Atezolizumab, venetoclax, and obinutuzumab combination in Richter transformation diffuse large B-cell lymphoma (MOLTO): a multicentre, single-arm, phase 2 trial

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Summary

Background The diffuse large B-cell lymphoma (DLBCL) variant of Richter transformation (DLBCL-RT) is typically chemoresistant with poor prognosis. Aiming to explore a chemotherapy-free treatment combination that triggers anti-tumour immune responses, we conducted a phase 2 study of atezolizumab (a PD-L1 inhibitor) in combination with venetoclax and obinutuzumab in patients with DLBCL-RT.

Methods This was a prospective, open-label, multicentre, single-arm, investigator-initiated, phase 2 study in 15 hospitals in Italy and Switzerland. Eligible patients had a confirmed diagnosis of chronic lymphocytic leukaemia or small lymphocytic lymphoma as per the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) 2008 criteria with biopsy-proven transformation to DLBCL; had not previously received treatment for DLBCL-RT, although they could have received chronic lymphocytic leukaemia therapies; were aged 18 years or older; and had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2. No previous treatment with any of the drugs in the triplet combination was allowed. Patients received 35 cycles of 21 days of intravenous obinutuzumab (100 mg on day 1, 900 mg on day 2, 1000 mg on day 8 and day 15 of cycle 1; 1000 mg on day 1 of cycles 2–8) and intravenous atezolizumab (1200 mg on day 2 of cycle 1 and 1200 mg on day 1 of cycles 2–18), and continuous oral venetoclax (ramp-up from 20 mg/day on day 15 of cycle 1 according to chronic lymphocytic leukaemia schedule, then 400 mg/day from day 1 of cycle 3 to day 21 of cycle 35). The primary endpoint was overall response rate at day 21 of cycle 6 in the intention-to-treat population. We considered an overall response rate of 67% or more to be clinically active, rejecting the null hypothesis of a response of 40% or less. The study is registered with ClinicalTrials.gov, NCT04082897, and has been completed.

Findings Between Oct 9, 2019, and Oct 19, 2022, 28 patients were enrolled (12 [43%] male patients and 16 [57%] female patients). Median follow-up was 16·8 months (IQR 7·8–32·0). At cycle 6, 19 of 28 patients showed a response, yielding an overall response rate of 67·9% (95% CI 47·6–84·1). Treatment-emergent adverse events that were grade 3 or worse were reported in 17 (61%; 95% CI 40·6–78·5) of 28 patients, with neutropenia being the most frequent (11 [39%; 21·5–59·4] of 28 patients). Serious treatment-emergent adverse events were reported in eight (29%; 14·2–48·7) patients, which were most commonly infections (five [18%; 6·1–36·9] of 28 patients). There were two (7%) deaths attributable to adverse events during the study: one from sepsis and one from fungal pneumonia, which were not considered as directly treatment-related by the investigators. Six (21·4%) patients had immune-related adverse events, none of which led to discontinuation. No tumour lysis syndrome was observed.

Interpretation The atezolizumab, venetoclax, and obinutuzumab triplet combination was shown to be active and safe, suggesting that this chemotherapy-free regimen could become a new first-line treatment approach in patients with DLBCL-RT.

Funding Roche.

Introduction

Richter transformation consists of chronic lymphocytic leukaemia or small lymphocytic lymphoma evolution into an aggressive lymphoma, most commonly diffuse large B-cell lymphoma (DLBCL).[1] Despite substantial improvements in outcomes in patients with chronic lymphocytic leukaemia with novel targeted agents (Bruton tyrosine kinase [BTK] or BCL-2), the treatment of patients with Richter transformation remains a crucial unmet need.[2] Patients with the DLBCL variant of Richter transformation (DLBCL-RT) have a poor prognosis with chemoimmunotherapy, with a median overall survival of 6–12 months and an overall response rate of less than 40%, primarily due to chemotherapy refractoriness. Additionally, the application of intensive chemo-immunotherapy regimens is constrained by factors such

