ARTICLE

CHRONIC LYMPHOCYTIC LEUKEMIA

Ibrutinib in combination with rituximab is highly effective in treatment of chronic lymphocytic leukemia patients with steroid refractory and relapsed autoimmune cytopenias

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Autoimmune hemolytic anemia (AIHA) and pure red cell aplasia (PRCA) are common complications of CLL. The optimal treatment of steroid refractory AIHA/PRCA is not well established. We conducted a multicenter study of ibrutinib and rituximab in patients with relapsed/refractory to steroids AIHA/PRCA and underlying CLL. Protocol included induction (ibrutinib 420 mg/day and rituximab, 8 weekly and 4 monthly infusions) and maintenance phase with ibrutinib alone until progression or unacceptable toxicity. Fifty patients were recruited (44—warm AIHA, 2—cold AIHA, 4—PRCA). After the induction 34 patients (74%) have achieved complete response, 10 (21.7%) partial response. Median time to hemoglobin normalization was 85 days. With regards to CLL response 9 (19%) patients have achieved CR, 2 (4%) patients—stabilization and 39 (78%)—PR. The median follow-up was 37.56 months. In AIHA group 2 patients had a relapse. Among 4 patients with PRCA 1 patient did not respond, and 1 patient had a relapse after CR, 2 remained in CR. The most common adverse events were neutropenia (62%), infections (72%), gastrointestinal complications (54%). In conclusion ibrutinib in combination with rituximab is an active second-line treatment option for patients with relapsed or refractory AIHA/PRCA and underlying CLL.

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INTRODUCTION

Autoimmune cytopenias (AIC) occur in 5–10% of patients with chronic lymphocytic leukaemia (CLL) [1]. Traditional approach to management of AIC implies the use of corticosteroid hormones as a first line of treatment. For patients not responding to corticosteroids, rituximab and splenectomy are reasonable treatment choices. In cases of refractory autoimmune cytopenias the optimal choice of treatment is therapy of underlying CLL, as control over CLL clone provides effective control of AIC [2]. Despite expanded therapeutic options, relapses are common, and some cases remain refractory to all treatment approaches. AIC can significantly complicate the management of CLL, worsen the quality of life, and sometimes can be fatal [3].

Ibrutinib, a selective covalent inhibitor of Bruton Tyrosine Kinase (BTK), has emerged as a breakthrough in targeted therapy for patients with CLL [4–6]. Mechanism of action of ibrutinib is complex and mirrors multiple functions of BTK in B-cells. In addition, ibrutinib inhibits interleukin-2 inducible tyrosine kinase (ITK), expressing in T- and NK-cells and mediating TCR-signaling, Th2-, Th9 and Th17 responses, as well as antibody-dependent cellular cytotoxicity (ADCC) [7]. Inhibition of ADCC, a component of RBC destruction, selective inactivation of Th2-cells, whose activity may contribute to both onset and maintenance of AIHA [8, 9] as well as suppression of CLL clone suggested promising activity of ibrutinib in treatment of AIC.

Several studies have shown high efficacy of monoclonal antibodies to CD20 both as monotherapy or in combination with chemotherapy in treatment of autoimmune complications of CLL [10–17]. Rituximab in combination with chemotherapy produces high rate of remissions, however the duration of responses is

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unsatisfactory (12–28 months), especially in patients with refractoriness to corticosteroids.

The objective of this study was to evaluate efficacy and safety of ibrutinib in combination with rituximab in patients with autoimmune hemolytic anemia (AIHA)/pure red cell aplasia (PRCA) and underlying CLL.

METHODS

Study design and participants

This phase 2, multicenter, single-arm study enrolled patients with CLL complicated with AIHA or PRCA from 4 centers in Russia (S1). Eligible patients were aged at least 18 years and had immunophenotypically confirmed CLL as well as proven diagnosis of autoimmune hemolytic anemia or pure red cell aplasia. The AIHA was defined as the presence of normocytic or macrocytic anemia, increase of LDH level above upper limit of normal and/or decrease of haptoglobin level below lower limit of normal and the presence of anti-RBC autoantibodies determined by positive direct antiglobulin test. PRCA was defined as the presence of transfusion dependent normocytic or macrocytic anemia, platelets level ≥100,000/mm^3 and marked decrease or absence of erythroblasts in the bone marrow. Eligible patients had a relapse of AIHA/PRCA after steroids, splenectomy or rituximab or refractoriness to glucocorticosteroid hormones, defined by no response to steroids, requirement of >20 mg of prednisone or equivalent to control hemoglobin level or relapse within 6 months after treatment with steroids. The patients should have received no more than 2 lines of antileukemic treatment and should have life expectancy >6 months. Detailed inclusion and exclusion criteria are presented in Supplementary Material (S2).

The protocol was approved by the Moscow city independent ethics committee (approved October 28, 2016).

Procedures

During induction phase patients received ibrutinib 420 mg orally once daily and rituximab (8 weekly with subsequent 4 monthly infusions, in total 12 infusions). Rituximab was given intravenously at the dose of 375 mg/m^2 during first infusion and at the dose of 500 mg/m^2 during subsequent infusions. In maintenance phase—only ibrutinib was given until relapse, progression or unacceptable toxicity. CT scan imaging was done at baseline, the end of the induction phase (day 225). CT was repeated in case of clinical suspicion of progression. Bone marrow assessment was mandatory at baseline as well as at the end of induction phase irrespective of the response. Direct antiglobulin test as well as biochemical markers of hemolysis were performed at each visit.

