Results of Polish Adult Leukemia Study Group (PALG) project assessing TP53 mutations with next-generation sequencing technology in relapsed and refractory chronic lymphocytic leukemia patients — an 18-month update

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Abstract

Introduction and methods: In chronic lymphocytic leukemia (CLL), molecular and cytogenetic diagnostics are crucial for the determination of accurate prognosis and treatment choice. Among different genetic aberrations, del(17p13) or TP53 mutations constitute high-risk factors, and early identification of such defects is a high priority for CLL patients. While cytogenetic diagnostics is well-established and accessible for the majority of CLL patients in Poland, molecular diagnostics of TP53 mutations is performed only in a few ERIC-certified centers (eight as of September 2020), and only two of these employ next-generation sequencing (NGS) for routine analysis of TP53 status in CLL patients. Here we report the interim results of a project assessing TP53 mutations with NGS technology in relapsed or refractory CLL patients with confirmed negative del(17p13) status.

249 patients from 32 clinical centers were included in the study.

Results: NGS analysis revealed TP53 mutations in 42/249 (17%) patients, half of whom (21/249, 8.5%) had subclonal mutations (VAF ≤10%). These results are in line with published data in relapsed/refractory CLL patients.

Conclusions: The results of the project demonstrated the feasibility and accuracy of NGS testing in CLL patients despite several initial logistical and technical obstacles. Our study also proved that, with appropriate funding, CLL patients from any hematological center in Poland can have access to state-of-the-art molecular diagnostics.

Key words: chronic lymphocytic leukemia, TP53 mutations, next-generation sequencing, targeted sequencing

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Introduction

Chronic lymphocytic leukemia (CLL) is the most commonly diagnosed leukemia in adults in the Western world, with a median age at diagnosis of 72 years. Incidence rates are similar in Europe and North America, ranging from 4 to 6 per 100,000 persons per year. In 2014, there were more than 12,000 new cases detected in the European Union (EU) (including approximately 2,000 cases in Poland) and more than 15,000 cases in United States (US) with more than 70% of patients being older than 65 at diagnosis. The disease is characterized by a very heterogeneous clinical course ranging from indolent cases which never require treatment, to rapidly progressing patients in whom therapy needs to be urgently initiated at diagnosis [1]. So far, numerous clinical and molecular prognosticators have been identified in CLL. However, molecular aberrations affecting the TP53 gene leading to dysfunction of p53 protein remain the most recognized factor associated with poor disease outcome [1, 2].

Common mechanisms of TP53 defects in CLL include the deletion of the short arm of chromosome 17, del(17p13), that encompasses the TP53 locus, as well as loss-of-function mutations. Del(17p13) is routinely tested by interphase fluorescence in situ hybridization (FISH) in CLL patients at treatment initiation, along with a panel of other recurrent cytogenetic abnormalities such as del(13q14), del(11q) and tris(12). Of these aberrations, del(17p13) is associated with the shortest median overall survival (OS), only 32 months when classical chemo-therapeutics are used as a treatment modality [3]. Del(17p13) is diagnosed in approximately 5–10% of treatment-naive CLL cases, however its incidence increases in relapsed or refractory CLL (RR-CLL) patients [3]. The introduction of anti-CD20 antibodies-based immuno-chemotherapy did not improve the prognosis of patients with p53 defects. Combination therapy with rituximab and purine nucleoside analogs rarely resulted in the achievement of complete remission (CR), while median progression-free survival (PFS) reached only 11–14 months [4]. Similarly, a rituximab and bendamustine regimen resulted in even poorer outcomes, with a median PFS of 8.7 months [5]. On the other hand, the prognosis of CLL patients with TP53 abnormalities has dramatically improved with the development of novel agents inhibiting B-cell receptor (BCR) signaling e.g. Bruton’s tyrosine kinase (BTK) inhibitor ibrutinib and the phosphoinositide 3-kinase (PI3K) delta inhibitor idelalisib [2, 6–9]. In addition, venetoclax, a B-cell lymphoma 2 (BCL2) antagonist, has also shown a remarkable activity in heavily pretreated RR-CLL including patients with p53 pathway defects [10].