Research in context

Evidence before this study

We searched PubMed from database inception to Feb 20, 2024, using combinations of the terms "venetoclax", "atezolizumab", "obinutuzumab", "PD-1", "programmed cell death protein 1", "PD-L1", "programmed death ligand 1", "BCL-2", and "clinical trials", for publications in any language. The search terms were also combined with "CLL", "chronic lymphocytic leukaemia", "SLL", "small lymphocytic lymphoma", "DLBCL", "diffuse large B-cell lymphoma", "Richter", or "Richter's transformation". At the time of study initiation (Oct 9, 2019), only preliminary safety data on the atezolizumab, venetoclax, and obinutuzumab combination were available for relapsed or refractory diffuse large B-cell lymphoma and follicular lymphoma (presented at the 15th International Conference on Malignant Lymphoma; June 18–22, 2019). We did not find publications on completed clinical trials evaluating the combination of venetoclax, atezolizumab, and obinutuzumab in patients with Richter transformation. We also searched abstracts from the American Society of Clinical Oncology, the American Society of Haematology annual meetings, and the European Hematology Association congress from Jan 1, 2017, to Dec 31, 2023. We identified one abstract from the 2021 American Society of Haematology meeting presenting interim phase 2 results on the activity and safety of venetoclax, atezolizumab, and obinutuzumab in eight patients with Richter transformation. Preliminary results from these studies showed encouraging activity for this combination in patients with Richter transformation.

Added value of this study

In the present study, the venetoclax, atezolizumab, and obinutuzumab combination was highly effective in patients with Richter transformation, outperforming the historical results with chemoimmunotherapy in this poor-prognosis setting. This triplet combination achieved prolonged response duration and overall survival with a safety profile that was consistent with the known profile of each agent and with no unexpected toxicities. Since most patients presented unfavourable biological and clinical prognostic features, our study population was broadly representative of the real world.

Implications of all the available evidence

The combination of venetoclax, atezolizumab, and obinutuzumab was shown to be active and safe for the treatment of patients with Richter transformation. The clinical activity of this chemotherapy-free regimen translated into durable remissions and a prolonged survival benefit, making the venetoclax, atezolizumab, and obinutuzumab triplet a potential first-line standard treatment for patients with Richter transformation.

as age, comorbidities, and bone marrow infiltration.[2] Intensifying chemoimmunotherapy has not been shown to improve outcomes, but instead to increase toxicity and treatment-related mortality.[3,4] Even with both covalent and non-covalent BTK inhibitors as standalone treatments, only short-term disease control is achieved.[5,6] Considering the logical therapeutic targets and vulnerabilities in the pathophysiology of DLBCL-RT, we aimed to develop a new treatment concept in DLBCL-RT without chemotherapy.

In many patients, DLBCL-RT shares a clonal relationship with the underlying chronic lymphocytic leukaemia.[7] Therefore, the chronic lymphocytic leukaemia clone has often undergone extensive exposure to previous therapies and often carries high-risk genetic abnormalities of *TP53* (a prevalence of roughly 60%), which could contribute to treatment resistance.[7] Venetoclax triggers apoptosis irrespective of *TP53* aberrations and has been shown to be active in DLBCL-RT, either alone or when combined with chemoimmunotherapy.[8–11] Furthermore, many patients with DLBCL-RT have been previously exposed to BTK inhibitors and DLBCL-RT clones seem to exhibit downmodulated BCR activity.[12] The inhibitory molecules regulating T-cell functions (PD-1 and PD-L1) often have increased concentrations in the DLBCL-RT microenvironment.[13,14] The clinical response to anti-PD-1 inhibitors, alone or in conjunction with BTK inhibitors, highlights the vulnerability of the immune checkpoint in the context of DLBCL-RT.[15–19] Moreover, CD20 expression is maintained in Richter transformation, and CD20-targeting monoclonal antibodies have an additive effect with venetoclax in chronic lymphocytic leukaemia and DLBCL.[20,21] In particular, obinutuzumab has been shown to have greater anti-tumour activity in chronic lymphocytic leukaemia than rituximab.[22]

We designed the MOLTO trial based on the combination of atezolizumab, venetoclax, and obinutuzumab in patients with DLBCL-RT who had been previously untreated for the disease transformation.

