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Original article

OBINUTUZUMAB FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: SINGLE CENTER EXPERIENCE

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Abstract

Monoclonal antibodies (mAbs) targeting CD20 molecule on B lymphocyte are of great importance in the treatment of B-cell malignancies. In recent years a great effort has been put in developing novel mAbs that can provide greater efficacy than the well-known rituximab. A second class of mAbs obinutuzumab has been presented as a more powerful tool in the treatment of this group of patients. In this retrospective study 70 patients with symptomatic CLL were included. CLL patients were diagnosed and treated at the University Clinic for Hematology between January 2018 and January 2022. All patients were evaluated for traditional clinical and laboratory prognostic factors and newer prognostic factors including IGHV mutation status and CLL prognostic and predictive genetic abnormalities. Most of the patients treated with obinutuzumab had Binet B stage (56%). Mutational status of the immunoglobulin variable region heavy chain (IGHV) in most of CLL patients treated with obinutuzumab was unmated IGHV gene. The most frequently encountered adverse events were tumor lysis syndrome and leukopenia. The analysis of the initial results of the application of obinutuzumab-based therapy allows us to conclude that this therapeutical modality is not associated with severe adverse events that would limit the administration of therapy.

Keywords: chronic lymphocytic leukemia, anti-CD20 monoclonal antibody, obinutuzumab, treatment

Introduction

The anti-CD20 monoclonal antibody (mAb) rituximab has impressively optimized the treatment of CD20+ lymphoproliferative disorders, including chronic lymphocytic leukemia (CLL). CD20 is an ideal goal for targeted therapy, being highly expressed on B cells[1], but not expressed on stem cells, precursor cells, or plasma cells.

Regardless of low-level expression of CD20 on CLL cells, rituximab added to intensive chemotherapy including fludarabine and cyclophosphamide (FCR) in previously untreated, young, fit patients with CLL and led to an overall survival (OS) advantage, the first such

demonstration of an OS advantage in any phase III clinical trial in CLL[2]. But rituximab like single agent has a modest impact on survival[3], and most patients with CLL eventually either fail to respond or relapse after rituximab-containing therapies.

Because CD20 mAbs are of such importance in the treatment of B-cell malignancies, great efforts have been put in developing novel mAbs that can provide greater efficacy than rituximab. Class I monoclonal antibodies such as rituximab work by stabilizing of CD20 on lipid rafts, resulting in strong complement (C1q) binding *in vitro* and effective induction of complement-dependent cytotoxicity (CDC) and significant antibody-dependent cellular cytotoxicity (ADCC)[4]. A second class of mAbs, the type II antibodies, do not require lipid rafts and thus leave CD20 distributed across the surface of the B cell. They have much lower *in vitro* complement binding and CDC, but result in significantly greater ADCC and direct cell death (DCD) compared to type I mAbs[4]. Obinutuzumab (GA101, RO5072759) is the first type II mAb investigated in CLL and has shown efficacy in *in vitro* studies, animal models and clinical trials, and is the focus of science.

Obinutuzumab is a humanized anti-CD20 mAb that has a glycoengineered Fc portion, selected to increase its affinity for FcγRIIIa receptors on immune effector cells. This amplified affinity for immune effector cells such as neutrophils and macrophages is intended to produce enhanced ADCC. Obinutuzumab also contains a modified elbow hinge region to provide superior antigen binding[5].

Mechanisms of action of obinutuzumab appear different from those of rituximab. Obinutuzumab activates neutrophils and mediates phagocytosis through CD16B on neutrophils more potently than rituximab. Additionally, the glycoengineered obinutuzumab causes neutrophil-induced phagocytosis much more effectively than the parental nonglycoengineered antibody. Because of these differences, in whole blood, efficient induction of phagocytosis was elicited by obinutuzumab whereas no significant phagocytosis was observed with rituximab[6].

