. Rosenberg, J.M. Hess, J. Edelmann, S. Kless, M. Kneba, M. Ritgen, A. Fink, K. Fischer, S. Gabriel, E.S. Lander, M.A. Nowak, H. Döhner, M. Hallek, D. Neuberg, G. Getz, S. Stilgenbauer, C.J. Wu, Mutations driving CLL and their evolution in progression and relapse, Nature 526 (2015) 525–530, https://doi.org/10.1038/nature15395.

[4] A. Puiggros, G. Blanco, B. Espinet, Genetic abnormalities in chronic lymphocytic leukemia: where we are and where we go, Biomed Res. Int. 2014 (2014) 435983, , https://doi.org/10.1155/2014/435983.

[5] A. Mina, J. Sandoval Sus, E. Sleiman, J. Pinilla-Ibarz, F.T. Awan, M.A. Kharfan-Dabaja, Using prognostic models in CLL to personalize approach to clinical care: are we there yet? Blood Rev. 32 (2018) 159–166, https://doi.org/10.1016/j.blre.2017.10.003.

[6] M.R. Grever, D.M. Lucas, G.W. Dewald, D.S. Neuberg, J.C. Reed, S. Kitada, I.W. Flinn, M.S. Tallman, F.R. Appelbaum, R.A. Larson, E. Paietta, D.F. Jelinek, J.G. Gribben, J.C. Byrd, Comprehensive assessment of genetic and molecular features predicting outcome in patients with chronic lymphocytic leukemia: results from the US Intergroup Phase III Trial E2997, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 25 (2007) 799–804, https://doi.org/10.1200/JCO.2006.08.3089.

[7] J. Delgado, B. Espinet, A.C. Oliveira, P. Abrisqueta, J. de la Serna, R. Collado, J. Loscertales, M. Lopez, J.A. Hernandez-Rivas, C. Ferra, A. Ramirez, J.M. Roncero, C. Lopez, A. Aventin, A. Puiggros, E. Abella, F. Carbonell, D. Costa, A. Carrio, M. Gonzalez, on behalf of the Grupo Español de Leucemia Linfatica Cronica (GELLC) y Grupo Español de Citogenetica Hematologica (GECGH), Chronic lymphocytic leukaemia with 17p deletion: a retrospective analysis of prognostic factors and therapy results, Br. J. Haematol. 157 (2012) 67–74, https://doi.org/10.1111/j.1365-2141.2011.09000.x.

[8] D. Rossi, B. Gerber, G. Stüssi, Predictive and prognostic biomarkers in the era of new targeted therapies for chronic lymphocytic leukemia, Leuk. Lymphoma 58 (2017) 1548–1560, https://doi.org/10.1080/10428194.2016.1250264.

[9] K. Fischer, J. Bahlo, A.M. Fink, V. Goede, C.D. Herling, P. Cramer, P. Langerbeins, J. von Tresckow, A. Engelke, C. Maurer, G. Kovacs, M. Herling, E. Tausch, K.-A. Kreuzer, B. Eichhorst, S. Böttcher, J.F. Seymour, P. Ghia, P. Marlton, M. Kneba, C.-M. Wendtner, H. Döhner, S. Stilgenbauer, M. Hallek, Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial, Blood 127 (2016) 208–215, https://doi.org/10.1182/blood-2015-06-651125.

[10] I. Cordone, S. Masi, F.R. Mauro, S. Soddu, O. Morsilli, T. Valentini, M.L. Vegna, C. Guglielmi, F. Mancini, S. Giuliacci, A. Sacchi, F. Mandelli, R. Foa, p53 expression in B-cell chronic lymphocytic leukemia: a marker of disease progression and poor prognosis, Blood 91 (1998) 4342–4349.

[11] H. Döhner, S. Stilgenbauer, A. Benner, E. Leupolt, A. Kröber, L. Bullinger, K. Döhner, M. Bentz, P. Lichter, Genomic aberrations and survival in chronic lymphocytic leukemia, N. Engl. J. Med. 343 (2000) 1910–1916, https://doi.org/10.1056/NEJM200012283432602.