In up to 90% of cases with del(17p13), a mutation in the second TP53 allele is also present [11, 12]. This mechanism leads to biallelic loss of the functional TP53 gene and thus to complete inactivation of p53 tumor suppressor pathway [11, 12]. Sole TP53 mutations without del(17p13) comprise approximately 30% of all CLL cases with p53 pathway defect, whereas sole del(17p13) without TP53 mutations are diagnosed only in 5% of such cases [11, 13, 14]. Likewise, sole del(17p13) or TP53 mutations are associated...
with CLL refractoriness or short responses to classical cytotoxic agents and anti-CD20-based immunochemotherapy, and poor outcomes [11, 15, 16].

Direct Sanger sequencing is the most commonly used method for TP53 mutations assessment in CLL. In the majority of cases, Sanger sequencing is capable of detecting TP53 mutation only if the mutant allele is present at a minimum level of 10–15%, and such mutations are denoted as clonal. Importantly, smaller amounts of TP53-mutated alleles (subclonal TP53 mutations) may thus remain not detected. Nevertheless, according to international guidelines, Sanger-based mutational TP53 analysis has been the recommended method in routine CLL diagnostics preceding treatment qualification along with FISH for detection of del(17p13) [1, 17, 18].

Recent years have brought immense progress in the diagnostics of CLL [1, 2]. Firstly, the introduction of next-generation sequencing (NGS) allowed for the identification of novel genes associated with CLL pathogenesis and prognosis. Secondly, NGS helped to recognize clonal evolution and emergence of drug-resistant clones evolving during treatment [19–22]. Most importantly, the application of NGS allows for the identification of small subclones of CLL cells with TP53 mutations, which would be missed using Sanger sequencing [23]. This seems clinically important as patients harboring small TP53 mutated subclones were characterized by poorer survival than patients carrying clonal TP53 lesions [23]. In addition, TP53 mutated subclones identified before treatment may become the predominant population at the time of CLL relapse and anticipate the development of chemorefractoriness [23]. Although NGS detects significantly more TP53 mutations compared to Sanger sequencing, the method has not yet been introduced in routine clinical work-up of CLL patients due to a lack of standardization and the debatable significance of subclonal TP53 mutations in clinical trials [1, 18].

While detection of del(17p13) by FISH is a part of the standard pretreatment work-up in CLL in most Polish hematology centers, diagnostics of TP53 mutations is much less commonly performed. Furthermore, access to NGS for the identification of prognostic mutations in routine practice is limited in Poland.

Therefore, to address these issues, the Polish Adult Leukemia Group (PALG) has initiated an observational study and educational project aimed primarily at providing Polish hematologists with the possibility of testing TP53 mutations by NGS in RR-CLL. Additional project objectives included sharing knowledge on TP53 mutations diagnostics and assessing the prognostic significance of subclonal TP53 mutations as well as of mutations of other selected genes with a potential role in CLL. Here, we report the preliminary results of the project from the first 18 months after its official launch in October 2018.

Material and methods

Study population

The prospective observational study entitled ‘Assessment of genetic and immunologic prognostic and predictive factors in relapsed and refractory chronic lymphocytic leukemia patients treated with B-cell receptor inhibitors: an observational study of the Polish Adult Leukemia Study Group (PALG)’ was designed by PALG and approved by the Ethics Committee of the Institute of Hematology and Transfusion Medicine in Warsaw (opinion no. 47/2018). The study was conducted in accordance with the provisions of the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice.

Blood or bone marrow samples were collected in participating hematological centers of PALG and sent to the central NGS laboratory (Department of Immunology and Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland). In a few cases, lymph node biopsies were used as biologic material. The inclusion criteria were 1) confirmed relapsed or refractory CLL with a previous one line of treatment and 2) a confirmed negative result towards del(17p13) utilizing FISH. Patients with confirmed del(17p13) upon FISH analysis and confirmed transformation to aggressive lymphoma were excluded from the study. The presence of del(17p13) was diagnosed by interphase fluorescence in situ hybridization (FISH) using commercially available kits such as the Vysis CLL FISH Probe Kit (Abbott Molecular, Chicago, IL, USA) at local cytogenetic laboratories.