Adverse events and serious adverse events were evaluated according to NCI-CTCAE criteria (version 5.0), categorized by treatment relatedness and reported continuously during the induction and maintenance phases until 28 days after the last treatment administration. Follow-up for relapse of CLL or AIC or other adverse events was continued every 3 months after the discontinuation of treatment for adverse events.

Ibrutinib dose was reduced or interrupted in cases of toxicity during treatment according to the manufacturer's instructions. Patients who did not tolerate the lowest applicable dose were discontinued from treatment. Rituximab dose was not reduced but postponed or missed in cases of toxicity until resolution of adverse events. During induction phase cotrimoxazole prophylaxis was mandatory. Folic acid supplementation was recommended until resolution of active hemolysis and hemoglobin (Hb) response. The use of anticoagulants was not permitted per initial protocol. In the amendment of protocol from 04.02.2019 the use of direct oral anticoagulants, excluding vitamin K antagonists was approved.

Response criteria were defined as followed: a partial response (PR) implied a Hb level >8 g/dL with at least a 2 g/dL increase from the pretreatment level (in the absence of any transfusion within 3 months). A complete response (CR) was defined by a Hb level >12 g/dL in women or 13 g/dL in men. Relapse in patients with CR was defined as a fall in hemoglobin below 10.0 g/dL along with decrease of haptoglobin in AIHA or decrease of reticulocyte in PRCA. Relapse in patients with PR was defined as a re-emergence of transfusion dependence and the requirement for new treatment.

The primary endpoint was the proportion of patients who achieved an overall response of AIC (CR, CR with positive Direct Antiglobulin Test (DAT), or PR) as per investigator assessments at each visit during first 6 months and at the end of the induction phase as well as the duration of the response (interval between time of first response to the first documented relapse of AIC).

Secondary endpoints were response of CLL according to iwCLL 2008 criteria, time to treatment failure (interval from the first day of treatment to the date of first documented relapse of AIHA/PRCA, CLL progression, discontinuation for unacceptable toxicity or death) (TTF), time to best response (interval between first day of treatment and best response of AIC), safety, overall survival (OS), and biomarker analysis.

Statistical analysis

Taking into account the design, study power and sample size were calculated under exponential model. Since the time-to-event is assumed to be exponentially distributed, the median survival time is determined by the hazard rate. As a result, comparing median survival times is equivalent to comparing hazard rates. We hypothesized that the primary endpoints (ie, the median duration of the response) would increase by 50%. Expected hazard rate in historical control group was calculated using pooled data (53 cases) for clinically similar trials. The median DR-AI time for pooled data was 22 months with hazard rate of 0.0315. For assumed median of $22 + 24 = 46$ months (hazard rate of 0.0151) with attrition rate 0.001, for alpha 0.05 and power >80% not less than 38 subjects subsequently required. Target enrolment was 50 patients to account for a 20% dropout rate.

The analysis was per-protocol, the efficacy population included all patients who received at least one month of treatment, and the safety population included all patients who received at least one dose of either investigational drug. Safety analyses were summarized descriptively. Responses were reported as percentages of patients, with 95% CIs. Time to-event data are presented as Kaplan-Meier plots for time to first event and as summary tables for fixed time points. Median time to event and response rates were calculated with 95% CIs. Chi square test analysed the effect of prognostic factors on response rates (post-hoc analyses), and Cox proportional-hazards regression model was used to estimate hazard ratios and 95% CIs.

Power and sample size were calculated using IBM SPSS SamplePower version 3.0.1. All other analyses were done with Prism 9 software, Version 9.2.0 (283). The study is registered with ClinicalTrials.gov, number NCT01582776, and is ongoing but closed to accrual.

RESULTS

Fifty patients with CLL and relapsed or refractory to steroids AIC were recruited from February 21, 2017 to November 18, 2019. Fifty patients were evaluable for activity and safety. Forty-seven (94%) completed induction phase (Fig. 1), and started maintenance treatment. Forty-three (86%) completed 24 months of treatment.

Demographic and clinical patient characteristics are described in Table 1. Median age was 65 years and 27 (46%) of 50 patients were men. Four patients had PRCA, 44 patients had warm type AIHA and 2 patients had cold type AIHA. In eight cases AIC and CLL were found simultaneously, in 42 cases AIC occurred during the course of CLL with a median time from CLL diagnosis to AIC 3, 4 years (range 0.15–14). Forty-four patients had IgG-positive (+/− C3d) DAT, and 2 patients with cold type antibody had C3d-only positive DAT and a cold agglutinin titer over 64. In total, 37 patients (74%) had received transfusions within 3 months prior to inclusion, and 2 patients could not be transfused due to difficulties with blood compatibility testing. The median number of transfusions within 3 months was 4 (range 1–18).

At baseline, 14 (28%) had active disease according to iwCLL2018 guidelines. The median number of previous lines of treatment both for CLL and AIC was 2 (range 1–5), 23 patients had previously received treatment for CLL and 35 patients had previously received rituximab (18 for AIC and 17 for active CLL + /−AIC).