[12] T. Zenz, D. Mertens, R. Küppers, H. Döhner, S. Stilgenbauer, From pathogenesis to treatment of chronic lymphocytic leukaemia, Nat. Rev. Cancer 10 (2010) 37–50, https://doi.org/10.1038/nrc2764.

[13] R. Marasca, R. Maffei, S. Martinelli, S. Fiorcari, J. Bulgarelli, G. Debbia, D. Rossi, F.M. Rossi, G.M. Rigolin, S. Martinelli, V. Gattei, G. Del Poeta, L. Laurenti, F. Forconi, M. Montillo, G. Gaidano, M. Luppi, Clinical heterogeneity of de novo 11q deletion chronic lymphocytic leukaemia: prognostic relevance of extent of 11q deleted nuclei inside leukemic clone, Hematol. Oncol. 31 (2013) 88–95, https://doi.org/10.1002/hon.2028.

[14] P. Starostik, T. Manshouri, S. O'Brien, E. Freireich, H. Kantarjian, M. Haidar, S. Lerner, M. Keating, M. Albitar, Deficiency of the ATM protein expression defines an aggressive subgroup of B-cell chronic lymphocytic leukemia, Cancer Res. 58 (1998) 4552–4557.

[15] P. Baliakas, M. Iskas, A. Gardiner, Z. Davis, K. Plevova, F. Nguyen-Khac, J. Malcikova, A. Anagnostopoulos, S. Glide, S. Mould, K. Stepanovska, M. Brejcha, C. Belessi, F. Davi, S. Pospisilova, A. Athanasiadou, K. Stamatopoulos, D. Oscier, Chromosomal translocations and karyotype complexity in chronic lymphocytic leukemia: a systematic reappraisal of classic cytogenetic data, Am. J. Hematol. 89 (2014) 249–255, https://doi.org/10.1002/ajh.23618.

[16] G.M. Rigolin, L. Formigaro, M. Cavallari, F.M. Quaglia, E. Lista, A. Urso, E. Guardalben, S. Martinelli, E. Saccenti, C. Bassi, L. Lupini, M.A. Bardi, E. Volta, E. Tammiso, A. Melandri, M. Negrini, F. Cavazzini, A. Cuneo, An extensive molecular cytogenetic characterization in high-risk chronic lymphocytic leukemia identifies karyotype aberrations and TP53 disruption as predictors of outcome and chemorefractoriness, Oncotarget 8 (2017) 28008–28020, https://doi.org/10.18632/oncotarget.15883.

[17] S.M. Jaglowski, A.S. Ruppert, N.A. Heerema, A. Bingman, J.M. Flynn, M.R. Grever, J.A. Jones, P. Elder, S.M. Devine, J.C. Byrd, L.A. Andritsos, Complex karyotype predicts for inferior outcomes following reduced-intensity conditioning allogeneic transplant for chronic lymphocytic leukaemia, Br. J. Haematol. 159 (2012) 82–87, https://doi.org/10.1111/j.1365-2141.2012.09239.x.

[18] P.A. Thompson, S.M. O'Brien, W.G. Wierda, A. Ferrajoli, F. Stingo, S.C. Smith, J.A. Burger, Z. Estrov, N. Jain, H.M. Kantarjian, M.J. Keating, Complex karyotype is a stronger predictor than del(17p) for an inferior outcome in relapsed or refractory chronic lymphocytic leukemia patients treated with ibrutinib-based regimens, Cancer 121 (2015) 3612–3621, https://doi.org/10.1002/cncr.29566.

[19] M. Jarosova, M. Hruba, A. Oltova, K. Plevova, L. Kruzova, E. Kriegova, R. Fillerova, E. Koritakova, M. Doubek, D. Lysak, V. Prochazka, M. Mraz, K. Indrak, T. Papajik, Chromosome 6q deletion correlates with poor prognosis and low relative expression of FOXO3 in chronic lymphocytic leukemia patients, Am. J. Hematol. 92 (2017) E604–E607, https://doi.org/10.1002/ajh.24852.

