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Current issues in the treatment of chronic lymphocytic leukemia, the role of chemoimmunotherapy in the era of targeted treatments

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The PhD defense will be held on 29 November, 2021 at 1 p.m.

Live online access will be provided via Webex. If you wish to join the discussion, please send an e-mail to the address szaszr@med.unideb.hu until 12 p.m. on the previous workday at latest (26 November, 2021). For technical reasons, after the deadline, it will be not possible to join the defense.

1. Introduction

Chronic lymphoid leukemia (CLL) is a malignant disease of the lymphoid system. The disease is characterized by the proliferation of incompetent monoclonal B lymphocytes.

Based on the incidence of the disease, we can count on approximately 420 new patients per year in Hungary, and at the same time, approx. 4,500 patients are diagnosed with CLL. CLL has an overall indolent course with a 10-year median survival, but survival is influenced by several factors and varies widely. CLL does not progress or only slowly progresses in approximately 30% of cases and therefore does not require treatment. The unusual treatment strategy for CLL in malignancies is based on the above facts. Treatment of patients is only recommended if there are clear signs of progression. Over the last ten years, dominant chemoimmunotherapies are increasingly being replaced by treatment with targeted small molecules targeting intracellular signaling mechanisms. Chemoimmunotherapy continues to play an essential role in shaping treatment strategy. While modern treatments provide a complete survival advantage over repeated chemotherapy in relapsing cases, the first line does not yet show a complete survival benefit, so chemoimmunotherapy can still be used.

Due to the choices, it is paramount to select the optimal target groups for the treatments. Retrospective data obtained from “real-life” data serve this purpose well. The combination of fludarabine, cyclophosphamide, and rituximab (FCR) has become widespread since the early 2000s and is still the most effective chemoimmunotherapy in CLL to date. Due to its efficacy, it has been used in many patients, thus recognizing treatment-related toxicity data. The older mean age in CLL and the associated comorbidities result in narrowing the target population for FCR treatment in light of newer treatment options. However, due to its efficacy, it remains an inevitable part of the repertoire of treatment options.

Dose intensity reduction is also a standard part of “real-life” treatment in elderly, vulnerable CLL patients. Unfortunately, the effect of dose reductions on progression is often hidden from the treating physician. Statistical evaluation of treatment habits fills this gap, helping to carefully select doses and, in particular, the appropriate therapeutic modality during treatment.

2. Literature review

2.1. Epidemiology, diagnosis and prognosis of CLL

Chronic lymphoid leukemia is a disease of old age, with a median onset of around 70 years. Only 15% of patients are under the age of 55 but can appear as young as twenty.

CLL is the most common leukemia in Western countries. It is more common in men than in women, with a gender distribution of 1.3-1.7: 1. The incidence of the disease in Europe is approximately 4-6 / 100,000.

Like in other tumors, the pathogenesis of CLL is a multi-step and multi-factor process, not all details of which are known. Numerous genetic abnormalities can be detected in CLL, but unlike some hematological malignancies, none of these are direct causes of the disease but rather affect its progression. The main factors in pathogenesis are increased B-cell receptor function, inhibition of apoptosis, the presence of epigenetic abnormalities, positive feedback from the microenvironment, and the appearance of "driver" mutations leading to disease progression. To the best of our knowledge, CLL is always preceded by monoclonal B-cell lymphocytosis (MBL). MBL is a condition in which a certain number of cells with the CLL phenotype can be detected, but the diagnostic criteria for CLL are not met. The role of apoptosis in pathogenesis has been previously emphasized, as 99% of CLL cells are in the non-dividing G0 phase, and their apoptosis is inhibited, ultimately leading to lymphocyte accumulation. Inhibition of apoptosis is due to the accumulation of BCL2 and other antiapoptotic proteins. It is now clear that CLL is a proliferative disease where the proliferation rate correlates well with disease progression. The B-cell receptor (BCR) signaling pathway's enhanced function uniformly characterizes CLL cells to varying degrees. Cell proliferation, differentiation, apoptosis, adhesion, and migration are all under the control of the BCR signaling pathway. Specific (auto) antigens may play a role in the enhanced activation of BCR.

The discovery that the B-cell receptor immunoglobulin of some CLL patients is "stereotyped" - i.e., almost identical - also suggests that (auto) antigen selection may play a role in the pathogenesis of the disease. Cell proliferation does not occur in the circulating leukemia population that underlines the importance of signals from the microenvironment. The role of epigenetic abnormalities is indicated by mutations or elevated microRNAs (miRs) (miR-21, miR-155) levels. MiRs are also associated with the TP53 system, BCL2 expression (miR-15a, miR-16-1), and some proteins of some BCR signaling pathways. Secondarily, T and NK cell functions are also impaired in CLL. The number of NK cells decreased to varying degrees. Defective B and T cell function results in the observed hypogammaglobulinemia and autoimmune hemolytic anemia, thrombocytopenia, or pure red cell aplasia. A serious consequence of defective immunity is an increased risk of infections and the appearance of secondary tumors.

CLL is most often noticed based on an elevated white blood cell count on routine blood sampling. A common manifestation is the appearance of painless lymph nodes. Less than 10% of patients present with a typical B symptom. Some patients present with a complication of CLL to their doctor: infections, autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), pure red cell aplasia (PRCA).

CLL diagnosis requires $>5x10^9/l$ B-lymphocyte in the peripheral blood, which persists for at least three months. The leukemic cells found in the blood smear are typically small, mature lymphocytes. The smeared nucleus remaining after cell disintegration, known as Gumprecht shadows, is an additional characteristic of CLL. The clonality of B lymphocytes should be confirmed by flow cytometry. CLL cells co-express CD5 surface antigen with CD19, CD20, and CD23 B-cell antigens. All cells in the leukemia clone carry the same κ or λ immunoglobulin light chain.

The course of chronic lymphocytic leukemia shows significant differences. Some patients die in 2-3 years, others show a more aggressive picture of the disease after an initial indolent course, but in the case of persistently indolent disease, survival can be up to 20 years.

Approx. one-third of the patient will never require treatment. Given that CLL is a disease of old age, 20–30% of deaths are not related to CLL. The average survival is approx. ten years.

Due to the long course of the disease, prognostic factors are of paramount importance. Some prognostic factors also have predictive value; predict the expected response or lack of response to various treatments. Immunoglobulin variable heavy chain (IGHV) genes from leukemia cells may or may not be somatically mutated. The survival of patients with leukemic cells carrying the non-mutated IGHV gene is shorter than that of those with mutated IGHV genes. Their disease progresses faster, recurs sooner, and frequently associates with other poor prognostic factors.