Thirty-two patients (624%) were steroid-refractory. Among them 4 patients had an early relapse after steroid withdrawal, 7 had relapse upon steroid tapering, 11 had a relapse as well as poor response to steroids and 10 had their first hemolytic episode and poor response to steroids. In the latter category of patients, the median time from the beginning of steroid treatment to first day

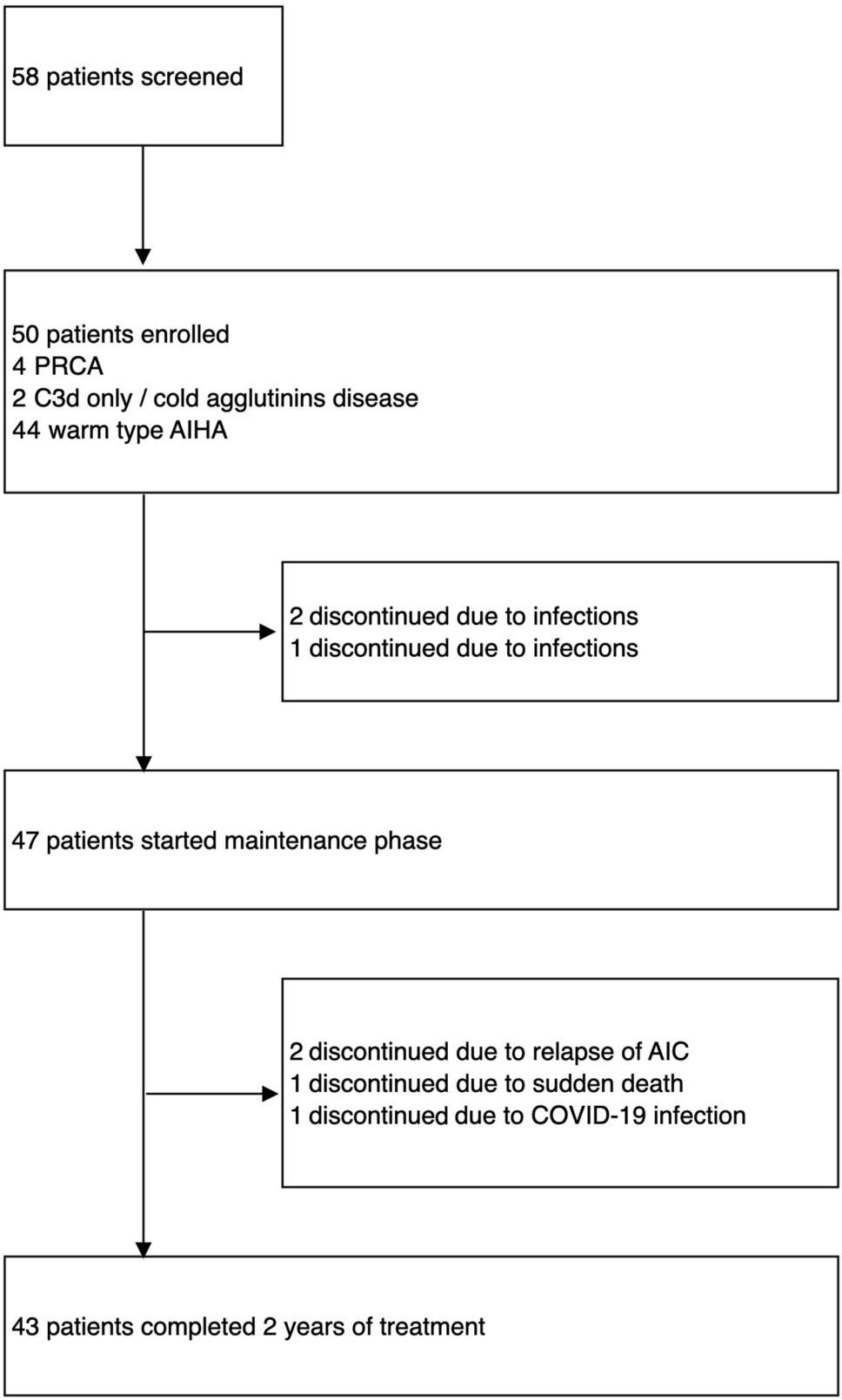

Fig. 1 CONSORT flow diagram of the study participants. PRCA pure red cell aphasia, AIHA autoimmune hemolytic anemia.

of protocol was 35 days (range 20–59 days). All steroid refractory patients remained on steroids when rituximab and ibrutinib were started, and the dose was tapered over 1 week. Thirteen patients, including 2 patients with cold type RBC autoantibodies, had a relapse after rituximab or immunochemotherapy. Finally, 5 patients had persisting AIC despite multiple lines of treatment with time of transfusion dependent anemia of 10, 16, 18, 64 and 94 months.

All patients in the safety population ($n = 50$) received a starting dose of ibrutinib of 420 mg in the induction phase as well as

Table 1. Patient characteristics.