Methods

Study design and participants

This was a prospective, open-label, multicentre, single-arm, investigator-initiated phase 2 study in 15 centres in Italy and Switzerland. We included patients aged 18 years or older who had a confirmed diagnosis of chronic lymphocytic leukaemia or small lymphocytic lymphoma as per the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) 2008 criteria, with biopsy-proven transformation to DLBCL as per local assessment; an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; and adequate renal and hepatic function (creatinine clearance ≥50 mL/min and aminotransferase values ≤2·5 times the upper limit of normal). Patients were excluded if they had previous treatment for Richter transformation, although they could have received chronic lymphocytic leukaemia therapies;

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previous exposure to venetoclax or anti-PD-1 or PD-L1 antibodies; or active or previous autoimmune disease (appendix p 2). Self-reported patient sex, race, and ethnicity were provided by the individual investigators.

See Online for appendix

Considering the need for immediate disease control, patients were allowed to be enrolled as per local histological diagnosis of DLBCL and to start treatment independently of central histological confirmation. Patients could continue protocol treatment, even if DLBCL diagnosis was not centrally confirmed, provided that, in the investigator's judgement, they were benefiting from therapy.

The study was conducted under the International Conference on Harmonization Good Clinical Practice guidelines in accordance with the Declaration of Helsinki and approved by the institutional review board. All patients provided written informed consent before study entry. The trial is registered with ClinicalTrials.gov, NCT04082897, and is complete.

Procedures

Treatment consisted of 35 cycles of 21 days of intravenous obinutuzumab (100 mg on day 1, 900 mg on day 2, and 1000 mg on days 8 and 15 of cycle 1 and 1000 mg on day 1 of cycles 2–8), intravenous atezolizumab (1200 mg on day 2 of cycle 1 and 1200 mg on day 1 of cycles 2–18), and continuous oral venetoclax (ramp-up phase from a starting dose of 20 mg/day on day 15 of cycle 1 according to chronic lymphocytic leukaemia treatment schedule, reaching a full dose of 400 mg/day from day 1 of cycle 3 to day 21 of cycle 35; appendix p 7). Tumour lysis syndrome management was done as per local practice according to venetoclax indications for chronic lymphocytic leukaemia. Hospitalisation was not mandatory for any patients. Accelerated ramp-up was allowed at investigator discretion in patients showing inadequate disease control. Prophylaxis against *Pneumocystis jiroveci* pneumonia and herpes viruses were mandatory in all patients. Growth factors and supportive care were provided according to institutional standards of care. CNS prophylaxis was administered according to guidelines.[23]

An initial safety run-in was done in the first nine enrolled participants to assess the potential toxicities of the combination during the first 9 weeks of treatment (three cycles; appendix p 2). No dose-finding steps were planned. Safety was evaluated by an Independent Data Monitoring Committee (IDMC). In the case of more than three non-infective and non-haematological grade 4 or worse adverse events, which, according to investigators' experience, were considered related to the treatment, the accrual was to be halted and the IDMC was to be convened to decide on an early enrolment termination. The treatment schedule applied to the initial safety run-in cohort was used for the remaining participants. The safety run-in cohort was included in the activity and safety analysis.

Patients were allowed to withdraw from the study at any time. If considered necessary, investigators could discontinue patients from the study at any time for any reason, including unsatisfactory response, unacceptable toxicity, pregnancy, non-compliance, or if in the patient's best interest. Patients showing a stable disease or progressive disease at the cycle 6 response evaluation were considered non-responders and discontinued treatment. Patients discontinuing treatment for any reason would continue to be followed up until consent withdrawal or death. Dose interruptions or reductions were applied for haematological and extra-haematological toxicity at investigators' discretion.

CT and PET scans were done locally for all patients at screening and on day 21 of cycle 6 (first response assessment), which was then repeated at day 21 of cycles 14, 22, 30, and 35 in patients who responded to treatment to capture improvements in response or disease progression. Bone marrow biopsy and aspirate were done locally to confirm complete remission. Response assessment and progression were locally evaluated. Laboratory assessments were done at day 1 of each cycle and at investigators' discretion (appendix pp 78–83).

Immunohistochemistry on tumour samples for biological tissue markers was evaluated at baseline and scored with a semiquantitative approach based on a modified H score. The modified H score (range 0–300, with 300 being the highest concentration of markers) was calculated by multiplying the proportion of positive malignant cells (0–100%) by the average intensity of staining (in which 0 was no staining, 1+ was weak staining; 2+ was moderate staining, and 3+ was strong staining).[24] PD-1 expression was evaluated on both tumour cells and T cells, and PD-L1 expression was evaluated on tumour cells and macrophages. Dynamic modifications in the immune cell populations of peripheral blood were assessed by flow cytometry before treatment initiation and at prespecified timepoints (appendix p 8).