Recognition of "stereotyped" B-cell receptor immunoglobulins supports the role of antigen selection. Based on the IGHV hypervariable region 3 (CDR3) shared sequence motifs, about one-third of the patients belong to the stereotyped group. Some of these subgroups appear to show a similar prognosis. For example, the prognosis of subset # 2 (stereotype 2 subgroup) characterized by IGHV3-21 gene use is unfavorable regardless of the mutational status of IGHV.

Genetic differences are traditionally examined by fluorescent in situ hybridization (FISH). FISH can be used to identify cytogenetic abnormalities in 80% of all CLL cases. The most common deletions involve the long arm of chromosome 13. Other common chromosomal abnormalities include chromosome 12 trisomy, deletion of the long arm of chromosome 11, and deletion of the short arm of chromosome 17. In the absence of other genetic abnormalities with poor prognosis, 13q deletion and trisomy 12 have a better prognosis than FISH negative cases. The 11q and 17p deletions, on the other hand, are

associated with a worse prognosis. Of these, the 17p deletion has the most important prognostic and predictive value. The TP53 mutation detected by sequencing results in a similar defect in the p53 protein. Dysfunction of the p53 protein is associated with partial or complete chemoresistance of leukemia. The complex karyotype (number of chromosomal abnormalities >5) detected by classical karyotyping represents a poor prognosis. Lymphocyte doubling time expresses the frequency of leukemia cell division. A doubling time of less than half a year is in favor of progressive disease. Additional markers without claiming completeness: β2-microglobulin, CD38, thymidine kinase, ZAP-70. IL-8, CD49d, bone marrow infiltration pattern.

There are two widely accepted systems of staging used in both patient care and clinical trials: Rai and Binet. Both classifications include three subgroups with different prognoses. Both rely solely on physical examination and standard laboratory tests.

The CLL International Prognostic Index (CLL-IPI) was introduced in addition to the most relevant prognostic biomarkers. Weighted scores of clinical stage, age, IGHV mutation status, serum β2-microglobulin, del (17p), and/or TP53 mutations are considered. CLL-IPI makes it easy to distinguish four different subgroups of CLL.

After treatment, the optimal endpoint is considered to be the complete remission. However, complete remission does not mean the absolute eradication of leukemia. Residual disease present in the blood and bone marrow is called minimal residual disease (MRD). Prospective studies have demonstrated that MRD-negative patients can expect more prolonged progression-free survival after treatment. In some chemoimmunotherapy clinical trials, MRD negative partial remission was associated with a better prognosis than complete remission with MRD positivity. For all these reasons, MRD can be used as an important endpoint after treatment. MRD-guided treatment is part of the therapeutic strategies under development. The most common method for detecting MRD is flow cytometry.

2.2. Indications for the treatment of CLL

The results of several randomized trials have demonstrated that early treatment does not improve patient survival. The current indication for treatment is Rai stage III-IV or progressive disease (bone marrow failure, massive lymph node enlargement, significant splenomegaly, progressive lymphocytosis, steroid-refractory/dependent autoimmune cytopenia, CLL-related complaints). It should be noted that these experiences date back to the period before modern treatments. Intervention for early leukemia, even with modern BCR inhibitors or BCL2 antagonists, or a combination of monoclonal antibodies, is only recommended in a clinical study.

The relapse of the disease does not mean that the patient needs to be re-treated. The indication for second- and multi-line treatments is the same as for first-line treatment.

2.3. Treatment of CLL

To the best of our knowledge, the disease is incurable with current standard medications, so remissions and relapses alternate in its course. It should be emphasized that patients have approx. 30% never require treatment, so adherence to the treatment indications set out in the recommendations is of paramount importance.

The treatment of patients with CLL is becoming increasingly individualized. There is no single therapeutic protocol that is considered exclusive, either for the first line or for the treatment of relapses. Knowledge about CLL treatment is expanding rapidly due to the large number of new drugs and new drug combinations, making it difficult to make long-term recommendations. International recommendations are significantly modified by the different availability of medicines in each country.

2.3.1. The most important drugs used

Since the 1960s, chlorambucil has been the accepted treatment for CLL for decades, sometimes supplemented with steroids. The response rate to treatment ranged from 30 to 50%, with complete remission of 5%. From the late 1980s, the results have improved with fludarabine and combined fludarabine and cyclophosphamide (FC) from the 1990s. The overall response rate with these treatments increased steadily (50-80% and then 75-90%), and the rate of complete remission also increased (20-30%, 35%). However, none of these agents have been considered standard treatment since the 2010s. Anti-CD20 monoclonal antibodies are essential partners of every treatment used in CLL to date. Three monoclonal antibodies have been approved for the treatment of CLL: obinutuzumab, ofatumumab and rituximab.

Bruton tyrosine kinase is an essential component of the B-cell receptor and cytokine receptor pathways. Activation of the B-cell receptor activates several fundamental functions of cells (cell proliferation, inhibition of apoptosis, expression of "homing" receptors). Ibrutinib irreversibly inhibits Bruton's tyrosine kinase, resulting in decreased survival and proliferation of malignant B cells. Side effects include hypertension, atrial fibrillation, and hemorrhage due to platelet dysfunction. The next-generation BTKi, acalabrutinib, is also an approved treatment for CLL with a slightly more favorable side effect profile.

Idelalisib inhibits the δ isoform (PI3Kδ) of phosphatidylinositol 3-kinase. Inhibition leads to apoptosis of malignant tumor cells. In addition, idelalisib inhibits several signaling pathways, including B-cell receptors and CXC chemokine receptors (CXCR4 and CXCR5). Idelalisib has been used to treat CLL since 2014. Duvelisib, another PI3K

inhibitor, results in dual inhibition of PI3Kδ and PI3Kγ. Inhibition of the δ isoform induces apoptosis in malignant tumor cells, whereas inhibition of the γ isoform reduces the differentiation and migration of supporting cells in the tumor microenvironment.

The BCL2 protein is overexpressed in CLL and results in the survival of leukemic B cells by inhibiting apoptosis. Venetoclax directly inhibits BCL2, activating proapoptotic proteins and restoring apoptosis. FDA first approved to treat 17p deletion CLL patients in 2016, but its indication range has been expanding rapidly since then.