Characteristic	Number of patients (%)
Age	
≤59 years	16 (32%)
60–69 years	20 (40%)
70–79 years	11 (22%)
≥80 years	3 (6%)
Male	27 (54%)
Autoimmune complication	
Warm type AIHA	44 (88%)
Cold type AIHA	2 (4%)
PRCA	4 (8%)
DAT IgG + /− C3d	44 (88%)
DAT C3d only	2 (4%)
Transfusions in the previous 4 weeks	37 (74%)
Active CLL according to iwCLL2008 guidelines	14 (28%)
Lines of prior therapies for AIC/CLL	
1	14 (28%)
2	15 (30%)
3	12 (24%)
≥4	9 (18%)
Previous therapies	
Prednisolone/Dexamethasone as a monotherapy	44 (88%)
R-CVP/RCD	17 (34%)
Rituximab as a monotherapy	6 (12%)
Splenectomy	3 (6%)
Obinutuzumab	2 (4%)
Alemtuzumab	1 (2%)
FCR	18 (36%)
BR	12 (24%)
Chlorambucil	2 (4%)
R-CHOP	2 (4%)
Venetoclax	1 (2%)
Cytogenetic abnormalities	
del(17p) and del(13q)	7 (14%)
del(17p), del(13q), del(11q)	1 (2%)
del(17p) as a sole abnormality	1 (2%)
del(11q) and del(13q)	11 (22%)
del(11q)	1 (2%)
Trisomy 12	3 (6%)
del(13q) as a sole abnormality	11 (22%)
No aberrations	15 (30%)
IGHV mutational status	
Unmutated CLL	35 (70%)
Mutated CLL	10 (20%)
Heterogeneous sequence/missing	5 (10%)
Immunoglobulin level	
IgG <7 g/L	25 (50%)
IgA <0.77 g/L	27 (54%)
IgM <0.48 g/L	21 (45%)
Haptoglobin in patients with AIHA	
<0.08 g/L (below detection limit)	37 (80%)
0.08–0.4 g/L (below normal value)	5 (10%)
>0.6 g/L (within normal values)	3 (4%)
Missing	1
Characteristic	**Median (range)**
Age, years	65 (48–82)
Lines of previous therapies for CLL/AIC	2 (1–6)
Number of transfusions within last 3 months	4 (1–18)
CIRS	4 (1–11)
Beta-2-microglobulin, mg/L	4.75 (2.49–11.0)
IgG, g/L (Norm: 7–18)	6.92 (2.24–17.52)
IgA, g/L (Norm: 0.77–3.5)	0.66 (0.11–5.57)
IgM, g/L (Norm: 0.48–3.24)	0.49 (0.11–2)
Hemoglobin at day of treatment initiation, g/L	7.9 (4.9–10.6)
Reticulocytes in patients with AIHA, K/μL	177 (25–906)
WBC, K/μL	30.1 (1.89–247)
ANC, K/μL	3.78 (1.0–10.9)
Platelets, K/μL	218 (61–771)
LDH, U/L	526 (167–1822)
Indirect bilirubin, mkmol/L	27 (11–146)

AIHA autoimmune hemolytic anemia, *PRCA* pure red cell aplasia, *DAT* direct anti globulin test, *R-CVP* rituximab, cyclophosphamide, vincristine, prednisolone, *RCD* rituximab, cyclophosphamide, dexamethasone, *FCR* fludarabine, cyclophosphamide, rituximab, *BR* bendamustine, rituximab, *R-CHOP* rituximab, cyclophosphamide, vincristine, doxorubicin, prednisolone, *ANC* absolute neutrophil count, *LDH* lactate dehydrogenase.

rituximab as planned. Fifteen patients had a missed or delayed dose of rituximab for active infections at the day of planned infusion.

Twenty-eight (56%) of patients had no dose modifications during the total period of study. Among 22 patients with modification the median dose intensity (total dose taken [mg] × 100/total dose expected [mg]) was 97% (range 58.2–99.5%). Reasons and dose intensities are presented in Table S3. Dose modification were observed in 16 (32%) patients during the first year of study, 7 (15%) patients in the second year. Eight patients had to reduce dose permanently.

Efficacy

The median follow-up at the data cut off was 37.56 months (IQR 2.7−58). The hemoglobin dynamics as well as DAT-response in AIHA patients are shown in Figs. 2 and 3. Hemoglobin response was evident in 69% of patients by week 4 of treatment and 95.6% of AIHA patients had at least a PR by week 8. All AIHA patients became transfusion-free by week 8. The proportion of patients who achieved an overall response by the end of induction phase (day 169) was 97.8%. Normalization of haptoglobin was noted in 56% of patients by day 29 and in 74% of patients by day 57. Thirteen patients with AIHA (29.5%) demonstrated initial elevation of LDH after initiation of treatment. The median time to elevation in these patients was 15 days (range 8–29 days). Normalization of LDH at 4, 8 and 12 weeks occurred in 56%, 67.5% and 81% of patients, respectively (Fig. S4).

At final assessment of the response after the induction phase (day 225) 12 patients (27%) have achieved DAT-negative CR, 22 (50%) DAT-positive CR, 1 DAT-negative PR and 9 (20%) DAT-positive PR.

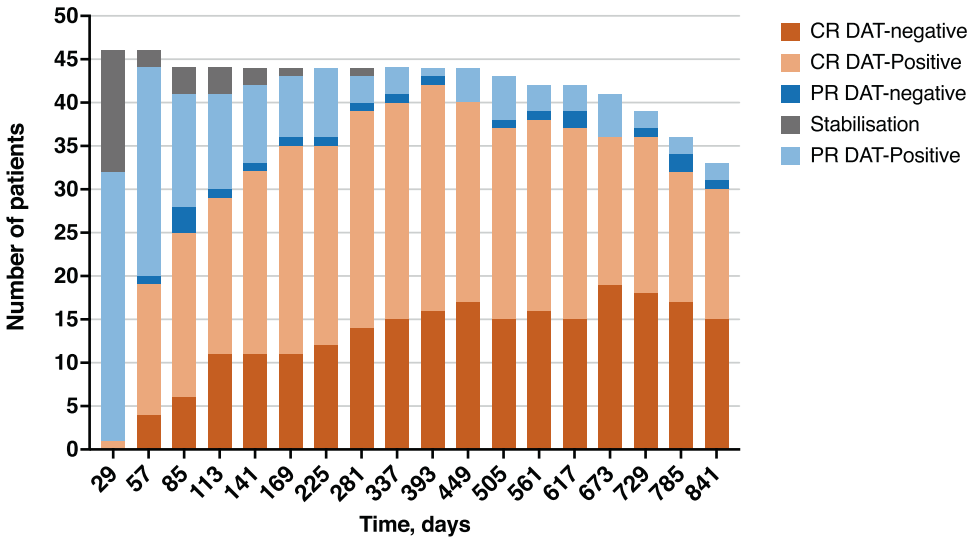

Fig. 2 Hemoglobin and DAT response in patients with AIHA within first 2 years. CR complete response, PR partial response, DAT direct antiglobulin test.