Analysis of clonal relation between DLBCL and chronic lymphocytic leukaemia was done locally comparing by real-time PCR of the *IGHV* rearrangement of genes on tissues from DLBCL-RT and chronic lymphocytic leukaemia.

Concomitant circulating chronic lymphocytic leukaemia component is common in DLBCL-RT. With the aim of assessing the response of the underlying chronic lymphocytic leukaemia fraction, minimal residual disease (MRD) was measured in peripheral blood at day 21 of cycle 6 using either eight-colour flow cytometry (sensitivity 10^{-4}), immunoglobulin high-throughput sequencing (Ig-HTS clonoSEQ; sensitivity 10^{-6}), or cancer personalised profiling by deep sequencing (CAPP-seq) of cell-free DNA (sensitivity 10^{-3}). Gene mutations were assessed by CAPP-seq in purified chronic lymphocytic leukaemia cells of the peripheral blood, in bulk DLBCL-RT biopsy, and in plasma

circulating tumour DNA (ctDNA). Further details are in the appendix (pp 3–5).

Adverse events were monitored and recorded throughout the study and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE; version 5.0). Based on the known safety profile of the three drugs, adverse events of special interest were also assessed (appendix pp 5–6).

Outcomes

The primary endpoint was overall response rate (defined as the proportion of patients in complete remission or partial remission) at day 21 of cycle 6, according to the Lugano classification for aggressive lymphomas[25] using CT and PET. Residual underlying chronic lymphocytic leukaemia could persist in node or bone marrow and still qualify as complete remission, denoting complete DLBCL-RT response. Secondary endpoints were complete response rate at day 21 of cycle 6, duration of response (time from first response assessment at day 21 of cycle 6 to disease progression or death in patients who had a response), progression-free survival (time from treatment initiation to disease progression or death from any cause, whichever occurred first), and overall survival (time from treatment initiation to death from any cause). Duration of response, progression-free survival, and overall survival were all reported at the 12-month timepoint. Safety secondary endpoints were the incidence of adverse events and incidence of adverse events of special interest. Exploratory endpoints were the rate of unmeasurable MRD at day 21 of cycle 6 in chronic lymphocytic leukaemia cell fraction, gene mutations in the peripheral blood and Richter transformation biopsy, and changes in immune cell quantity and quality (ie, the immunomodulatory effects of the treatment combination; appendix pp 3–4). We also assessed the correlation between response rate and progression-free survival with PD-1 and PD-L1 concentration, clonal relationship between chronic lymphocytic leukaemia and DLBCL-type Richter transformation, *TP53* status, c-*MYC* status, *BCL-2* status, mutational profile, microenvironment immune profile by gene expression, and cell of origin by Hans algorithm.

Statistical analysis

This study had a single-stage design, defined as follows: a proportion of objective responses (p; intended to represent the achievement of at least a partial remission) equal to or greater than 67%[3] was considered the minimum response rate clinically satisfactory to warrant further studies, whereas a p equal to or less than 40% was considered not sufficient to define the treatment as clinically acceptable. With an α error of 0·05 and with a statistical power equal to 0·903, the study required 28 patients to decide whether the p was 0·40 or less (null hypothesis) or 0·67 or greater (alternative hypothesis). If the number of objective responses was 16 or more, the hypothesis that p was 0·40 or less would be rejected with an actual error rate of 0·05. If the number of objective responses was 15 or fewer, the hypothesis that p was 0·67 or greater would be rejected with an actual error rate of 0·097.

All patients enrolled in the trial were included in the activity and safety analyses (intention-to-treat population).

A logistic regression model was used to estimate the overall response rate and complete response rate together with the corresponding 95% CIs. Variables characterising the study sample were described, reporting median values with IQRs for continuous variables and the absolute and relative frequency for categorical variables.