2.3.2. First line treatments

Supplementation of fludarabine and cyclophosphamide with rituximab resulted in more favorable progression-free and overall survival. In the FCR300 study, a 95% response rate (ORR), a 72% complete response rate (CR), and a 6.4-year mean progression-free survival (PFS) were observed. Other studies have confirmed the result. The long-term results of study FCR300 showed that half of the patients with mutated IGHV who were negative for minimal residual disease at the end of treatment achieved an unprecedented excellent result with 79.8% PFS observed at 12.8 years. Based on these data, FCR is currently considered the best choice for young, fit, IGHV mutated patients without TP53 aberration.

FCR therapy is not well tolerated in patients over 65 years of age or those with comorbidities, mainly due to the higher incidence of side effects such as prolonged myelosuppression and infections. Better tolerability and similar efficacy have been demonstrated with bendamustine and rituximab in elderly patients. CLL10, a phase 3, randomized trial, compared first-line FCR versus BR. Patients with a 17p deletion were excluded from the study. The results showed that FCR was more effective than BR (PFS 55.2 months vs. 41.7 months), but patients older than 65 had an increased incidence of cytopenia and infection in the FCR arm. The subgroup analysis showed no difference in PFS between FCR and BR in patients older than 65 years. Therefore, BR is preferred in this group of patients.

The efficacy of ibrutinib and chlorambucil in patients older than 65 years without a 17p deletion was first compared in the RESONATE 2 study. This phase 3, open-label, randomized study demonstrated that ibrutinib was more effective than chlorambucil and had a more favorable toxicity profile. Ibrutinib has been included in the first-line options based on this study. However, other therapeutic options have been developed to treat patients over 65 years of age or younger patients not eligible for high-dose chemotherapy.

FCR and BR are high-dose chemotherapies, although they have been used in elderly patients with comorbidities with various dose adjustments. Due to the significant risk of toxicity, large clinical trials excluded frail patients with multiple comorbidities. For these

patients, the treatment of disease-related symptoms and the maintenance of the quality of life are the primary treatment goals. Chlorambucil in combination with monoclonal antibodies is preferred in these situations. The first combination studies were performed with rituximab, with an ORR of 82–84% and a median PFS of 24–35 months. Subsequently, ofatumumab and obinutuzumab were studied in combination with chlorambucil, with ORRs of 82% and 78% and PFS of 23 months and 29 months, respectively. In the CLL11 study, obinutuzumab outperformed rituximab in terms of PFS (28.9 months vs. 15.7 months), with a significant difference in overall survival after 60 months of follow-up. Median OS was achieved only in the rituximab arm, which was 73.1 months. All of these combinations can be used to treat patients with relapsed generalized CLL.

The CLL14 study included non-fit patients with CIRS scores> 6 and/or creatinine clearance <70 ml/min. Based on recently reported 2-year results, the combination of obinutuzumab, venetoclax provides better progression-free survival than obinutuzumab combined with chlorambucil. Median survival without progression has not yet been achieved in the venetoclax, obinutuzumab group compared to 35.6 months in the chlorambucil, obinutuzumab group.

2.3.3. Treatment of patients with relapsed / refractory CLL

Almost all previously treated CLL patients relapse at some point, but this is not an immediate indication for treatment. In selecting the appropriate therapy, we consider the success and tolerability of previous therapies, the time of remission, the results of repeated prognostic studies, the patient's characteristics, and the general condition.

Chemoimmunotherapy is becoming less and less relevant in the treatment of relapses. It is by no means recommended if the patient relapses early, within 24 to 36 months after chemoimmunotherapy, as this is usually associated with aggressive disease and short overall survival with standard chemoimmunotherapies. It is only used to treat late relapses only where the availability of modern drugs is limited.

A phase 2, open-label, multicenter study of RESONATE-17 evaluated the efficacy of ibrutinib for relapsed/refractory conditions. The study enrolled 144 patients with 17p deletion, all of whom received ibrutinib until disease progression or until unacceptable toxicity. After a mean follow-up of 11.5 months, 64% of patients responded, and 83% at a median follow-up of 27.7 months. The rate of PFS was 63%, and the rate of OS was 75% at 24 months, which is an excellent result in this high-risk group compared to median PFS less than one year with chemoimmunotherapy. The RESONATE, randomized, phase 3 study compared ibrutinib and ofatumumab in relapsed CLL, enrolled 195 patients in the ibrutinib arm, and 56 patients had a 17p deletion. The ORRs in the ibrutinib and ofatumumab arms were 90% and 25%, respectively. The median PFS

was 44.1 months in the ibrutinib arm compared to 8.1 months in the ofatumumab arm. The benefit of ibrutinib was observed in all subgroups, including unmutated IGHV, the 17p deletion, and the 11q deletion.

Idelalisib in combination with rituximab was compared with rituximab monotherapy. This study showed a significantly better response rate in the idelalisib arm. ORRs were 81% and 13%, OS 92% and 80%, and PFS at 24 weeks were 93% and 46% in the idelalisib and placebo arms, respectively, the latter representing a median PFS of 5.5 months. Efficacy in the idelalisib plus rituximab arm did not differ significantly in different prognostic groups, including patients with poor prognosis due to the 17p deletion, TP53 mutation, and 11q deletion. The efficacy of idelalisib is overshadowed by significant toxicity, particularly life-threatening colitis, pneumonia, and hepatitis. Idelalisib is recommended for relapsed patients who do not tolerate or are resistant to ibrutinib and venetoclax.

The phase 3 DUO study compared duvelisib with ofatumumab. The study showed a significant PFS advantage in the duvelisib arm (13.3 and 9.9 months), including patients with a 17p deletion and/or TP53 mutation. Based on this, the FDA approved duvelisib for all patients with relapsed/refractory CLL who had received at least two prior therapies.

Venetoclax monotherapy induces a rapid decrease in tumor mass. The measured ORR was 77%, and complete remission was 30% in multiple relapsed patients with advanced disease. The main side effects were neutropenia and tumor lysis syndrome, which can be eliminated by continuous dose escalation and appropriate determination of tumor lysis risk. In another study, venetoclax showed an ORR of 79.4 at a median follow-up of 12.1 months in patients with relapsed/refractory CLL who had a 17p deletion. This study resulted in the approval of venetoclax monotherapy in patients with relapsed / refractory CLL with a 17p deletion.

The recently completed phase 3 MURANO study compared the combination of venetoclax, rituximab, and bendamustine, rituximab in patients with relapsed/refractory CLL. PFS was 53.6 months in the venetoclax and rituximab combination arm compared with 17 months in the bendamustine and rituximab arm.