The mechanism of cell killing is independent of classic apoptosis pathways so it has been postulated that using lysosomal-induced cell death, obinutuzumab may be able to overcome resistance to chemotherapy-induced apoptosis[7]. Obinutuzumab has also been shown to induce apoptosis in a caspase-dependent manner, with mitochondria important to early caspase activity[4].

Another advantage of the new class of monoclonal antibody, class II, is precisely the ability to cross over resistance to class I monoclonal antibodies, including CD20 ‘shaving’ in which rituximab/CD20 complexes are removed from the B-cell surface by monocytes through an endocytic reaction called trogocytosis[9], aberrant lipid raft composition of some malignant B cells, complement depletion[10], polymorphisms in the FcγRIIIa receptor reducing the affinity of the Fc receptor for rituximab, downregulation of proapoptotic proteins and reduction in CD20 antigen expression levels after treatment with rituximab[11]. However, to date, studies have not determined how many of these mechanisms might also apply to type II mAbs like obinutuzumab or whether obinutuzumab will be able to overcome these resistance mechanisms.

In the clinical application, this new group of monoclonal antibodies was implemented several years ago, precisely in our country in 2016 in the clinical study GREEN (A Safety Study of Obinutuzumab Alone or in Combination With Chemotherapy in Patients With Chronic Lymphocytic Leukemia-GREEN MO28543 ClinicalTrials.gov: NCT01905943). Patients with CLL were treated with obinutuzumab-chemotherapy like bendamustine or FC. Since 2018 obinutuzumab has been included in the regimen for treatment of CLL patients in the Republic of North Macedonia.

Material and methods

This retrospective study included 70 patients with symptomatic CLL diagnosed and treated at the University Clinic for Hematology in the period from January 2018 to January 2022. The median follow-up was 30 months (1-60 months). The diagnosis of patients with CLL was established according to the recommendations of the International Working Group on CLL (IWCLL)[12]. All patients were evaluated for traditional clinical and laboratory prognostic factors and newer prognostic factors including IGHV mutation status and CLL prognostic and predictive genetic abnormalities.

Traditional prognostic factors and clinical and laboratory variables included sex, age, Binet stage, physical examination with evaluation of the number of involved lymph node sites (cervical, axillary, and inguinal), measurement of liver and spleen size, white blood cell count (WBC), absolute lymphocyte count (ALC), hemoglobin level, platelet count, Beta-2 microglobulin (B-2M). Mutational status of the immunoglobulin variable region heavy chain (IGHV) gene and prognostic molecular markers like: deletion 17p/TP53 mutation, deletion 11q, trisomy 12, deletion 13q, SF3B1, NOTCH1, MYD88 were detected by the direct sequencing method. Patients were categorized as unmutated (IGHV ≥ 98% germline homology) or mutated (< 98% homology). IGHV mutation status and detection of genetic abnormalities were performed by the Center for Biomolecular Pharmaceutical Analyses at the Faculty of Pharmacy, Skopje, Republic of North Macedonia. Patients received obinutuzumab of 1000 mg alone or with chemotherapy (investigator's choice of fludarabine-cyclophosphamide for fit patients, chlorambucil for unfit patients, or bendamustine for any patient) on days 1, 8 and 15 of cycle 1, and day 1 of cycles 2-6 (28-day cycles), with the cycle 1/day 1 dose administered over two days. Statistical analysis was performed using the SPSS software package, version 21.0. The value of $p<0.05$ was considered significant for all analyses.

Results

We analyzed data of 70 CLL patients, diagnosed and treated at University Clinic for Hematology within 12 months of diagnosis and who had complete data available for all parameters. Since the implementation of obinutuzumab in CLL treatment in our country, in 2019 the largest number of patients were treated (Figure 1).