Progression-free survival, overall survival, and duration of response were estimated using the Kaplan–Meier method, and the association of categorical baseline characteristics with these outcomes was performed using the log-rank test in a post-hoc analysis. Progression-free survival and duration of response data were censored at the time of the last tumour assessment in patients who did not have an event or, if an assessment was done after the cutoff, at time of data cutoff. For overall survival, data were censored at the last available follow-up in patients who did not have an event. For patients who died, the censor time corresponded to the date of death. No informative censoring on categorical characteristics assessed was done.

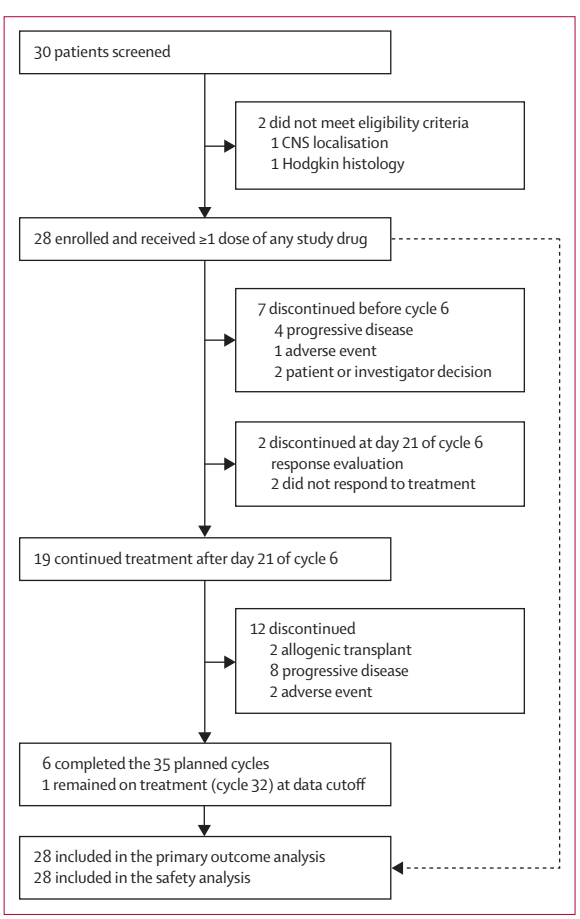

Figure 1: **Study profile**

	All patients (N=28)
Age, years	70 (32–81; IQR 66–74)
Patients aged ≥70 years	15 (54%)
Sex	
Male	12 (43%)
Female	16 (57%)
Race	
White	28 (100%)
Chronic lymphocytic leukaemia treatment status	
Untreated	8 (29%)
Previously treated	20 (71%)
Median number of previous chronic lymphocytic leukaemia treatments (range)	1 (0–3)
Previous chronic lymphocytic leukaemia treatment	
BTK inhibitor only	7 (25%)
Chemoimmunotherapy only	9 (32%)
BTK inhibitor and chemoimmunotherapy	4 (14%)
Time from chronic lymphocytic leukaemia diagnosis to Richter transformation, months	48·1 (0–242·5)
Time from BTK inhibitor start to Richter transformation, months*	22·8 (1·4–64·3)
ECOG performance status	
0	12 (43%)
1	10 (36%)
2	6 (21%)
Presence of B-symptoms†	9 (32%)
Richter prognostic score‡	
Low	17 (61%)
Intermediate low	3 (11%)
Intermediate high	4 (14%)
High	4 (14%)
Median absolute lymphocyte count before treatment start, cells per µL	22 (0–367)
Patients with lactate dehydrogenase concentrations greater than the upper limit of normal	13 (46%)
Patients with Binet stage C before treatment start	8 (29%)
Tumour lysis syndrome risk	
Low	8 (29%)
Intermediate	13 (46%)
High	7 (25%)
Bulky disease	16 (57%)
Ann Arbor stage	
I–II	6 (21%)
III–IV	22 (79%)
(Continued from previous column)	
FISH	
17p deletion	10 (36%)
11q deletion	0
12 trisomy	8 (29%)
No FISH aberrations	6 (21%)
13q deletion	6 (21%)
17p deletion or *TP53* aberrations	12 (43%)
17p deletion only	1 (4%)
TP53 mutation only	2 (7%)
17p deletion and *TP53* mutation	9 (32%)
Complex karyotype‡	9/19 (47%)
IGHV unmutated	24 (86%)
PD-1 expression, n/N (%); median H score	
Tumour cells	11/19 (58%); 50
T cells	12/19 (63%); 100
PD-L1 expression, n/N (%); median H score	
Tumour cells	1/19 (5%); 220
Macrophages	15/19 (79%); 200
Cell of origin (Hans algorithm phenotype)	
Germinal centre B cell	3/16 (19%)
Non-germinal centre B cell	13/16 (81%)
BCL-2 expression, n/N (%); median H score	19/19 (100%); 300
BCL-6 expression, n/N (%); median H score	5/19 (26%); 70
c-MYC expression, n/N (%); median H score	17/19 (89%); 50
c-MYC and BCL-2 double expressor§	7/16 (44%)
Ki67 median expression (range)	70 (25–90)
Chronic lymphocytic leukaemia–Richter transformation clonal relationship	
Related	20/24 (83%)
Unrelated	4/24 (17%)