Despite the advancement of targeted treatments, chemoimmunotherapy can still be considered a standard first-line treatment in CLL. However, chemoimmunotherapy is no longer recommended for the treatment of patients with relapsed/refractory disease.

3. Objectives

- National data on chemoimmunotherapy used in daily clinical practice in Hungary are currently not available. Our goal was to collect nationwide "real-life" data on chemotherapy combined with rituximab in patients with chronic lymphocytic leukemia. Data collection was used to determine the overall response rate. We also aimed to determine the ORRs measured in subgroups according to age, CIRS score, and chromosome mutations (17p and 11q deletions).
- The secondary objective of the national study was to assess the frequency of pre-treatment genetic testing.

- With collecting the data of patients treated according to the FCR protocol in our clinic, our primary goal was to determine the frequency of persistent cytopenias as a function of the fitness and doses utilized. With the rise of targeted treatments, the FCR protocol is limited to absolutely fit patients. In order to obtain realistic toxicity data, we wanted to compare the incidence of long-term cytopenias in patients in the period before and during the availability of targeted treatments.
- The secondary goal was to determine the rate of remission and time to next treatment.

4. Patients and methods

4.1. The study in patients receiving first-line chemotherapy in combination with rituximab

This study was an open-label, non-randomized, single-arm, nationwide, multicenter, observational study in previously untreated patients who had started standard chemotherapy supplemented with rituximab.

We planned to involve up to 200 patients from 9 Hungarian centers. The study was later expanded to include six additional centers, and the planned number of patients was increased to 150. Patients were treated according to local protocols used in daily practice. Data collection lasted from April 2014 to December 2016.

During the study, patients were followed according to routine clinical practice. Data collection was performed on a web interface suitable for clinical trials. Data were collected during each treatment cycle after inclusion and two months after the end of treatment (demographics, treatment indication, diagnosis data, FISH, treatment data). Data on rituximab therapy and concomitant chemotherapy, as well as adverse events, were documented at each visit. Therapeutic response to induction treatment was recorded within two months of the last cycle.

The primary endpoint was the ORR. Secondary variables in the study were ORRs measured in subgroups by age, CIRS score, and chromosome mutations (17p and 11q deletions). This subgroup analysis was ultimately not published due to the biasing effect of low-patient subgroups.

Data from all patients who received at least one dose of rituximab during the study were evaluated in the safety analysis.

The compliance of the data recorded in the electronic data sheets with the source documents (medical records, finds) was ensured by regular monitoring. The data was processed following the ethical guidelines of the Declaration of Helsinki.

4.2. Study with FCR combination

Between January 2012 and January 2016, we treated 120 CLL patients with FCR, whose data were collected retrospectively using the electronic clinical database. Their age, eGFR levels, and comorbidity were considered to determine their suitability for FCR treatment. To standardize comorbidities, we used the CIRS score.

We determined the treatment outcome based on the blood count and physical examination. The treating physician's opinion was decisive in determining complete and partial remission or stable disease and progression. Loss of response was not always well

documented, so treatment-requiring progression was counted, which can be accurately determined from retrospective data and is more relevant.

For comparison, another smaller cohort of 14 patients was added to the study. They were treated between February 2016 and January 2019 when ibrutinib became available with expanding indications (TP53 aberration in all treatment lines, second line without restriction, and most recently, first-line for IGHV unmutated patients).

The study's main aim was to investigate the incidence of persistent cytopenias as a function of comorbidity, age, and renal function. Cytopenia was considered persistent if it continued beyond two months. Only cytopenias of grade 3 or 4 after completion of treatment, attributable to chemotherapy (neutrophil count below 1 G/l, hemoglobin below 80 g/l, and platelet count below 50 G/l) were taken into account.

In Hungary, an oral version of FCR treatment is used with the corresponding equivalent doses (fludarabine po: 40 mg/m^2 for 3 days, cyclophosphamide po: 250 mg/m^2 for 3 days, rituximab: 375 mg/m^2 in the 1^{st} cycle, then 500 mg/m^2 in cycles 2-6).

Patients unsuitable for full-dose FCR treatment may have received reduced-dose FCR therapy. If the patient's creatinine clearance is below 70 ml/min, it is recommended to reduce the dose of fludarabine by at least 50%.

5. Statistical evaluation

Data on chemotherapy with rituximab and the response rates of the overall patient population and the individual subgroups were assessed using descriptive statistical methods. In addition to the percentage data, the 95% confidence intervals of the listed primary and secondary end-points were also defined.

Descriptive statistical methods were used as well to report data on FCR treatment. Pearson's chi-square test was used to compare categorical variables and evaluate independence, and the time to next treatment was plotted with a Kaplan-Meier curve.

6. Results

6.1. First line chemoimmunotherapy combined with rituximab

6.1.1. Patients' characteristics in the study group

The per-protocol (PP) population consisted of 78 patients. The vast majority of excluded patients (68 patients) did not receive all six cycles of treatment due to adverse events (24 patients), death from other causes (5 patients), withdrawal of consent (5 patients), disease progression (4 patients), or for other reasons not detailed (30 patients). Although receiving all six treatment cycles, an additional four patients were also excluded from the PP population due to significant protocol violations. Thus, a total of

82 patients (54.67% of the total population) received six treatment cycles, of which 78 patients (52%) entered the PP population.

The ITT population consisted of 92 men (61.3%) and 58 women (38.7%), while the PP population consisted of 54 (69.23%) men and 24 (30.77%) women, respectively. The mean age of the patients was nearly the same in the two study populations (ITT: 67.26 years; PP: 67.87 years). Patients 65 years of age or older and 65 years of age showed an approximately 60-40% distribution.

The majority of patients in both the ITT and PP populations were classified as Binet B (ITT: 45.33%, PP: 42.31%) and Binet C (ITT: 44.00%, PP: 48.72%). However, 10.67% of the patients involved were in the Binet A stage.

The mean CIRS score in the ITT population (4,278 ± 4,511) was minimally lower than that measured in the PP population (5,583 ± 4,986).

Patients were classified into subgroups based on CIRS scores. It is noteworthy that the investigating physician did not record the CIRS score in 42 patients. Of the 87 patients with a CIRS score of 6 or less, 45 did not receive the planned six cycles (51.7%), while 21 of the patients with a CIRS score greater than 6 had only 14%.

Regarding chromosome mutations, 60% of the population presented evaluable data. The 17p deletion and the 11q deletion were detected in only a small proportion of patients (2-8 patients, 2.00-7.69%).