DNA isolation and next-generation sequencing

TP53 mutation screening was performed using DNA isolated from blood, bone marrow aspirates, frozen pellet leukocytes or lymph node biopsies. Most blood and bone marrow samples were delivered by a courier company at ambient temperature, stored at 4°C after delivery, and processed twice a week. Frozen cells and tissues were processed immediately after delivery. A DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used to isolate DNA from blood and bone marrow and a Gentra Puregene Tissue Kit (Qiagen) was used to isolate DNA from leukocytes and tissues.

TP53 mutation status was assessed by custom targeted NGS panel. Typically, 500–1,000 ng DNA was converted into genomic libraries using KAPA Hyper Plus (Roche, Basel, Switzerland), subsequently multiplexed up to 24-plex pools and enriched using two different SeqCap EZ (Roche) probe sets, covering 1.8Mb and 1.1Mb genomic sequences, including entire TP53 coding sequence also with flanking intronic regions covering splice acceptor/
donor sites. Captured multiplex libraries were paired-end sequenced on MiSeq FGx, HiSeq1500 or NovaSeq6000 (Illumina, San Diego, CA, USA) instruments (depending on the current availability) using 2 × 150 bp or 2 × 100 bp reads. Mean coverages in range 65.1–674.85 (median: 120.02) and \%ge20 in range 87.2–99.7 (median: 96.6) were achieved for samples used to analyze TP53 mutation status.

Variant discovery was performed according to GATK Best Practices. Variants were filtered using gnomAD database and the internal database of the Department of Medical Genetics and Department of Immunology (Medical University of Warsaw) to remove common genetic variations and sequencing artifacts as described previously by us [24].

The detection limit varied between 1% and 5% depending on the depth of sequencing and sequencing platform which was used (MiSeq FGx, HiSeq 1500 or NovaSeq 6000). Information regarding limit of detection was updated for each sequencing run and included in the final report for each patient. Moreover, all subclonal TP53 mutations (with VAF ≤10%), as well as selected clonal variants, were validated by deep amplicon sequencing and paired-end sequenced as described above (Nextera XT DNA Library Prep Kit, Illumina), and/or Sanger sequencing.

According to the ERIC-TP53 recommendations [18], we adopted codon numbering according to NM_000546.5 (LRG_321t1) reference transcript and used IARC-TP53 and ClinVar databases to assess pathogenicity of detected variants. For variants with unclear pathogenicity status, other databases were also employed such as COSMIC and VarSome.

**Results**

**Patient recruitment**

As of the end of March 2020, within the PALG project we had received biological material from 254 patients with RR-CLL. Several samples were excluded from the study due to del(17p13) (two patients, FISH results delivered after NGS was performed at a later date), Richter’s transformation (two patients, information provided by physicians at a later date) and TP53 analysis using NGS performed two months earlier in another center (one patient). For three patients, NGS was performed twice during the project duration but using two different samples from different time points. For eight patients, the NGS had to be performed twice due to poor coverage in the initial sequencing. In total, 249 patients (260 samples) were included in the TP53 mutation study. Samples included in the study came from 32 centers in Poland (Table I), the cumulative number of samples sent between November 2018 and March 2020 being set out in Figure 1A.
Pre-laboratory errors and hurdles
The most common pre-laboratory errors included: lack of all required documents (in particular results of blood morphology), lack of doctor’s or patient’s signature on the PALG form, expired FISH results (12 months or older before sample collection), and delay in sample delivery (three samples delivered 48 hours after blood collection). Despite these problems and pre-laboratory errors, sequencing was successfully performed even for samples with late delivery (it did not influence the quality of DNA significantly, as subclonal mutations were detected and confirmed in such samples).

NGS findings
NGS analysis showed TP53 mutations in 42/249 (17%) patients. Clonal mutations (VAF >10%) were detected in 21 (8.5%) patients, while another 21 patients (8.5%) harbored subclonal mutations (VAF ≤10%) (Figures 1B and 1C). Eleven patients had two or more mutations, of whom six patients had both clonal and subclonal mutations. A total of 57 pathogenic or probably pathogenic mutations were detected (Figure 2). The most frequent nonsynonymous polymorphism c.215C>G, p.Pro72Arg was detected in 231/249 (93%) patients, but according to the ERIC guidelines [18] it was not reported due to its probably
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benign role and high prevalence. Mutations with VAF lower than 10% detected in 21 patients and mutations in two randomly selected patients with VAF 11–20% for a total of 23 samples were confirmed by deep amplicon sequencing (Nextera, Illumina). In selected patients, the presence of mutations was also confirmed by Sanger sequencing. Representative results of TP53 mutations detected using NGS and Sanger sequencing are shown in Figure 3.