Data are median (range), n (%), or n/N (%), unless stated otherwise. BTK=Bruton tyrosine kinase. ECOG=Eastern Cooperative Oncology Group. FISH=fluorescence in-situ hybridisation. *11 patients. †Defined as unexplained fever, drenching sweats, unexplained weigth loss of more than 10% of the usual body weight in the 6 months prior to diagnosis ‡At least three chromosome lesions in the same clone assessed on stimulated karyotype with oligodeoxynucleotides on peripheral blood or bone marrow chronic lymphocytic leukaemia cells. §Double expressor status was defined as per WHO criteria (c-MYC immunohistochemistry ≥40% of cells and BCL-2 immunohistochemistry ≥50% of cells).

Table 1: **Patient demographics and baseline characteristics**

We also assessed best complete response rate (defined as the achievement of a complete response at any point during the course of therapy) as a post-hoc analysis.

The Cox proportional hazards regression model was used to assess the association of progression-free survival, overall survival, and duration of response with continuous baseline characteristics in a post-hoc analysis (age and Ki67 values). No multivariable regression models were done. Post-hoc analyses were conducted to analyse the association of categorical responses (complete remission *vs* partial remission) and chronic lymphocytic leukaemia fraction of MRD with progression-free survival, overall survival, and duration of response, and the association between baseline PD-1^{+}CD8^{+} T cells with overall response, progression-free survival, overall survival, and duration of response. The association between clinical and disease characteristics with overall response rate and complete remission was assessed using logistic regression. Incidence rate of adverse events was estimated with 95% CIs.

No planned interim or subgroup analyses were done. The level of significance was set at the threshold of 0·05. We used Stata (version 16.0) for all statistical

analyses. Further details can be found in the appendix (p 6). This study was registered with ClinicalTrials.gov, NCT04082897.

Role of the funding source

The sponsors (ASST Grande Ospedale Metropolitano Niguarda on behalf of Rete Ematologica Lombarda and the International Extranodal Lymphoma Study Group) designed the study, collected, analysed and interpreted the data, and wrote the Article. The study funder, Roche, supported the study and supplied the study drugs but was not involved in the study design, data collection, analysis or interpretation, writing of the report, or editorial assistance.

Results

From Oct 9, 2019, to Oct 19, 2022, 30 patients were screened and 28 patients (12 [43%] male and 16 [57%] female) were enrolled (figure 1; appendix p 9), representing the intention-to-treat population analysed for safety and activity. No treatment-emergent adverse events that prevented further accrual occurred during the safety run-in phase.

The median age of the patients was 70 years (range 32–81; IQR 66–74), 20 (71%) patients had previously been given treatment for chronic lymphocytic leukaemia, and 11 (39%) had received BTK inhibitors (table 1). Richter prognostic score[26] was intermediate high or high in eight (29%) patients.

Analysis of clonal relation between DLBCL and chronic lymphocytic leukaemia was done in 24 patients, and 20 (83%) of 24 analysed patients had clonally related disease. Five (25%) of the 20 patients with clonally related disease and three (75%) of the four patients with unrelated disease had not received previous treatment for chronic lymphocytic leukaemia.