Patients showed a heterogeneous picture with respect to concomitant chemotherapy. In both study populations, rituximab was most commonly combined with fludarabine/cyclophosphamide (64 patients, 42.67%), cyclophosphamide /vincristine/prednisolone (CVP) (26 patients, 17.33%), and chlorambucil (21 patients, 14.00%).

6.1.2. Treatment results

For the whole study, the ORR was 88.24% in the ITT population (CI95%: 81.6-93.12%) and 94.59% in the PP population (CI (95%): 86.73-98.51%).

6.1.3. Total response rate by age

Slightly higher ORRs were observed in patients over 65 years of age, both in the ITT and PP populations. The lowest ORR was measured in patients under 65 years of age in the ITT population (86.54%), while the highest ORR (97.62%) was measured in patients over 65 years of age in the PP population.

6.1.4. Total response rate by chromosome mutations

In the ITT population, both the 17p deletion and the 11q deletion resulted in a lower ORR than patient groups in which these mutations were not detectable. However, the negative effect of the 17p deletion was not apparent in the PP population. The 11q deletion also resulted in a lower ORR in the PP population (80% vs. 97.22%). These results cannot be interpreted due to the small number of patients with confirmed chromosomal mutations (2-8 patients).

6.1.5. ORR by CIRS score

In both study groups, most patients had a CIRS score of 6 or less. Based on this, the number of comorbidities in these subgroups at the time of inclusion was small. However, there is no clear trend between the CIRS score and the ORRs measured in the corresponding subgroups. In the ITT population, the lowest ORR (62.5%, CI95%: 24.49-91.48%) was observed in the CIRS = 5 patient group, while in the PP population, the CIRS = 1 and CIRS = 6 patient groups had the lowest ORR (80%, CI95%: 28.36-99.49%). Regardless of the population, 100% ORR was observed in all CIRS ≥ 7 subgroups. However, when evaluating the above results, the distorting effect caused by the small number of patients should be taken into account, which is also reflected in the wide confidence intervals.

6.1.6. Adverse events

A total of 144 adverse events were reported in 75 patients, of which 48 (33.3%) were considered serious by the investigators.

One non-serious adverse reaction was identified, reported in a total of 29 cases in 23 patients, namely neutropenia. This adverse reaction affected more than 5% of the total patient population (N = 150). Of the adverse events, 15 (10.42%) were associated with rituximab treatment. Of all adverse events, 24 (16.67%) led to the discontinuation of therapy.

A total of 48 serious adverse events were reported during the study, of which four events were associated with rituximab therapy. Seven patients reported eight serious adverse events that were fatal.

In the study, 24 patients reported rituximab-related abnormalities in a total of 32 cases, of which 4 were medication errors (inappropriate dosing) and 28 were off-label.

6.2. Results of patients treated with FCR protocol

6.2.1. Demographic data, dose adjustments

Of the 120 patients, 86 patients received FCR treatment in first-line and 34 patients in any subsequent treatment lines.

Of all patients, 74 were under 65 years of age, 23 were under 65-70 years, and 23 were over 70 years of age. The distribution was similar between those treated in the first and non-first line.

Patients ranged in age from 33 to 85 years, with a mean age of 59 years. The patients in the post-2016 group were between 45 and 66, with a mean age of 53.5 years. Of the 120 patients, only 82 (68.3%) had a creatinine clearance of 70 ml/min or greater. Although all patients with impaired renal function received a reduced dose of fludarabine, none of the dose reductions reached the recommended 50%.

The CIRS score for 15 patients was higher than the currently accepted maximum of 6.

Based on age, GFR, and CIRS, a total of 45 patients (37.5%) would have been eligible for full-dose FCR treatment according to today's principles. This distribution was similar in the first and the subsequent lines. None of the patients had dose reduction, but 14 patients did not receive the planned number of cycles, so 68.8% of the patients received the planned total dose. The reason for the reduction in the number of cycles in fit patients was the early achievement of a good response and not toxicity.

However, 26 of the 75 non-fit patients were also scheduled for full-dose FCR treatment. Of these, 13 received the planned entire treatment (50%). The number of cycles was reduced in six patients, and the dose was reduced in seven patients due to cytopenia.

The 49 non-fit patients who received a reduced dose of FCR from the outset required ten additional dose reductions. The planned six cycles were given to 27 patients without further dose reduction (55%).

6.2.2. Treatment responses

Of the 86 patients treated in the first line, 68 achieved complete hematologic remission (CR 79.07%), 14 entered partial remission (PR 16.28%), and 4 patients had stable or progressive disease (SD + PD 4.65%). . This ratio was significantly worse than those not treated in the first line (CR 47.05%, PR 35.29%, SD + PD 17.64%).

In the 2016–2019 cohort, the rate of total remission was 86% and the remaining 14% achieved partial remission. One patient discontinued treatment after 3 cycles due to hematological toxicity. There was no dose reduction.

Reducing the number of cycles and reducing the dose is a deviation from the original therapeutic plan. Both dose intensity adjustments result in a significant reduction in the rate of complete remissions ($\chi^2 2$ (2) = 6.429, p = 0.04 for any dose adjustment, $\chi^2 2$ (2) = 6.824, p = 0.033 for fewer cycles).

The median time to next treatment was 49 months for first-line patients. Patients treated in multiple lines required re-treatment much earlier, on average after 24 months.

6.2.3. Incidence of persistent cytopenias

We observed persistent cytopenia in 17 (14%) of the 120 patients treated with FCR. In the three most severe cases requiring multiple hospitalizations, anemia was the primary abnormality associated with neutropenia in one case and thrombocytopenia in another case. The latter patient was the only one to die from persistent cytopenia; cytopenia was observed for seven months. We did not see neutropenia during this period; however, the cause of death was eventually confirmed in sepsis. In the other 14 cases, persistent cytopenia was neutropenia, which was observed for 3 to 8 months. Neutropenia was associated with febrile neutropenia in 10 cases; however, only one patient required hospitalization. Among patients eligible for full-dose chemotherapy, four persistent cytopenias occurred, but only two received first-line FCR treatment. The rate of persistent cytopenia was higher in patients unsuitable for high-dose treatment who were nevertheless scheduled to receive full-dose FCR (7/26, 27%). When patients received the appropriate dose, the rate of persistent cytopenia was 10%. Furthermore, among fit patients treated in the first line, the incidence of persistent cytopenia was only 4.6%.