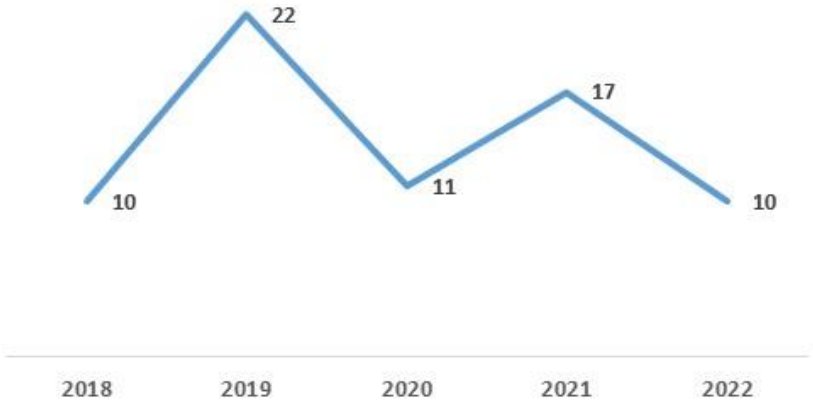

Fig. 1. Distribution of patients with CLL treated with obinutuzumab

According to gender distribution of CLL patients treated with obinutuzumab, there was male predilection (Figure 2). The average age was 64 years (41-85) (Figure 3).

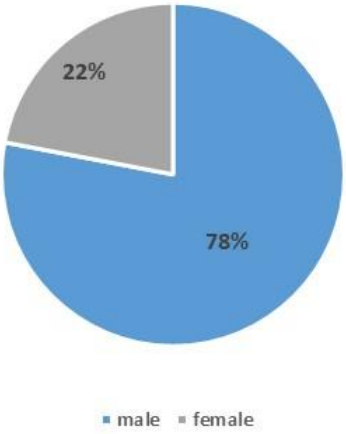

Fig. 2. Gender distribution of CLL patients treated with Obinutuzumab

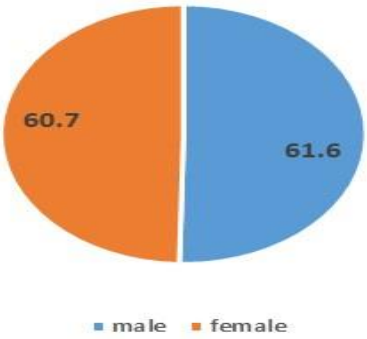

Fig. 3. Age distribution of CLL patients treated with obinutuzumab

Most of the patients treated with obinutuzumab had Binet B stage (Figure 4).

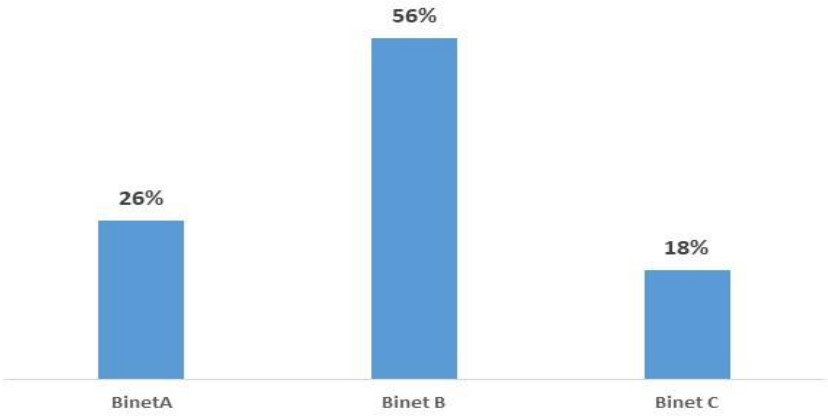

Fig. 4. Distribution of CLL patients treated with obinutuzumab according to Binet stage

A higher percentage of patients treated with obinutuzumab had evaluated Cumulative Illness Rating score (CIRS) (Figure 5), and majority of patients had CIRS 4 (21%) and CIRS 1 and 3 (17%) (Figure 6).