Overall, 19 tumour samples were adequate for central histological revision, which confirmed DLBCL in 18 patients, with one patient categorised as having accelerated chronic lymphocytic leukaemia. Of the remaining nine tumour samples, five were unavailable and four did not have sufficient material for centralised evaluation after local analyses. CD5 expression was lost in five (26%) of 19 tumour samples and CD23 expression in 12 (63%) of 19 tumour samples. CD20 was expressed in 18 (95%) of 19 tumour samples. None of the 19 tumour samples were positive for Epstein–Barr virus.

At the data cut-off on Oct 1, 2023, one patient was still undergoing treatment. Median follow-up was 16·8 months (IQR 7·8–32·0).

As per the intention-to-treat approach, all 28 enrolled patients were included in the analysis. At first response assessment (day 21 of cycle 6), 21 patients were still on treatment. Seven patients prematurely discontinued treatment due to progressive disease (four patients), *E coli* sepsis (adverse event; one patient), and investigator decisions (two patients; figure 1) and were considered as non-responders. 19 (67·9%) of 28 patients showed a response, with an overall response rate of 67·9% (95% CI 47·6–84·1), rejecting the null hypothesis of a response 40% or less in favour of the alternative hypothesis of a response 67% or more and indicating the activity of the treatment regimen (figure 2A). Complete response was observed in eight (28·6%; 95% CI 11·8–45·3) of 28 patients. A conversion from partial remission to complete remission occurred after cycle 6 in two patients, thus the best complete response rate achieved was 35·7%. None of the clinical and disease characteristics assessed in our post-hoc subgroup analysis were considered to have an effect on response or complete remission (appendix p 10).

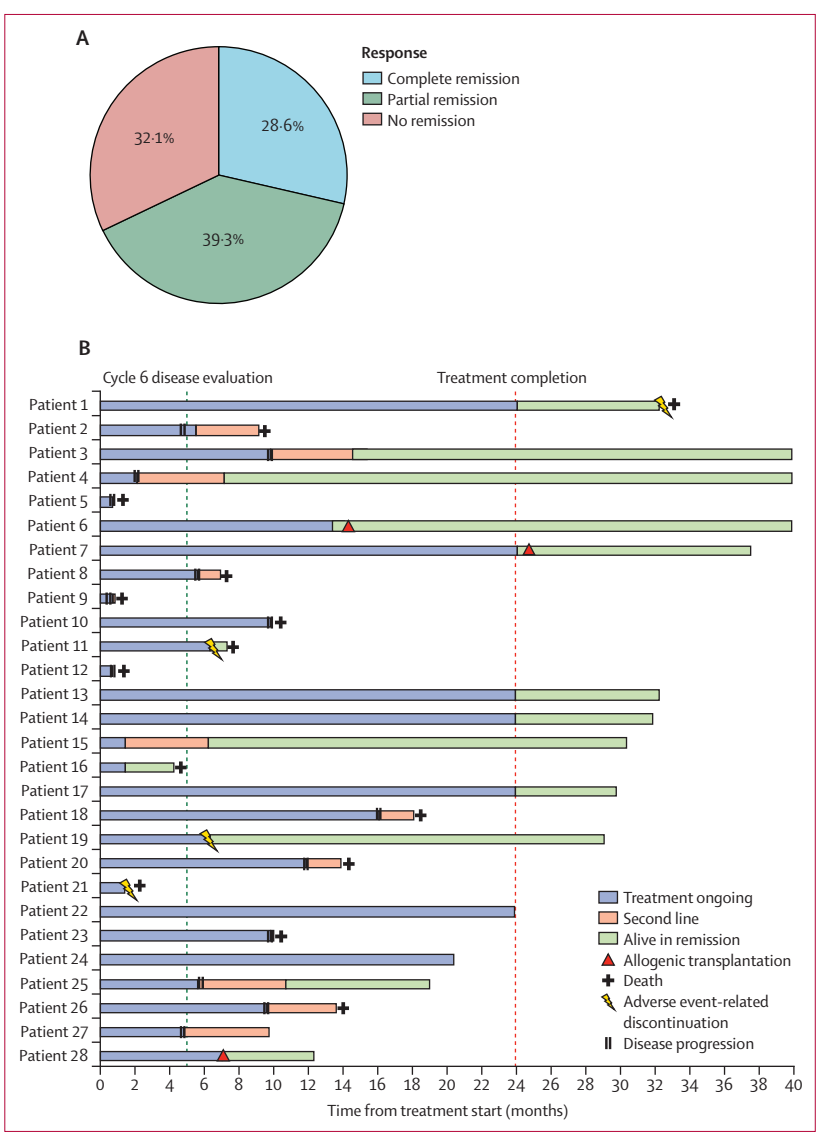

Figure 2: **Treatment response at day 21 of cycle 6 according to intention-to-treat analysis**
(A) Proportion of patients with complete remission, partial remission, or no remission. (B) Swimmer plot of responses and patient outcomes.