In a later cohort, when ibrutinib was available as second-line treatment or first-line for patients with TP53 aberration, FCR was given only to patients younger than 65 without significant comorbidities and with creatinine clearance of at least 70 ml/min. Only one long-term cytopenia occurred in this group of patients. It should be noted that the patient's suitability was not adequately assessed; the creatinine clearance was 57 ml/min. Treatment was finally finished after three cycles. The patient had severe transfusion-dependent anemia for five months. In terms of treatment efficacy, after three cycles of FCR, the patient maintained an MRD positive complete remission for 28 months, and there was no sign of progression at the last visit.

Fitness calculated before FCR treatment was clearly correlated with post-treatment persistent cytopenias in the overall patient population as well as in first-line patients (χ^2 (1) = 6.001, p = 0.014 in the total patient population, (χ^2 (1) = 5.705, p = 0.017 for first-line patients).

7. Discussion

Chemoimmunotherapy has been continuously present in CLL treatment since the introduction of rituximab, and from the early 2000s to the emergence of targeted small molecules, it dominated the treatment palette. From the second half of 2010, agents acting on the BCR signaling pathway and BCL2 inhibition are gaining ground. These agents are referred to as targeted treatments because their mechanism of action is based on the pathological function of CLL cells.

The diversity of chemoimmunotherapeutic combinations and the advancement of targeted treatments provide a significant choice for physicians. The choice of therapy is influenced by the results of clinical studies, their target groups, the general condition of the patient, comorbidity, the stage of the disease, and prognostic markers. The different availability of drugs further complicates the decision-making mechanism. By learning about domestic and domestic clinical data, we can help support decision-making mechanisms. “Real life” data also confront us about our deviations from standards, revealing our gaps and outdated innervations.

Epidemiological data in patients enrolled in a concomitant chemotherapy study with rituximab did not differ from those expected from international data. The ratio of men to women reflected the known ratio of 1.3-1.7: 1; in our case, it was 1.58: 1. Of the patients enrolled, 68 did not complete the planned six cycles for various reasons, which did not change this proportion.

The majority of patients enrolled in the study were Binet B and Binet C stage. However, 10.67% of patients were treated in stage Binet A, which is not in line with current guidelines. Patients with Binet stage A usually show no signs of progression and do not require treatment. One of the main treatment strategies for CLL, which has been investigated in chemoimmunotherapies, is that treatment of patients at an early stage has no beneficial effect on overall survival.

The mean age of the patients was also consistent with the literature data. In developed countries, the median age of treated patients is 70 years, which is 69.86 years in the ITT population and 68.55 years in the per-protocol population. In the study populations, patients 65 years of age or older and 65 years of age showed a distribution of approximately 60-40%. The proportion of patients younger than 65 was slightly higher than the 33% published by the National Cancer Institute.

The mean age of patients completing six cycles and the mean age of the total patient population were the same, assuming that age did not play a role in earlier completion of the planned treatment. This observation needs to be explained, as the primary reason for discontinuation of treatment is intolerable side effects or death from any cause. Their occurrence is usually associated with age and comorbidity. Since the mean ages of the two populations were the same, we also examined the comorbidities. Based on the

examination of the CIRS score, it can be stated that the average CIRS value of the patients included in the study was 4,278, while the CIRS value of the patients who completed six cycles was 5,583. It can also be concluded that the reason for early termination of treatment was usually not high CIRS or age, so elderly and comorbid patients received rituximab with a less toxic chemotherapeutic partner appropriate to their fitness well chosen by the treating physician.

The above is partly explained by the discontinuation of treatment, which was adverse event in only 24 of the 68 patients, and death in 5 patients. In addition to the four patients with progression and the five patients who withdrew their study consent, 30 patients had stopped treatment for other reasons. Thus, in our study, the most common reason for discontinuation of treatment was not detecting undesirable side effects. Although the other reason was not recorded in the study, a common reason for discontinuation of treatment in CLL practice is the early achievement of a favorable response.

Patients in the study populations were subgrouped based on the fixed CIRS score. Among the relevant data, 42 patients did not have a CIRS score, which means that rituximab partner chemotherapy was determined without accurate recording of comorbidity. The calculation of the CIRS score may be superfluous if the patient's single parameter determines that he or she cannot receive high-dose chemotherapy, for example, due to decreased creatinine clearance. The complexity of calculating the CIRS score limits its use in routine clinical practice.

Of the 87 patients with a CIRS score of 6 or less, 45 did not receive the planned six cycles (51.7%), compared with only 14% of the 21 patients with a CIRS score greater than 6. Choice of chemotherapy partner and comorbidity are interrelated. Younger people with low CIRS scores are more likely to choose more toxic, high-dose treatment.

In our survey, the proportion of patients with a 17p or 11q deletion was lower than in previous clinical trials. The frequency of the 17p deletion was 2%, and the frequency of the 11q deletion 5.33%. The fact that the FISH test was performed in only 60% of the population revealed obvious shortcomings in routine clinical practice in Hungary. In parallel with the increased use of ibrutinib, initiatives have been taken to improve the availability of FISH tests. Testing is only available in the laboratories of academic centers, but with the help of sample transport, this ratio has hopefully improved significantly.

The pathogenetic role of the TP53 mutation in chronic lymphoid leukemia was investigated in the early 1990s. Its association with chemoresistance and its role in Richter transformation through genetic instability has also long been known. With the increase of gene sequencing, it became clear that the clinical prognosis of TP35 mutated CLL was similar to that of 17p deleted CLL. At the time of the study, sequencing of the TP53 gene was not part of clinical practice, but this test was also introduced in academy

centers with the recognition of its practical significance. One of the centers is also ERIC (European Research Initiative on CLL) accredited.

Testing for IGHV mutation status is also recommended in the iwCLL2018 guideline, a change from the previous recommendation resulting from long-term results from several chemoimmunotherapy studies and that ibrutinib has been approved in all lines of CLL treatment. In Hungary, testing for the mutational status of IGHV has become routinely available in large academic centers. At the time of the study, testing for IGHV mutation status was not yet recommended.

The chemotherapy partners selected for rituximab in the study showed significant heterogeneity. The most commonly used protocols were FCR (42.67%) and RCVP (17.33%), and chlorambucil monotherapy (14%). While FCR, R-bendamustine, and R-chlorambucil were considered standard first-line protocols at the time of the study according to clinical recommendations, R-CVP, R-CHOP, R-CAP were not included in the recommendations. Overall, 73.3% of patients received standard CLL treatment. Among the non-standard treatments, R-CVP was the dominant CLL protocol of the previous decade in Hungary, received by 17.33% of patients.