[20] A. Obr, V. Procházka, A. Jirkuvová, H. Urbánková, E. Kriegova, P. Schneiderová, M. Vatolíková, T. Papajík, TP53 mutation and complex karyotype portends a dismal prognosis in mantle cell lymphoma, Clin. Lymphoma Myeloma Leuk. 18 (2018) 762–768, https://doi.org/10.1016/j.clml.2018.07.282.

[21] L. Raskova Kafkova, V. Navrkalova, M. Jarosova, T. Loja, J. Chovancova,

J. Kucerova, E. Kriegova, V. Prochazka, Z. Novak, D. Simkova, S. Pospisilova, V. Divoky, Ability to downregulate the level of cyclin-dependent kinase inhibitor p27Kip1 after DNA damage is retained in chronic lymphocytic leukemia cells with functional ATM/p53 signaling pathway, Leuk. Lymphoma 58 (2017) 199–203, https://doi.org/10.1080/10428194.2016.1187276.

[22] E. Ochodkova, S. Zehnalova, M. Kudelka, Y. Cao, J. Chen (Eds.), Graph Construction Based on Local Representativeness, Comput. Comb., Springer International Publishing, 2017, pp. 654–665.

[23] S. van den Elzen, J.J. van Wijk, Multivariate network exploration and presentation: from detail to overview via selections and aggregations, IEEE Trans. Vis. Comput. Graph. 20 (2014) 2310–2319, https://doi.org/10.1109/TVCG.2014.2346441.

[24] V.D. Blondel, J.-L. Guillaume, R. Lambiotte, E. Lefebvre, Fast unfolding of communities in large networks, J. Stat. Mech. Theory Exp. 2008 (2008), https://doi.org/10.1088/1742-5468/2008/10/P10008 P10008.

[25] P.J. Rousseeuw, Silhouettes: a graphical aid to the interpretation and validation of cluster analysis, J. Comput. Appl. Math. 20 (1987) 53–65, https://doi.org/10.1016/0377-0427(87)90125-7.

[26] J. Pagès, Multiple Factor Analysis by Example Using R, Chapman & Hall/CRC. The R Series London, 2014 272 pp..

[27] International CLL-IPI working group, An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data, Lancet Oncol. 17 (2016) 779–790, https://doi.org/10.1016/S1470-2045(16)30029-8.

[28] S. Martinelli, A. Cuneo, L. Formigaro, M. Cavallari, E. Lista, F.M. Quaglia, M. Ciccone, A. Bardi, E. Volta, E. Tammiso, E. Saccenti, O. Sofritti, G. Daghia, M. Negrini, M. Dabusti, P. Tomasi, S. Moretti, F. Cavazzini, G.M. Rigolin, Identifying high-risk chronic lymphocytic leukemia: a pathogenesis-oriented appraisal of prognostic and predictive factors in patients treated with chemotherapy with or without immunotherapy, Mediterr. J. Hematol. Infect. Dis. 8 (2016) e2016047, , https://doi.org/10.4084/MJHID.2016.047.

[29] S. Pai, G.D. Bader, Patient similarity networks for precision medicine, J. Mol. Biol. 430 (2018) 2924–2938, https://doi.org/10.1016/j.jmb.2018.05.037.

[30] R. Fillerova, J. Gallo, M. Radvansky, V. Kraiczova, M. Kudelka, E. Kriegova, Excellent diagnostic characteristics for ultrafast gene profiling of DEFA1-IL1B-LTF in detection of prosthetic joint infections, J. Clin. Microbiol. 55 (2017) 2686–2697, https://doi.org/10.1128/JCM.00558-17.

[31] D. Starostka, E. Kriegova, M. Kudelka, P. Mikula, S. Zehnalova, M. Radvansky, T. Papajik, D. Kolacek, K. Chasakova, H. Talianova, Quantitative assessment of informative immunophenotypic markers increases the diagnostic value of immunophenotyping in mature CD5-positive B-cell neoplasms, Cytometry B Clin. Cytom. 94 (2018) 576–587, https://doi.org/10.1002/cyto.b.21607.