Figure 2. Details of all 57 pathogenic mutations in TP53 gene detected in study: A. Distribution of TP53 mutations among individual exons (pathogenic variants were not detected in exons 2, 3 and 11, testing of these exons was performed although not required); B. Frequency of detected mutations according to their functional consequences. *A pathogenic synonymous variant (c.375G>A, p.Thr125Thr) affecting splicing of TP53 was found in one patient

Figure 3. Representative results of TP53 mutations detected using next-generation sequencing (A and C) and confirmed by Sanger (B) and amplicon (D) sequencing, respectively. A, C, D. Variant allele frequency (VAF, %) and numbers of reads supporting mutated variants are marked with green boxes in IGV screenshots; B. Fragment of chromatogram presented as screenshot from FinchTV software. Red arrow indicates starting point of a frameshift mutation
Educational part of project
As part of the PALG project, a workshop for laboratory diagnosticians was carried out in October 2018, attended by nine participants from nine laboratories (including two from Warsaw, two from Poznan, and one each from Brzozow, Bydgoszcz, Katowice, Krakow, and Wroclaw). The major scope of the workshop focused on the ERIC (European Research Initiative on CLL) guidelines for the detection of TP53 mutations. The aim of this training was to prepare other laboratories to successfully apply for ERIC certification. The workshop provided not only comprehensive tuition in the methods used for the detection of TP53 mutations, but was also partly dedicated to the analysis and interpretation of detected variants.

Discussion
In this prospective, observational study, we evaluated TP53 mutation status using NGS-based approach in RR-CLL patients devoid of del(17p13) by cytogenetics. We identified TP53 mutations in 42 out of 249 (17%) patients. Clonal mutations (VAF >10%) and subclonal mutations (VAF ≤10%) were detected with an equal frequency of 8.5% (21 patients). Numerous studies have demonstrated that sole TP53 mutations in the absence of del(17p13) are present in approximately 5–12% of treatment-naïve CLL cases [25]. Considering the enrichment in p53 pathway defects with an increasing number of administered treatments, del(17p13) and TP53 mutations were identified in up to 44% of fludarabine refractory CLL patients. In this highly pretreated patient cohort, TP53 mutations were found in 37% of patients utilizing Sanger sequencing. Sole TP53 mutations were detected in 12 out of 67 (18%) patients without del(17p13) [15]. This ratio is higher compared to the number of detected clonal TP53 mutations in our study (8.5%), most probably due to initial exclusion from this study of patients harboring del(17p13). However, other factors might be involved and a further analysis will be possible due to the ongoing collection of our patients’ clinical data and utilization of multi-gene sequencing panels in this study.

Interestingly, in our study clonal and subclonal mutations were distributed equally (8.5%) which is comparable to data reported in other studies. Using an ultra-deep NGS sequencing Rossi et al. identified 85 TP53 mutations in 46 out of 309 (14.8%) newly diagnosed CLL patients. In this cohort, 35 mutations were clonal and detectable using Sanger sequencing, but an additional 50 mutations were missed by this method due to low abundance. These subclonal mutations were only detectable by NGS [23]. In another group of 290 treatment-naïve CLL patients, 41 (14%) patients had clonal and 31 (11%) had subclonal TP53 mutations [26].