Median time to Hb normalization was 85 days (95% CI 57–113), median time to DAT-negative response was not reached (Fig. 4A).

Longer time to hemoglobin normalization in patients with AIHA was not correlated with disease status, number of previous treatment lines, transfusion burden, LDH and beta-2-microglobulin levels at screening, absolute reticulocyte count or corrected reticulocyte count at screening, age and sex. Significant associations were found for 17p deletion (hazard ratio (HR) 0.54, 95% CI 0.29–1.01, $p = 0.026$), hemoglobin level at screening (⩽7.5 g/dL vs >7.5 g/dL, HR 0.54, 95% CI 0.3–0.99, $p = 0.017$), AIC status (steroid refractoriness vs relapse, HR 0.54, 95% CI 0.24–1.23, $p = 0.02$) as well as CLL response status by day 225 (any response as opposed CR or nodular PR, HR 2, 95% CI 0.8–4.6, $p = 0.012$) (Fig. S5).

Longer time to DAT-negative response correlated with steroid refractoriness as opposed to relapse (HR 3.67, 95% CI 1–12, $p < 0.001$) and LDH level above 500 U/L (HR 2.3, 95% CI 0.97–5.3, $p = 0,048$) (Fig. S6). Borderline correlation was found for the hemoglobin level (below 7.5 g/dL). No other correlations with the above-mentioned factors were found.

In a cohort of patients with PRCA one patient did not respond by the time of treatment discontinuation (day 146) and retained transfusion dependence, while three other patients achieved hemoglobin normalization by 85, 113 and 141 days, respectively.

Fluctuations of Hb level during the follow up period occurred in the context of infections and were explained by anemia of chronic disease, iron deficiency or other causes and resolved without modifications of ibrutinib dosage with resolution of infection or supportive measures.

With regards to CLL response by the end of induction phase 9 (19%) patients have achieved a CR, 2 (4%) patients—stabilization and the rest of the patients—PR. The reasons of partial remissions were residual enlargement of the spleen in 16 (34%) patients, residual enlargement of lymph nodes in 3 (6%), residual bone marrow involvement—10 (21%), PR with lymphocytosis—6 (12.7%), multiple sites −1. Bone marrow was investigated in all patients. Only 1 patient with CR had an undetectable minimal residual disease (MRD) in the bone marrow, all other patients were MRD positive.

TTF, OS and time to discontinuation for any reason is shown in Fig. 4B–D. In total, 4 AIC/CLL events have occurred at the data cut off. One patient had a relapse of wAIHA, which occurred in the context of COVID-19 infection, 1 patient had progression of CLL as well as relapse of wAIHA. Among 3 patients with PRCA who demonstrated response to treatment one patient had a relapse after CR by day 415. Two other PRCA patients remained in CR by last follow up (days 1005 and 1373).

Median TTF and duration of response have not been reached. At 3 years, probability of response preservation in patients with AIHA was 95% (95% CI 82–98%) and therefore met its prespecified threshold (i.e., lower bound of 95% CI excluding 50%). Furthermore, duration of response to ibrutinib was longer compared to previous episodes in the same patients. In 32 relapsed patients before inclusion into the study the median time between the first episode to next treatment was 15.6 months, while after the inclusion to protocol the median was not reached (hazard ratio (log rank) 24.7, 95% CI 12.1–50.6).

Safety

Adverse events observed in >3 patients, rated by investigators as at least possibly related to study drugs, as well as all events of at least grade 3 are listed in Table 2. The most common adverse events (all grades) were neutropenia (62%), infections (72%) and gastrointestinal complications (54%). G-CSF for neutropenia was used in 14 (28%) patients. There was no significant decrease in the level of immunoglobulins during the induction phase (Fig. S7). The median normalized monthly rate of infections during the first 8 months was 10 (range 6–22), while from 9 to 24 months—4.5 (1–7, $p < 0.001$). Among 13 non-COVID-19 pneumonias 8 (62%) occurred during the induction phase.

No patients had serious bleeding and no patients developed second malignancies during observation period. Cardiac arrhythmias occurred in 12 patients. Ten patients (20%) had atrial fibrillation/supraventricular tachycardia, 1—atrioventricular block, 1—symptomatic sinus bradycardia. In 3 patients atrial fibrillation occurred in the context of infections.

In total 33 serious adverse events were reported during the study by 19 (38%) of 50 patients, which included 13 (39%) serious adverse events during the induction phase. Serious adverse events were neutropenia ($n = 8$, 24%), COVID-19 ($n = 9$, 27%), infections, other than COVID-19 ($n = 8$, 3%), and arrhythmias ($n = 8$, 24%).

In total 14 patients discontinued treatment. Two patients discontinued for progression (1—relapse of AIHA, 1—relapse of CLL), 3 for toxicity (1—recurrent infections and poor compliance, 1—drug related maculo-papular rash, 1—atrial fibrillation). There were two sudden deaths and 1 previously splenectomized patient with PRCA died from septic shock. By the time of SAE response of PRCA was not achieved. One sudden death occurred in a man of