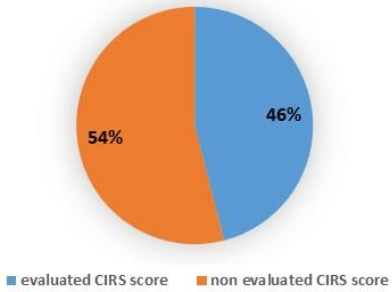

Fig. 5. Distribution of CLL patients treated with obinutuzumab according to evaluated CIRS

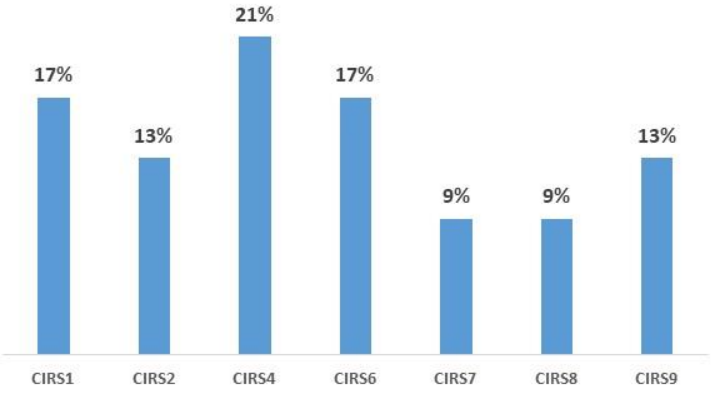

Fig. 5.a. Distribution of CLL patients treated with obinutuzumab according to CIRS

Laboratory characteristics of CLL patients treated with obinutuzumab are presented in Table 1. The average hemoglobin value in patients with CLL treated with obinutuzumab was 115.0 ± 25.7 g / l, which was within normal range (110 to 160 g / l) (Table 1). The average leukocyte count in patients with CLL was 99.8 ± 63.3, and absolute lymphocyte count was 87.5 (Table 1). The mean platelet count in patients with CLL was 177.5 ± 86.2x10^9 / l, which was within normal range (150 to 450) (Table 1). The average albumin value in patients with CLL was 40.6. 6.6 and it was within normal range (35-50) (Table 1). The average ECOG score was 1, and only 5.7% of patients with CLL treated with obinutuzumab had autoimmune hemolytic anemia (AIHA).

Table 1. Laboratory characteristics of CLL patients treated with obinutuzumab

Hemoglobin (g/l)	115
WBC (x 10 ^{3}/dl)	99.8
Platelets (x10 ^{9}/l)	177.5
Absolute lymphocyte count (x 10 ^{3}/dl)	87.5
Albumin level g/l	40.6

Regarding the predictive biomarker mutational status of the immunoglobulin variable region heavy chain gene (IGHV), patients were categorized as unmutated (UnM IGHV) (IGHV ≥ 98% germline homology) or mutated (M IGHV) (< 98% homology) subgroups. IGHV mutation status and detection of genetic abnormalities were performed by the Center for Biomolecular Pharmaceutical Analyses at the Faculty of Pharmacy, Skopje, Republic of North Macedonia. Distribution of patients according to mutational status of the immunoglobulin variable region heavy chain (IGHV) gene is presented in Figure 6, and majority of CLL patients treated with obinutuzumab had unmated IGHV genes.

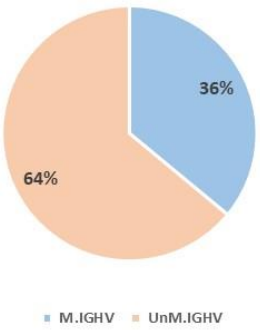

Fig. 6. Distribution of CLL patients treated with obinutuzumab patients according to mutational status of the immunoglobulin variable region heavy chain (IGHV) gene.

Most frequent V gene was 3-30 in M IGHV gene group, and 1-69 in UnM IGHV gene group of CLL patients (Table 2).