To date, the prognostic and predictive impact of subclonal TP53 mutations has been unclear and therefore not reflected in clinical guidelines. The recommended level of mutation threshold for clinical decision is currently 10% [18]. However, recent data from the ERIC consortium and different laboratories suggests that this threshold is too high, and should be between 1% and 10% depending on the ability of a given laboratory to detect such mutations. Nevertheless, according to the guidelines, subclonal changes in the TP53 within the range of 5–10% detected utilizing NGS may be optionally reported even though the clinical significance of these mutations is still under debate [21, 23, 25]. In patients treated within the CLL8 trial, the presence of clonal TP53 mutations has been shown to confer resistance to fludarabine-cyclophosphamide (FC) and fludarabine-cyclophosphamide-rituximab (FCR) regimens owing to short PFS and OS [27]. The majority of studies point to the poor prognostic role of TP53 mutations identified using NGS [20, 21, 23, 28–30]. However, in the CLL4 study, subclonal TP53 mutations did not have an impact on patient PFS and OS [26]. On the contrary, the recent study by Brieghel et al. [26] confirmed the prognostic impact of TP53 mutations for patients with a level of detection greater than 1%. This is in line with the currently accepted consensus that the NGS error rate resulting from library preparation, polymerase chain reaction (PCR) enrichment and sequencing ranges from 0.1% to 1%, affecting the maximal sensitivity of detection of such mutations [31]. Considering the results of the above-mentioned studies, further research aimed at identifying the unified cut-off level for subclonal TP53 mutations seems necessary to incorporate these findings into clinical guidelines. On the other hand, subclonality may be misidentified if the level of CLL cells is low in the blood or bone marrow sample. In such cases, even a low level of the detected TP53 mutation may originate from a dominant clone.

In the last decade, NGS technology has not only become a highly effective tool in the discovery of new genetic aberrations in human malignancies, but has also become widely used in comprehensive molecular diagnostics of human tumors. One of the major obstacles to employing this technology more widely is the prohibitive cost of equipment and the level of expertise required to perform the analysis and validate the results. However, in recent years the cost of sequencing has dropped substantially and the availability of NGS platforms become more widespread, especially in universities and research institutes, including in our country. Unfortunately, in Poland most centers employ NGS almost solely for research purposes and only a few clinical departments have constant access to diagnostics with NGS. This raises another issue regarding a lack of awareness of the advantages and limitations of NGS in clinical environments, leading in turn to limited trust in NGS results as a valuable diagnostic method among clinical specialists.

Our study has proved that large scale NGS testing in Poland is feasible and effective. Although it was limited to
the detection of pathogenic variants in one (TP53) gene in a single disease (CLL), the variability of the biological samples, as well as complex mutational landscape of TP53 gene encompassing almost the whole coding sequence (exon 2 to exon 11), raised the difficulty level far beyond a typical one-gene assay. Our study generated results with high sensitivity and high confidence, which were consistent with those already published in similar cohorts of patients. Importantly, the project met with great interest among hematologists and we were able to recruit patients from the majority of hematology departments and hospitals in Poland, 32 in total (see detailed list in Table I).

The cost of the analysis (2,000 PLN which equals approx. 440 Euro) was lower or similar compared to major laboratories offering similar NGS testing in Europe. This was possible by pooling several samples in one genomic DNA library and one sequencing run. However, the downside of such an approach was a longer time from the patient’s sample delivery to the final results, ranging from 2 to 6 weeks. Last but not least, an important benefit of the project was enabling a significant number of patients with poor prognosis the access to targeted therapy with BTK inhibitor, while it is still restricted in Poland to RR-CLL patients with TP53 defects.

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Authors’ contributions

MP — prepared NGS libraries, carried out Sanger sequencing, analyzed NGS data and wrote the manuscript. BP — collected clinical samples, monitored and treated patients analyzed clinical data and wrote the manuscript. MMM — prepared NGS libraries and analyzed NGS data. EMS, AU, ISG, ZSG, AS, DK, RW, DZ, EB, KKT, KW, MOS, JW, MD, WT, DWO, PA, AL — provided clinical samples and analyzed patient data. MR, AP — sequenced NGS libraries. TS — analyzed NGS data and supervised MR and AP. TS — conceived idea of NGS testing, analyzed data, supervised MP and MMM, and wrote the manuscript. KJ — conceived idea of project, coordinated clinical part, and supervised MP and MMM, and wrote the manuscript. All authors — provided critical feedback and contributed to final manuscript.

Conflict of interest

TS — Janssen: Speaker honoraria. KJ — Janssen: Speaker honoraria, advisory board participation, research funding. BP — Janssen: Speaker honoraria, advisory board participation, research funding. TS — Janssen: Speaker honoraria. KJ — Janssen: Speaker honoraria, advisory board participation, research funding.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for Manuscripts submitted to Biomedical Journals.