Many phase II and III studies examined the efficacy of rituximab in combination with various chemotherapy regimens in previously untreated CLL patients. In combination with fludarabine, rituximab treatment resulted in 87% and 90% response rates, respectively, while the ORR was 88% for the rituximab-bendamustine combination. FCR showed an ORR of 95% in an early study (CI95%: 92% –98%), although later, a larger, randomized, phase III study reported a slightly lower 90% ORR. These previously reported results are consistent with the overall response rate observed in the entire population of our study. The ORR was 88.24% (CI95%: 81.6-93.12%) in the ITT population and 94.59% (CI95%: 86.73-98.51%) in the PP population. Analysis of responses in different age groups showed a slightly higher ORR in patients 65 and older. Hallek et al. made a similar observation; they found the ORR to be 93% and 89% in the ≥65 and <65 age groups, respectively. This finding is probably related to the more clinically aggressive CLL in younger people. However, there were common difficulties in interpreting the response rates of subgroups determined by chromosome mutation status or CIRS scores indicating comorbidity. Although the ORR appeared to be as high as 100% in several subgroups, the distorting effect of a small number of patients is reflected in the broad confidence intervals. However, it can be concluded that elderly comorbid patients received the appropriate chemotherapeutic partner.

Compared to data from previous clinical trials, the present study results support the efficacy and safety of rituximab in standard chemotherapy in previously untreated CLL patients. A total of 144 adverse events were reported in 75 patients during the study, of which 48 (33.3%) were considered serious adverse events by the investigators. In total, eight serious adverse events with fatal outcomes were reported in seven patients and 15

(10.42%) non-serious adverse events were associated with rituximab treatment. Patients tolerated rituximab therapy well, and no unexpected safety events were reported with rituximab.

The main benefit of FCR treatment is that it prolongs progression-free survival and overall survival, which is why it was widely used in almost all patient populations during the study period, from 2012 to 2016, as alternative treatments (R-bendamustine, ibrutinib) access was limited. The known benefit of FCR in terms of progression-free survival is overshadowed by increased toxicity. The most important of these are Richter transformation, the development of secondary malignancies, and long-term cytopenias. The mechanism of the Richter transformation remains the subject of active research. Direct toxic effects, therapy-related immunosuppression, and consequent EBV reactivation are among the possible explanations. The incidence of Richter transformation after FCR treatment is lower than post-FC (13.1% vs. 17.4%). A deeper remission can be achieved with FCR, highlighting that the development of transformation may be related to the progression of CLL itself. The prevalence of Richter transformation in relapsed/refractory patients treated with the new agents is comparable to historical data on transformation after chemoimmunotherapy, which also confirms that the main factor in the development of Richter transformation is the progressive disease itself.

The incidence of solid tumors did not increase compared to the general population after FCR therapy. Among the secondary hematological malignancies, MDS / AML was 1.6-2.8-4.6% based on various studies.

Persistent cytopenia lasting more than two months can lead to increased hospital visits and treatments, more frequent and severe infections, or even death, which offsets the progression benefits of FCR treatment. The personal experience of many hematologists is that this is an unexpected and unpredictable side effect that has led to unnecessary abandonment of FCR treatment in recent years.

In our study, persistent cytopenia was observed in 17 cases (14%), which is comparable to the large clinical trials of FCR, where the incidence of prolonged cytopenias was 17% and 19%, respectively. Most of them had prolonged neutropenia, and only two patients required hospitalization for infections, indicating that granulocyte colony-stimulating factors effectively avoid serious complications. Currently, the indication for FCR is limited to fit and young patients, and chemoimmunotherapy is no longer recommended for relapsed/refractory patients. The studies that established the position of FCR in the first-line treatment of CLL included patients who did not meet today's criteria. In both studies, there were a significant number of patients over 65 years of age. In the study, led by the MD Anderson Cancer Center, the upper limit of expected renal function for admission was 176 μmol/l, which is almost always associated with a

creatinine clearance below 70 ml/min. Comorbidities were assessed by CIRS score in the CLL8 study, with only a general condition assessment in the MD Anderson Cancer Center study. The putative cause of prolonged cytopenia was age and advanced Rai stage in the latter study, but age was only mentioned in the interim publication. In our study, age and creatinine clearance (7-7 patients) were associated with persistent cytopenias. If unfit patients had not been treated with FCR at all and the FCR protocol was used only in the first line, persistent cytopenia would have been observed in 4.6% of cases. In our subsequent cohort from 2016 to 2019, we used this approach, and no long-term cytopenia occurred in fit patients, the only exception being the patient whose fitness was poorly assessed because the GFR was below 60 ml/min.

The effectiveness of first-line FCR treatment is indisputable. The overall response rate was 90-95%, and the overall remission rate was 44-72%. Our own experience has confirmed the effectiveness of FCR treatment. The overall remission rate for those treated in the first line was 79%. However, it should be noted that in clinical trials, complete remission was demonstrated using CT and bone marrow biopsy. The IWCLL guidance does not recommend these studies in routine practice, only in clinical trials. Although long-term progression-free survivors can also be found among our patients, these data cannot be compared with international data due to the shorter follow-up time and lack of knowledge of IGHV mutation status.

If toxicity is observed, treatment may be continued by reducing the dose and/or increasing the length of cycles. We used this option in 14.1% and 10.8% of the cases examined. The number of cycles was reduced in 33% of patients. 50% of these patients were in complete remission upon discontinuation of treatment. Nevertheless, when looking at the overall cohort, dose reduction is associated with a significant reduction in remission rate ($\chi^2(2) = 6.429$, $p = 0.04$ for any dose intensity reduction, $\chi^2(2) = 6.824$, $p = 0.033$ for cycle reduction).

The number of patients treated with FCR had decreased significantly since January 2016, when ibrutinib became available in Hungary. Also, at this time, a combination of alternative chemoimmunotherapies such as BR, obinutuzumab, and chlorambucil became widely available. Although first-line ibrutinib was limited to the few patients with TP53 aberration, second-line treatment changed the first-line treatment strategy. With effective second-line treatment, the use of less effective first-line chemoimmunotherapy is more acceptable.

We have found that FCR treatment is feasible in a wide range of CLL patients. After learning of the importance of long-term toxicity, dose reduction is widespread. The development of persistent cytopenias can also be avoided by reducing the number of cycles. FCR treatment of non-fit patients is no longer recommended, with the highest rate of persistent cytopenias among them.