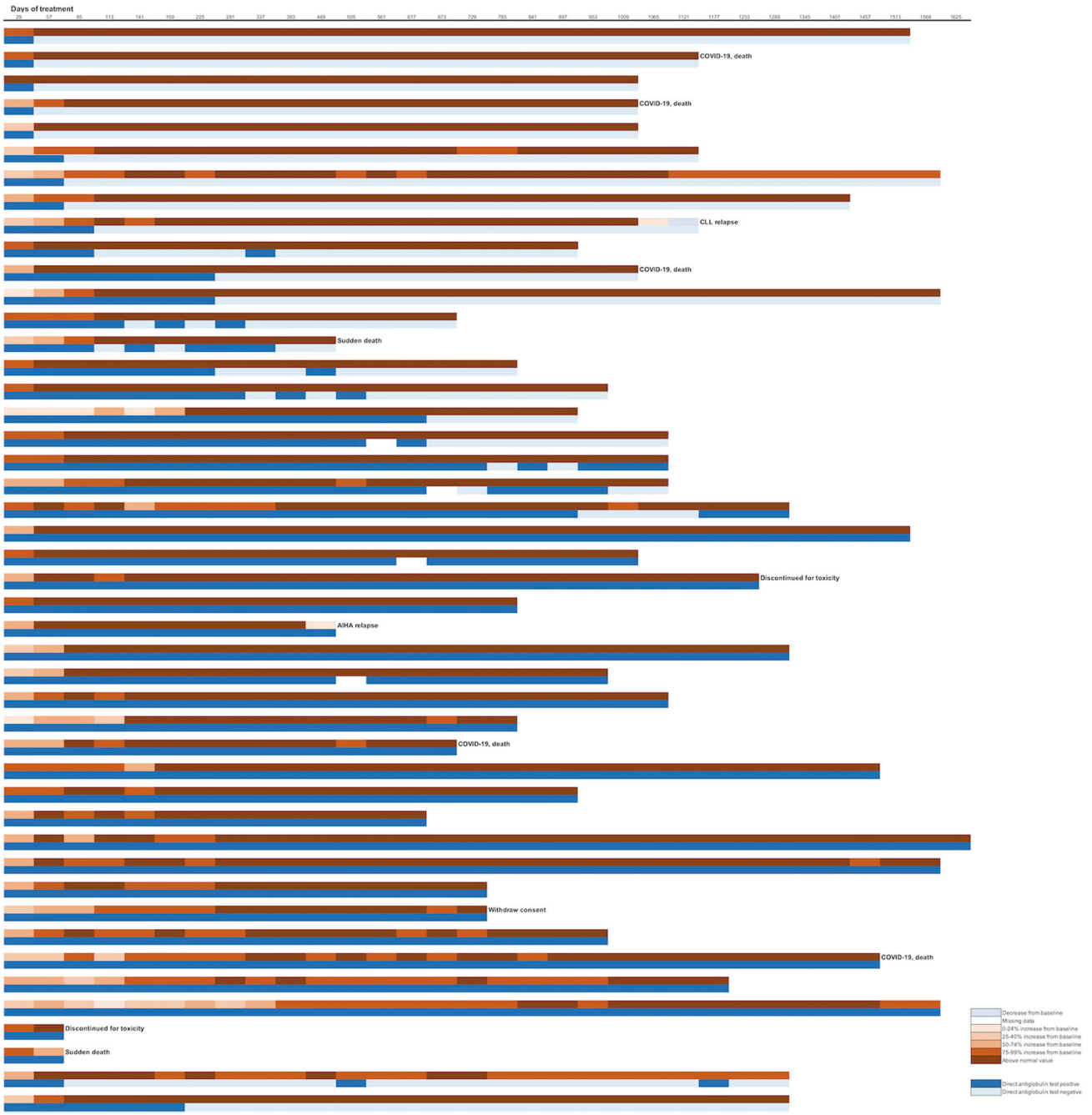

Fig. 3 Hemoglobin and DAT changes over time in patients with AIHA. Each patient is depicted as a separate timeline. For each patient hemoglobin level (top line) and direct antiglobulin test results (bottom line) are represented in dynamics by day.

73 y. o. on the day 82 of treatment. The patient had baseline asymptomatic bifascicular block and received ACE inhibitor for arterial hypertension. The second sudden death occurred in a man of 67 years on the day 439. The patient had right bundle branch block without symptoms, and his medications were cotrimoxazole and omeprazole. Finally, six patients, all in CR of AIHA, died from COVID-19.

DISCUSSION

In this work we present the results of the first prospective study on the use of BTK inhibitors in combination with rituximab in the treatment of refractory autoimmune complications in the context of CLL.

Although the overall survival of patients with and without autoimmune complications in CLL is not different [18], refractory AIHA often present a complex clinical problem. Prolonged use of corticosteroid hormones is associated with opportunistic infections [19], especially in relapsed CLL patients [20, 21], and seems to be less safe, than in patients with primary wAIHA. Rituximab as a monotherapy or in combination with cyclophosphamide and dexamethasone produces responses in 70–100% of patients, although the median duration of response among responders varies in the range of 12–28 months [10, 11, 13, 22–24].

The results of this study show that induction therapy with rituximab and ibrutinib followed by ibrutinib maintenance therapy is effective for patients with CLL and relapsed or refractory autoimmune complications. By the end of induction phase the