[32] E. Kriegova, G. Manukyan, Z. Mikulkova, G. Gabcova, M. Kudelka, P. Gajdos, J. Gallo, Gender-related differences observed among immune cells in synovial fluid in knee osteoarthritis, Osteoarthritis Cartilage 26 (2018) 1247–1256, https://doi.org/10.1016/j.joca.2018.04.016.

[33] J. Goy, T.L. Gillan, H. Bruyere, S.J.T. Huang, M. Hrynchak, A. Karsan, K. Ramadan, J. Connors, C.L. Toze, A.S. Gerrie, Chronic lymphocytic leukemia patients with deletion 11q have a short time to requirement of first-line therapy, but long overall survival: results of a population-based cohort in British Columbia, Canada, Clin. Lymphoma Myeloma Leuk 17 (2017) 382–389, https://doi.org/10.1016/j.clml.2017.04.001.

[34] C.D. Herling, M. Klaumünzer, C.K. Rocha, J. Altmüller, H. Thiele, J. Bahlo, S. Kluth, G. Crispatzu, M. Herling, J. Schiller, A. Engelke, E. Tausch, H. Döhner, K. Fischer, V. Goede, P. Nürnberg, H.C. Reinhardt, S. Stilgenbauer, M. Hallek, K.-A. Kreuzer, Complex karyotypes and KRAS and POT1 mutations impact outcome in CLL after chlorambucil-based chemotherapy or chemoimmunotherapy, Blood 128 (2016) 395–404, https://doi.org/10.1182/blood-2016-01-691550.

[35] G. Roos, A. Kröber, P. Grabowski, D. Kienle, A. Bühler, H. Döhner, R. Rosenquist, S. Stilgenbauer, Short telomeres are associated with genetic complexity, high-risk genomic aberrations, and short survival in chronic lymphocytic leukemia, Blood 111 (2008) 2246–2252, https://doi.org/10.1182/blood-2007-05-092759.

[36] G.M. Rigolin, I. del Giudice, L. Formigaro, E. Saccenti, S. Martinelli, M. Cavallari, E. Lista, E. Tammiso, E. Volta, L. Lupini, C. Bassi, A. Bardi, O. Sofritti, G. Daghia, F. Cavazzini, M. Marinelli, S. Tavolaro, A. Guarini, M. Negrini, R. Foà, A. Cuneo, Chromosome aberrations detected by conventional karyotyping using novel mitogens in chronic lymphocytic leukemia: clinical and biologic correlations, Genes Chromosomes Cancer 54 (2015) 818–826, https://doi.org/10.1002/gcc.22293.

[37] G.M. Rigolin, E. Saccenti, E. Guardalben, M. Cavallari, L. Formigaro, B. Zagatti, A. Visentin, F.R. Mauro, E. Lista, C. Bassi, L. Lupini, F.M. Quaglia, A. Urso, M.A. Bardi, L. Bonaldi, E. Volta, E. Tammiso, C. Ilari, L. Cafforio, A. Melandri, F. Cavazzini, M. Negrini, G. Semenzato, L. Trentin, R. Foà, A. Cuneo, In chronic lymphocytic leukaemia with complex karyotype, major structural abnormalities identify a subset of patients with inferior outcome and distinct biological characteristics, Br. J. Haematol. 181 (2018) 229–233, https://doi.org/10.1111/bjh.15174.

[38] S. Jeromin, S. Weissmann, C. Haferlach, F. Dicker, K. Bayer, V. Grossmann, T. Alpermann, A. Roller, A. Kohlmann, T. Haferlach, W. Kern, S. Schnittger, *SF3B1* mutations correlated to cytogenetics and mutations in *NOTCH1, FBXW7, MYD88, XPO1* and *TP53* in 1160 untreated CLL patients, Leukemia 28 (2014) 108–117, https://doi.org/10.1038/leu.2013.263.