Table 2. Gene profile of CLL patients treated with obinutuzumab

Gene	M IGHV	UnM IGHV
V	3-30	1-69
D	3-3	3-3
J	6	4

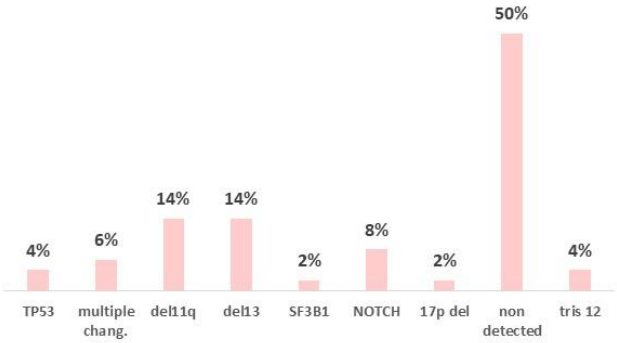

Fig. 7. Distribution of CLL patients treated with obinutuzumab according to prognostic molecular markers

Prognostic molecular markers like: deletion 17p/TP53 mutation, deletion 11q, trisomy 12, deletion 13q, SF3B1, NOTCH1, MYD88 were characterized by the direct sequencing method (Figure 7).

Most of the patients were treated with one line of therapy (64%) (Figure 8), and the most frequently administrated regimen was fludarabine cyclophosphamide rituximab (FCR) administrated in 32% of patients (Figure 9).

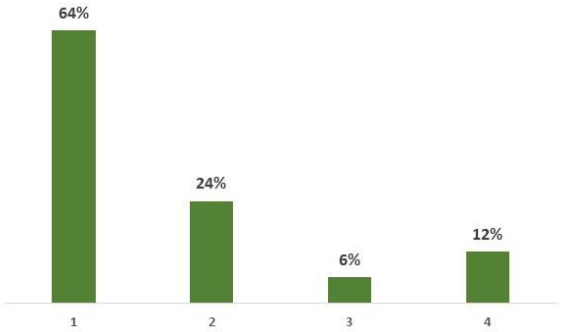

Fig. 8. Distribution of CLL patients treated with Obinutuzumab according to number of regimens administrated

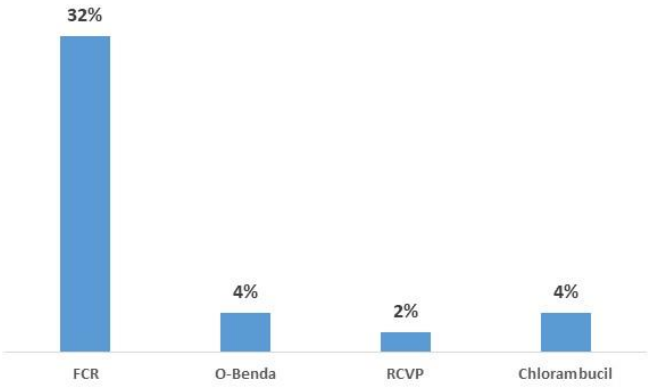

Fig. 9. Distribution of CLL patients treated with obinutuzumab according to type of regimens administrated

The most frequently administrated obinutuzumab-based regimen was obinutuzumab plus chlorambucil (66%) (Figure 10). Figure 11 illustrates distribution of patients according to number of therapy line of administrated obinutuzumab plus chlorambucil. Figure 12 shows distribution of patients according to number of therapy line of administrated obinutuzumab plus fludarabine and cyclophosphamide.