In properly selected patients, unpredictable, persistent cytopenias after FCR therapy are rare. The majority of prolonged cytopenias can be avoided by examining age, creatinine clearance, and comorbidities.

8. Conclusions, the future of chemoimmunotherapy

The rapid development of CLL treatment will significantly influence the future use of chemoimmunotherapies, including FCR. The BCR inhibitor ibrutinib is used in all lines of CLL. Numerous Phase 3 studies seek the previous site of the BCL2 inhibitor venetoclax in the therapeutic algorithm. The combination of obinutuzumab and venetoclax is now available as a first-line, fixed one-year treatment for significantly comorbid patients. The combination of chemoimmunotherapy and new agents is also under investigation. Drug innovation, the results of clinical studies, and last but not least, patient expectations may also lead to the rise of chemotherapy-free treatments in the first-line treatment of chronic lymphocytic leukemia.

Duvelisib, a new phosphatidylinositol 3-kinase inhibitor, and acalabrutinib, another Brutone kinase inhibitor, have recently been added to the CLL treatment portfolio, which is currently dominated by obinutuzumab, venetoclax, and ibrutinib. Several phase 3 studies are testing first-line combinations of new drugs to achieve long-term efficacy while reducing toxicity. Fixed-term therapies and MRD-guided treatment are the main pillars of these strategies. The results of clinical trials, and last but not least, the expectations of patients, are likely to result in the exclusivity of treatments without chemotherapy in the future.

Today, first-line FCR treatment is still recommended for patients younger than 65 who do not have significant comorbidity, good renal function, and no TP53 aberration. FCR “survival” may be ensured by data from long-term follow-up of patients in initial FCR studies. Recently, Keating and colleagues published data from an average of 12.8 years of follow-up of 300 patients. PFS was 53.9% among those whose disease had a good prognosis based on the mutational status of the immunoglobulin heavy chain variable (IGHV) region, i.e., mutated (IGHV-M). In contrast, only 8.9% of patients with unmutated IGHV (IGHV-UM) did not progress during this period. Furthermore, 50.7% of patients with IGHV-M CLL became MRD negative after treatment. The PFS of these patients was even more favorable at 79.8%. The PFS curve shows a ‘plateau’ that was unthinkable in previous CLL studies. The last relapse observed occurred at 10.4 years. No relapse was observed during the additional mean 2.5-year follow-up period in 42 patients. These patients can be considered functionally cured. The same conclusion can be drawn from the subgroup analysis of IGHV-M patients in the CLL8 study.

The advancement of targeted therapies has resulted in a steady shift in international recommendations in recent years. The CLL14 study comparing venetoclax in

combination with obinutuzumab to chlorambucil and obinutuzumab reported excellent results, with the one-year fixed-duration combination of venetoclax and obinutuzumab becoming a new first-line standard of care for patients with underlying conditions. Based on the results of the Resonate-2 study, ibrutinib is also widely used in the first line. The long-term benefits of first-line targeted treatments over sequential therapy have not yet been demonstrated in overall survival.

There is growing evidence to support the benefit of first-line ibrutinib in poor-prognosis CLL characterized by 11q deletion, IGHV-UM, and a complex karyotype. Important for FCR is the ECOG1912 study, which compares the combination of ibrutinib and rituximab with standard FCR to treat patients less than 70 years of age. Patients with a 17p deletion were excluded. After a 45-month follow-up period, PFS was better in the ibrutinib plus rituximab arm (89% vs. 71%). This difference is primarily due to outcomes in IGHV-UM patients (89% vs. 65%). PFS scores in IGHV-M patients did not differ significantly (88% vs. 82%). Based on PFS values, patients who are fit but with poor prognosis should also be treated primarily with ibrutinib. Proper interpretation of overall survival data requires a longer observation period, while FCR remains an option.

In most countries, including Hungary, the availability of targeted therapies is still limited in the first place. In clinical practice, FCR remains an important treatment option for young, fit patients. Even with the combination of modern agents, it is a great challenge to surpass the excellent results achieved with FCR, and recording the overall survival benefit requires a long observation time. Approximately 25% of patients with CLL are younger than 65, so the proportion of patients potentially receiving FCR treatment is already low. It is crucial to determine who can benefit most from the long-term advantages of FCR, so testing for IGHV mutation status is mandatory according to the iwCLL 2018 guidance. As a result, the number of patients receiving FCR treatment is expected to decline further; however, it is still a well-tolerated and exceptionally effective fixed-term treatment for appropriately selected patients.

9. New findings

In the Hungarian survey, the epidemiological characteristics of patients treated with chemoimmunotherapy did not differ from what was expected based on international data. 61.3% of the patients were male, and 38.7% were female, reflecting the known ratio of 1.3-1.7: 1; in our study, it was 1.58.

In our study, 10% of patients treated were Binet stage A, who generally show no signs of progression and often do not require treatment. General knowledge of iwCLL recommendations can improve the appropriate selection of patients in need of treatment.

In the national survey, the reason for the planned premature discontinuation of chemoimmunotherapy was not the high CIRS value or the advanced age, so elderly patients received a chemotherapy partner appropriate for their fitness in addition to rituximab. More toxic chemotherapies with low CIRS were repeatedly associated with early treatment discontinuation. In the Hungarian survey, only 73.3% of patients received treatment according to the standard first-line recommendation at the time of the study.

In our survey, the proportion of patients with a 17p or 11q deletion was lower than in international studies. The fact that the FISH test was performed in only 60% of the population revealed obvious shortcomings in routine clinical practice in Hungary. Since the study was completed, the tests have become more widely available.

Long-term cytopenia was observed in 14% of our patients treated according to the FCR protocol, which does not differ from the international data. In our study, age over 65 years and creatinine clearance below 70 ml/min were associated with the development of persistent cytopenias. In the FCR treatment of patients selected according to today's guidelines, we found the rate of persistent cytopenias to be as low as 4.6% between 2012-2016 and 0% after 2016.

The response rate achieved with FCR treatment, both in the national study and in our patient population, is in line with international data. The FCR protocol remains the most effective treatment with proven long-term progression-free survival in fit, young CLL patients with non-mutated IGHV and without TP53 aberration.

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List of publications related to the dissertation

1. **Szász, R.**, Telek, B., Illés, Á.: Fludarabine-Cyclophosphamide-Rituximab Treatment in Chronic Lymphocytic Leukemia, Focusing on Long Term Cytopenias Before and After the Era of Targeted Therapies.
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