References

1. Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood. 2018; 131(25): 2745–2760, doi: 10.1182/blood-2017-09-806398, indexed in Pubmed: 29540348.
2. Jamrozik K, Pula B, Walewski J. Current treatment of chronic lymphocytic leukemia. Curr Treat Options Oncol. 2017; 18(1): 5, doi: 10.1007/s11864-017-0448-2, indexed in Pubmed: 28185174.
3. Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000; 343(26): 1910–1916, doi: 10.1056/NEJM20001228343432602, indexed in Pubmed: 11136261.
4. Hallek M, Fischer K, Fingerle-Rowson G, et al. International Group of Investigators, German Chronic Lymphocytic Leukemia Study Group. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukemia: a randomised, open-label, phase 3 trial. Lancet. 2010; 376(9747): 1164–1174, doi: 10.1016/S0140-6736(10)61381-5, indexed in Pubmed: 20988994.
5. Fischer K, Cramer P, Busch R, et al. Bendamustine in combination with rituximab for previously untreated patients with chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. J Clin Oncol. 2012; 30(26): 3209–3216, doi: 10.1200/JCO.2011.39.2688, indexed in Pubmed: 22699884.
6. Byrd JC, Brown JR, O’Brien S, et al. RESONATE Investigators. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leuke- mia. N Engl J Med. 2014; 371(3): 213–223, doi: 10.1056/NEJMoa1400376, indexed in Pubmed: 24881631.
7. Sharman JP, Coutre SE, Furman RR, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med. 2013; 369(1): 32–42, doi: 10.1056/NEJMoa1215637, indexed in Pubmed: 23782158.
8. Byrd JC, Hillmen P, O’Brien S, et al. Long-term follow-up of the RESO- NATE phase 3 trial of ibrutinib vs ofatumumab. Blood. 2019; 133(19): 2031–2042, doi: 10.1182/blood-2018-08-870238, indexed in Pubmed: 30842083.
9. O’Brien S, Jones JA, Coutre SE, et al. Ibrutinib for patients with relapsed or refractory chronic lymphocytic leukemia with 17p deletion (RESONATE-17): a phase 2, open-label, multicentre study. Lancet Oncol. 2016; 17(10): 1409–1418, doi: 10.1016/S1470-2045(16)30212-1, indexed in Pubmed: 27637985.
10. Roberts AW, Davids MS, Page JM, et al. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. N Engl J Med. 2016; 374(4): 311–322, doi: 10.1056/NEJMoa1513257, indexed in Pubmed: 26639348.
11. Zenz T, Vollmer D, Trbusek M, et al. European Research Initiative on CLL (ERIC), TP53 mutation profile in chronic lymphocytic leukemia: evidence for a disease specific profile from a comprehensive analysis of 268 mutations. Leukemia. 2010; 24(12): 2072–2079, doi: 10.1038/leu.2010.208, indexed in Pubmed: 20861914.

12. Zenz T, Eichhorst B, Busch R, et al. TP53 mutation and survival in chronic lymphocytic leukemia. J Clin Oncol. 2010; 28(29): 4473–4479, doi: 10.1200/JCO.2009.27.8762, indexed in Pubmed: 20697090.

13. Malickova J, Smardova J, Rocnova L, et al. Monoallelic and biallelic inactivation of TP53 gene in chronic lymphocytic leukemia: selection, impact on survival, and response to DNA damage. Blood. 2009; 114(26): 5307–5314, doi: 10.1182/blood-2009-07-234708, indexed in Pubmed: 19850740.

14. Malickova J, Pavlova S, Kozubik KS, et al. TP53 mutation analysis in clinical practice: lessons from chronic lymphocytic leukemia. Hum Mutat. 2014; 35(6): 663–671, doi: 10.1002/humu.22508, indexed in Pubmed: 24415659.

15. Zenz T, Häbe S, Denzel T, et al. Detailed analysis of p53 pathway defects in fludarabine-refractory chronic lymphocytic leukemia (CLL): dissecting the contribution of 17p deletion, TP53 mutation, p53-p21 dysfunction, and miR34a in a prospective clinical trial. Blood. 2009; 114(13): 2589–2597, doi: 10.1182/blood-2009-05-224071, indexed in Pubmed: 19643983.