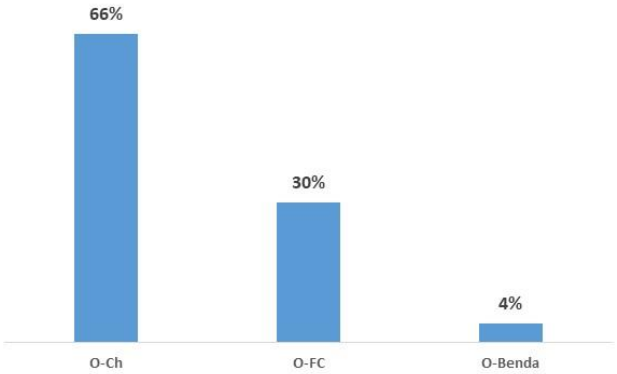

Fig. 10. Distribution of CLL patients treated with obinutuzumab according to type of regimens based on Obinutuzumab

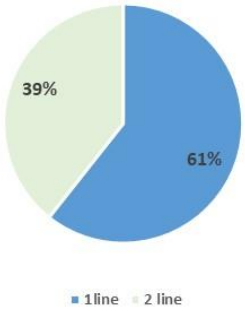

Fig. 11. Distribution of CLL patients according to number of therapy line of administrated obinutuzumab plus chlorambucil

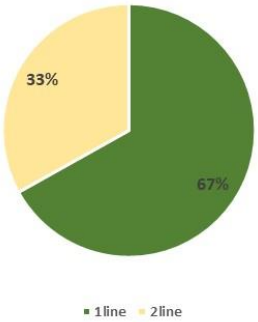

Fig. 12. Distribution of CLL patients according to number of therapy line of administrated obinutuzumab plus fludarabine and cyclophosphamide

Therapy based on obinutuzumab was associated with adverse events, presented in Figure 13, with the most frequently expressed leukopenia.

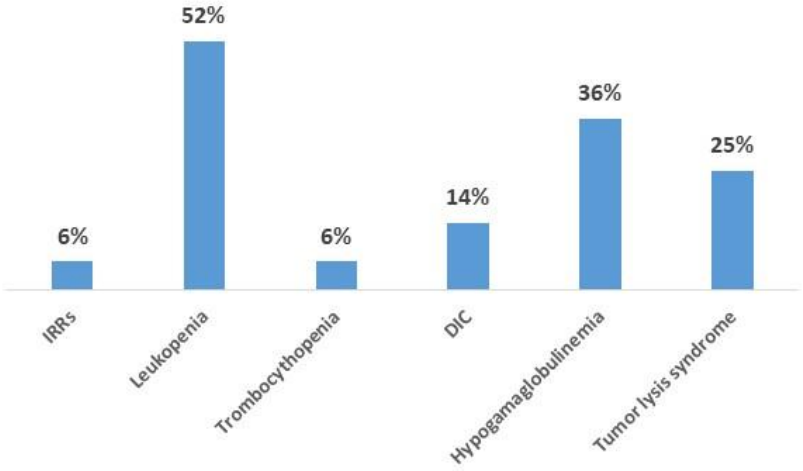

Fig. 13. Distribution of CLL patients treated with obinutuzumab according to adverse events

According to response of therapy, the largest number of patients (42%) achieved a complete response (CR) (Figure 14). Distribution of patients according to Covid-19 infection is presented in Figure 15.

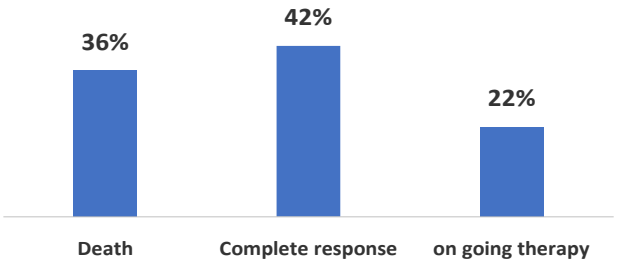

Fig. 14. Distribution of CLL patients treated with obinutuzumab according to response of therapy

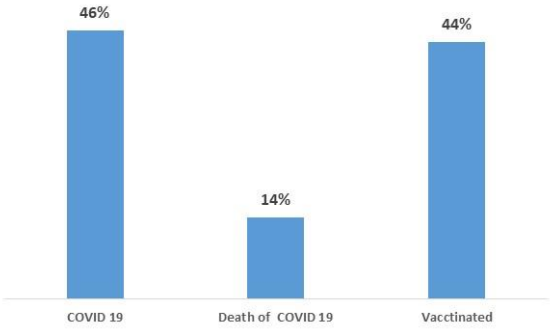

Fig. 15. Distribution of CLL patients treated with obinutuzumab according to Covid-19 infection.