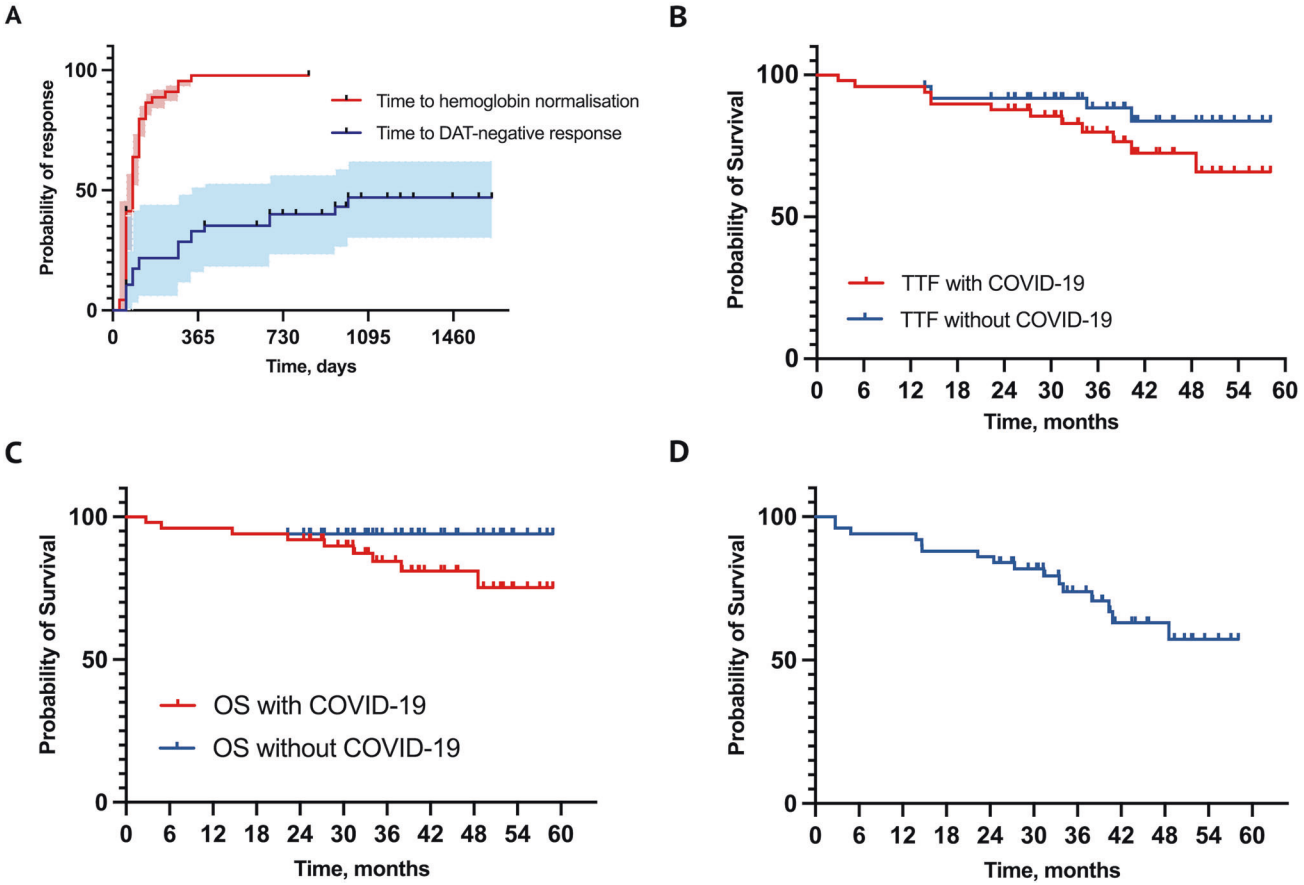

Fig. 4 **A** Time to hemoglobin normalization and time to DAT "-"ve response (95% CI within error bars) in patients with AIHA. **B** Time to treatment failure (TTF). **C** Overall survival (OS) with exclusion and inclusion of deaths related to COVID-19. **D** Time to discontinuation.

overall response in the efficacy population was 96%. With a median follow-up of 37.5 months, only 2 warm type AIHA recurrences occurred, one of them in the context of CLL progression. Thus, prolonged therapy with ibrutinib significantly outperformed rituximab alone or in combination with chemotherapy in terms of the rate and duration of responses according to pooled data from previous studies and significantly exceeded the prespecified thresholds. Marked difference in duration of response can be explained by the fact, that rituximab monotherapy or RCD are not likely to provide long term control of CLL. More effective CLL-directed therapy, like bendamustine and rituximab provide longer remissions of AIC [17]. A number of previous cohort and retrospective studies have shown efficacy of ibrutinib in CLL patients with AIHA as well as low rate of treatment emergent AIC in patients with previous episodes [25, 26]. Efficacy of ibrutinib in treatment of cold agglutinin syndrome and underlying CLL have been also reported recently [27].

As previously published cohorts of patients with AIC, our patient sample is enriched with unfavorable prognosis risk factors [17]. Targeted drugs are more efficacious in high risk subgroups of patients compared to immuno-chemotherapy. In this regard they are reasonable choice for AIC complicated CLL. A number of reports on the efficacy in treatment of active AIC of venetoclax, a drug with other mechanism of action, supports this conclusion.

The mechanism by which AIHA is controlled by ibrutinib is not fully understood. More than half of the patients have persistence RBC-bound antibodies despite prolonged treatment period. Persistence of B-cells, producing detectable amounts of autoreactive antibodies may indicate probability of relapse, but most patients maintain the response. In a mouse model of AIHA Rogers et. al. showed that treatment with acalabrutinib led to significantly greater decrease of RBC-bound antibodies, compared to ibrutinib and this was not associated with faster RBC clearance, but with a decrease in antibody production [28]. This difference between ibrutinib and acalabrutinib remains unexplained. Numerous studies showed altered T-cells composition in CLL patients. Due to selective inactivation of Th2 cells, ibrutinib induces a shift in the Th2/Th1 ratio [29, 30]. Much evidence suggests that TH2 cells may be involved in the pathogenesis of autoimmune cytopenia. In addition, ibrutinib treatment causes significant reduction of Th17-cells and T follicular helper cells [29, 31]. Both populations have been shown to be implicated in the pathogenesis of AIHA in murine models [32, 33] and both are elevated in patients with autoimmune cytopenias [33–35]. In total, these data suggest, that ibrutinib may eliminate environment contributing to AIC.