[39] B. Eichhorst, A.-M. Fink, J. Bahlo, R. Busch, G. Kovacs, C. Maurer, E. Lange, H. Köppler, M. Kiehl, M. Sökler, R. Schlag, U. Vehling-Kaiser, G. Köchling, C. Plöger, M. Gregor, T. Plesner, M. Trneny, K. Fischer, H. Döhner, M. Kneba, C.-M. Wendtner, W. Klapper, K.-A. Kreuzer, S. Stilgenbauer, S. Böttcher, M. Hallek, First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial, Lancet Oncol. 17 (2016) 928–942, https://doi.org/10.1016/S1470-2045(16)30051-1.

[40] M. Hallek, K. Fischer, G. Fingerle-Rowson, A.M. Fink, R. Busch, J. Mayer, M. Hensel, G. Hopfinger, G. Hess, U. von Grünhagen, M. Bergmann, J. Catalano, P.L. Zinzani, F. Caligaris-Cappio, J.F. Seymour, A. Berrebi, U. Jäger, B. Cazin, M. Trneny, A. Westermann, C.M. Wendtner, B.F. Eichhorst, P. Staib, A. Bühler, D. Winkler, T. Zenz, S. Böttcher, M. Ritgen, M. Mendila, M. Kneba, H. Döhner, S. Stilgenbauer, Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial, Lancet 376 (2010) 1164–1174, https://doi.org/10.1016/S0140-6736(10)61381-5.

[41] A. Puiggros, R. Collado, M.J. Calasanz, M. Ortega, N. Ruiz-Xivillé, A. Rivas-Delgado, E. Luño, T. González, B. Navarro, M. García-Malo, A. Valiente, J.Á. Hernández, M.T. Ardanaz, M.Á. Piñan, M.L. Blanco, M. Hernández-Sánchez, A. Batlle-López, R. Salgado, M. Salido, A. Ferrer, P. Abrisqueta, E. Gimeno, E. Abella, C. Ferrá, M.J. Terol, F. Ortuño, D. Costa, C. Moreno, F. Carbonell, F. Bosch, J. Delgado, B. Espinet, Patients with chronic lymphocytic leukemia and complex karyotype show an adverse outcome even in absence of TP53/ATM FISH deletions, Oncotarget 8 (2017) 54297–54303, https://doi.org/10.18632/oncotarget.17350.

[42] A.-M. Tsimberidou, M.J. Keating, Treatment of fludarabine-refractory chronic lymphocytic leukemia, Cancer 115 (2009) 2824–2836, https://doi.org/10.1002/cncr.24329.

[43] S. Stilgenbauer, A. Schnaiter, P. Paschka, T. Zenz, M. Rossi, K. Döhner, A. Bühler, S. Böttcher, M. Ritgen, M. Kneba, D. Winkler, E. Tausch, P. Hoth, J. Edelmann, D. Mertens, L. Bullinger, M. Bergmann, S. Kless, S. Mack, U. Jäger, N. Patten, L. Wu, M.K. Wenger, G. Fingerle-Rowson, P. Lichter, M. Cazzola, C.M. Wendtner, A.M. Fink, K. Fischer, R. Busch, M. Hallek, H. Döhner, Gene mutations and treatment outcome in chronic lymphocytic leukemia: results from the CLL8 trial, Blood 123 (2014) 3247–3254, https://doi.org/10.1182/blood-2014-01-546150.

[44] D.L. Van Dyke, L. Werner, L.Z. Rassenti, D. Neuberg, E. Ghia, N.A. Heerema, P. Dal Cin, M. Dell Aquila, C. Sreekantaiah, A.W. Greaves, T.J. Kipps, N.E. Kay, The Dohner fluorescence in situ hybridization prognostic classification of chronic lymphocytic leukaemia (CLL): the CLL Research Consortium experience, Br. J. Haematol. 173 (2016) 105–113, https://doi.org/10.1111/bjh.13933.