16. Gonzalez D, Martinez P, Wade R, et al. Mutational status of the TP53 gene as a predictor of response and survival in patients with chronic lymphocytic leukemia: results from the LRF CLL4 trial. J Clin Oncol. 2018; 32(5): 1070–1080, doi: 10.1002/jco.2018.187583, indexed in Pubmed: 30442727.

17. Eichhorst B, Robak T, Montserrat E, et al. ESMO Guidelines Committee, appendix G: Chronic lymphocytic leukaemia: eUpdate published online September 2016 (http://www.esmo.org/Guidelines/Haematological-Malignancies). Ann Oncol. 2016; 27(Suppl 5): v143–v144, doi: 10.1093/annonc/mdw359, indexed in Pubmed: 27664254.

18. Malickova J, Tausch E, Rossi D, et al. European Research Initiative on Chronic Lymphocytic Leukemia (ERIC) — TP53 network. ERC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-update on methodological approaches and results interpretation. Leukemia. 2018; 32(5): 1070–1080, doi: 10.1038/s41375-017-0007-7, indexed in Pubmed: 29467486.

19. Burger JA, Landau DA, Taylor-Weiner A, et al. Clonal evolution in patients with chronic lymphocytic leukaemia developing resistance to BTK inhibition. Nat Commun. 2016; 7: 11589, doi: 10.1038/ncomms11589, indexed in Pubmed: 27199251.

20. Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. Nature. 2015; 526(7574): 525–530, doi: 10.1038/nature15395, indexed in Pubmed: 26466571.

21. Landau DA, Carter SL, Stojanov P, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. Cell. 2013; 152(4): 714–726, doi: 10.1016/j.cell.2013.01.019, indexed in Pubmed: 23415222.

22. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature. 2011; 475(7354): 101–105, doi: 10.1038/nature10113, indexed in Pubmed: 21642962.

23. Rossi D, Khiabanian H, Spina V, et al. Clinical impact of small TP53 mutated subclones in chronic lymphocytic leukemia. Blood. 2014; 123(14): 2139–2147, doi: 10.1182/blood-2013-11-539726, indexed in Pubmed: 24501221.

24. Kurkowiak M, Pepek M, Machnicki M, et al. Genomic landscape of human erythroleukemia K562 cell line, as determined by next-generation sequencing and cytogenetics. Acta Haematol Pol. 2017; 48(4): 343–349, doi: 10.1016/j.ajaem.2017.06.002.

25. Campo E, Cymbalista F, Ghia P, et al. aberrations in chronic lymphocytic leukemia: an overview of the clinical implications of improved diagnostics. Haematologica. 2018; 103(12): 1956–1968, doi: 10.3324/haematol.2018.187583, indexed in Pubmed: 30442727.

26. Brieghel C, Kinalis S, Yde OW, et al. Deep targeted sequencing of chronic lymphocytic leukemia: clinical impact at diagnosis and at time of treatment. Haematologica. 2019; 104(4): 789–796, doi: 10.3324/haematol.2018.195818, indexed in Pubmed: 30514802.

27. Stijlenbauer S, Schnaiter A, Paschka P, et al. Gene mutations and treatment outcome in chronic lymphocytic leukemia: results from the CLL8 trial. Blood. 2014; 123(21): 3247–3254, doi: 10.1182/blood-2014-01-546150, indexed in Pubmed: 24652989.

28. Nadeu F, Delgado J, Royo C, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. Blood. 2016; 127(17): 2122–2130, doi: 10.1182/blood-2015-07-659144, indexed in Pubmed: 26837699.

29. Yu L, Kim HT, Kasar S, et al. Survival of del17p CLL depends on genomic complexity and somatic mutation. Clin Cancer Res. 2017; 23(3): 735–745, doi: 10.1158/1078-0432.CCR-16-0594, indexed in Pubmed: 27503198.

30. Stengel A, Kern W, Haferlach T, et al. The impact of TP53 mutations and TP53 deletions on survival varies between AML, ALL, MDS and CLL: an analysis of 3307 cases. Leukemia. 2017; 31(3): 705–711, doi: 10.1038/leu.2016.263, indexed in Pubmed: 27880515.

31. Ma X, Shao Y, Tian L, et al. Analysis of error profiles in deep next-generation sequencing data. Genome Biol. 2019; 20(1): 50, doi: 10.1186/s13059-019-1659-6, indexed in Pubmed: 30867008.