The safety profile of ibrutinib and rituximab combination was acceptable, with no unexpected toxicity. The most common adverse events, including grade 3 or more were similar to those observed in ibrutinib plus rituximab arms in the previously published trials [36–38]. The rate of infections during induction phase was similar to ibrutinib arm in Resonate trial and significantly decreased thereafter. Although reporting differences make cross-study comparisons difficult, the rate of atrial fibrillation in our study tend to be higher (18% within the first 12 months), compared to published data [39–41]. We suggest that one of the contributing factors could be anemia and this observation requires further research.

A dose-intensive regimen of rituximab was used in our study. Dose intense regimens of rituximab have been used in treatment of non-Hodgkin lymphomas, CLL and autoimmune complications with no new safety signals [42–47]. We observed significantly more infections during induction phase. Increased rate of

Table 2. Summary of adverse events.

Characteristic, *n* (%)	Total	Grade 1/2	Grade 3/4
Investigations			
Neutrophil count decreased	31 (62%)	8 (16%)	23 (46%)
Infections and infestations			
Upper respiratory infection	20 (40%)	20 (40%)	
Bronchial infection	13 (26%)	13 (26%)	
Lung infection	10 (20%)	5 (10%)	5 (10%)
Herpes simplex infection	9 (18%)	9 (18%)	
Skin infection (including paronychia, periorbital infection)	8 (16%)	6 (12%)	2 (4%)
Pharyngitis	7 (14%)	7 (14%)	
Rhinitis infective	7 (14%)	7 (14%)	
Soft tissue infection	6 (12%)	2 (4%)	4 (8%)
Urinary tract infection	6 (12%)	6 (12%)	
Gastrointestinal disorders			
Diarrhea	16 (32%)	16 (12%)	
Dyspepsia	12 (24%)	11 (22%)	1 (2%)
Nausea	4 (8%)	3 (6%)	1 (2%)
Musculoskeletal and connective tissue disorders			
Muscle cramp	14 (28%)	13 (26%)	1 (2%)
Arthralgia	11 (22%)	10 (20%)	1 (2%)
Myalgia	10 (20%)	10 (20%)	
Nervous system disorders			
Headache	14 (28%)	13 (26%)	1 (2%)
Dizziness	10 (20%)	10 (20%)	
Hemorrhagic complications			
Hematoma	12 (24%)	12 (24%)	
Purpura	11 (22%)	11 (22%)	
Epistaxis	5 (10%)	5 (10%)	
Oral hemorrhage	3 (6%)	3 (6%)	
Hematuria	2 (4%)	2 (4%)	
Cardiac disorders			
Atrial fibrillation/ supraventricular tachicardias	10 (20%)	4 (8%)	6 (12%)
Other arrhythmias	2 (4%)	1 (2%)	1 (2%)
Vascular disorders			
Hypertension	8 (16%)	3 (6%)	5 (10%)
Psychiatric disorders			
Insomnia	8 (16%)	8 (16%)	
General disorders and administration site conditions			
Edema limbs	7 (14%)	7 (14%)	
Fever	8 (16%)	7 (14%)	1 (2%)
Rituximab infusion related reaction	5 (10%)	5 (10%)	
Skin and subcutaneous tissue disorders			
Rash maculo-papular	7 (14%)	7 (14%)	
Metabolism and nutrition disorders			
Hyperglycemia	4 (8%)	3 (6%)	1 (2%)

infections during first months of ibrutinib monotherapy is a well-documented fact, although rituximab could additionally contribute the rate of infections [48].

No randomized trials have shown that addition of rituximab to ibrutinib improves progression free survival (PFS) compared to ibrutinib alone [36, 38]. Furthermore, there is a number of possible antagonistic interactions between ibrutinib and rituximab [49]. The lack of difference in PFS raises the question if the addition of CD20 antibody inappropriate or is just not needed. In treatment of AIC the speed of response is usually desirable. As addition of rituximab speeds up the response of CLL [36] we believe, that antibody to CD20 is a valuable component of AIC treatment, especially in cases of severe anemia with poor response to steroids. Furthermore, a "flare" of autoimmune phenomena was described following ibrutinib initiation [50]. None of our patients with AIHA demonstrated decrease of Hb level compared to baseline. Nevertheless, since the two-drug combination is likely to be more immunosuppressive, there is no proven benefit of adding of rituximab to ibrutinib in the treatment of CLL, future prospective trials should address the value of BTK inhibitors as single agents in treatment of AIC. A number of cohort and retrospective studies have shown that ibrutinib alone is efficacious in treatment of both warm and cold types AIHA [25–27].

Thus, our study shows high efficacy of ibrutinib in combination with rituximab in treatment of AIC. All AIHA patients became transfusion-free within 2 months, all achieved at least a partial response by the end of induction phase. Maintenance therapy with ibrutinib provides long-term control of the autoimmune complication despite the persistence of RBC-bound antibodies. In conclusion, ibrutinib in combination with rituximab is an active second-line treatment option, with a manageable safety profile for patients with relapsed or refractory AIC in the context of CLL.

DATA AVAILABILITY

The anonymized data collected are available as open data via open-source research platform: https://www.synapse.org/israel.

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EN: Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing (original draft, review, and editing), visualization, supervision, project administration, funding acquisition; MK: Conceptualization, methodology, validation, formal analysis, data curation, writing (original draft, review, and editing), visualization; VB, BB, AS, TO: Investigation, writing (review and editing); DM, AS, JS, GV, IM, MS, AS, TK, PM, ED, OS, KK, TK: Investigation, resources, writing (review and editing); OM, IP, VP: Conceptualization, writing (review and editing), funding acquisition.

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EN reports research funding from Janssen during the conduct of the study. Other authors declare no competing interests.

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