Discussion

Chronic lymphocytic leukemia is the most common lymphoproliferative disorder in the Western world and predominantly affects older people. Until recently, most studies in CLL focused on younger patients in whom intensive therapy with the addition of rituximab to fludarabine and cyclophosphamide was shown to improve survival. Obinutuzumab is a novel type II anti-CD20 monoclonal antibody that recently demonstrated an overall survival advantage when combined with chemotherapy in previously untreated older patients with CLL and comorbidities. Obinutuzumab was superior to rituximab in terms of response rates and progression-free survival[2]. Several preclinical and early phase clinical studies also support the efficacy of obinutuzumab.

The most frequent adverse event noted with obinutuzumab is infusion-related reactions (IRRs), which occur more frequently than with rituximab and are typically restricted to the first cycle of therapy. The GALTON phase 1b study examined obinutuzumab in combination with FC (fludarabine and cyclophophamide) or B (bendamustine) using investigator choice of chemotherapy backbone in patients with previously untreated CLL. The results revealed high levels of grade 3–4 hematological toxicities as expected with these chemotherapy regimens, as well as high rates of IRRs, which were not dose limiting. The ORR was 62% in the FC-containing arm and 90% in the B arm, though several patients had treatment discontinued due to adverse events (AEs). The conclusion of the authors was that obinutuzumab could safely be administered with intensive chemotherapy to previously untreated patients with CLL[13]. In our study, the most common side effect was leukopenia and tumor lysis syndrome found in 25% of patients who did not require discontinuation of therapy or modification of doses. Similar results were found in[14] a study which was a multicenter, open-label, randomized, three-arm phase III study investigating the efficacy and safety of obinutuzumab plus chlorambucil (CLB) *versus* rituximab plus CLB *versus* CLB monotherapy in previously untreated patients with CLL of advanced age with comorbidities. IRRs (five of six patients) were generally limited to the first infusion and grade 3–4 neutropenias that were not associated with fever, infection or requirement for antibiotics[14].

The GAUGIN phase II study concluded that obinutuzumab was safe in patients with advanced CLL but that the single-agent activity was modest and combined with chemoimmunotherapy was likely to be necessary for most patients with CLL, especially those with higher tumor burdens[15].

In our study, most of the patients had UnM IGHV with 1-69 V gene, which is associated with adverse survival, and these patients would benefit from therapy with type II MoAb. But we have to emphasize that there is still a high percentage of patients with stage Binet A, which started obinutuzumab therapy, despite the fact that group of patients should be followed longer without therapy only on watch and wait strategy.

The main limitation of our study is the small group of patients according to the applied obinutuzumab therapy, which could not provide a statistical comparison with patients who were treated with rituximab-based regimens.

In our study, the frequency of prevalent infection with Covid-19 was shown, especially present in a large percentage of fully vaccinated patients, but immunocompromised status was the biggest culprit. Half of our analyzed patients treated with obinutuzumab had a fatal Covid-19 infection. These data were also confirmed in a recent publication by Shafat T *et al.*[16], which analyzed patients infected with the omicron variant COVID-19 (B.1.1.529) and treated with obinutuzumab.

Conclusion

The analysis of the initial results of the application of obinutuzumab-based therapy allows us to conclude that this therapeutical modality is not linked to severe adverse events that would limit the administration of the therapy, and allows a better response with longer survival. But, longer-term follow-up of larger number of included patients is necessary for adequate statistical conclusions.

Conflict of interest statement. None declared.

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