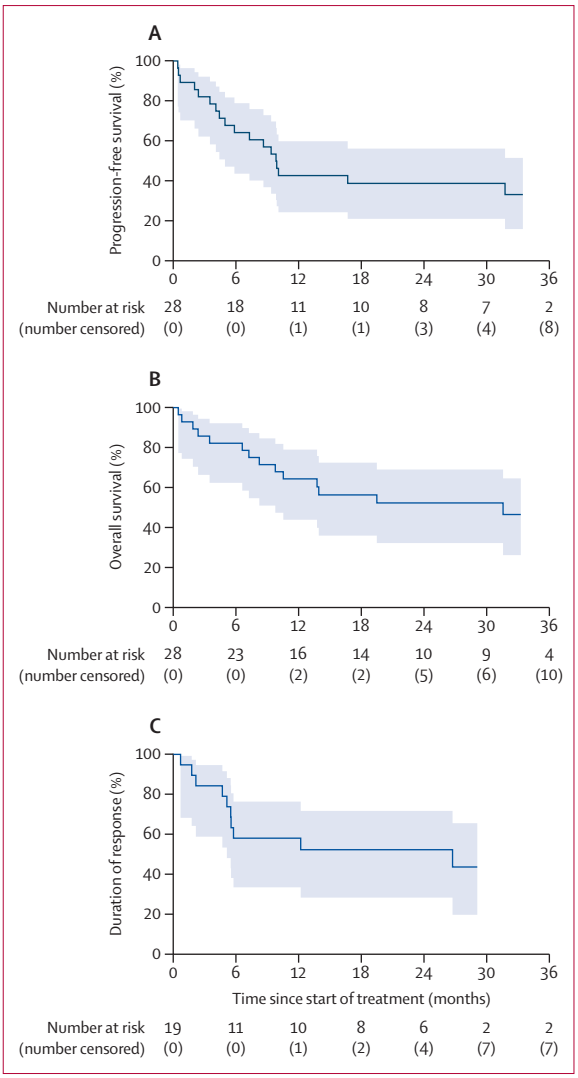

***Figure 3*: Kaplan–Meier plots of progression-free survival (A), overall survival (B), and duration of response (C)**
Shaded areas represent 95% CIs.

After cycle 6, the 19 responding patients continued treatment for a median of 11 additional cycles (range 0–29, figure 2B). Nine (32%) patients were alive in remission at data cut-off, of whom three received consolidative allogeneic stem-cell transplantation (two while on treatment after ten and 18 cycles and one after treatment completion). Two patients died in remission due to infection. Overall, eight (29%) patients had disease progression while on treatment after a median of seven (IQR 5–9) further cycles and two (5%) of eight patients were alive after salvage rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) or R-CHOP-like chemoimmunotherapy (appendix p 11).

At 12 months, progression-free survival was 42·9% (95% CI 24·6–60·0; figure 3A) and, in post-hoc subgroup analyses was influenced only by ECOG performance status (0 *vs* 1–2; p=0·047; appendix p 12). Overall, 14 patients (50%) of 28 had disease progression (four patients before the first response assessment, two who did not respond to treatment at day 21 of cycle 6, and eight after having initially had a response). Overall survival was 64·3% (95% CI 43·8–78·9) at 12 months (figure 3B) and in post-hoc subgroup analyses was associated with previous treatment for chronic lymphocytic leukaemia (p=0·033), previous treatment with BTK inhibitors (p=0·007), previous BTK inhibitor treatment only (p=0·032), number of previous treatments for chronic lymphocytic leukaemia (p=0·039), and ECOG performance status (p=0·036; appendix p 12). Overall, 14 patients died (two patients due to infections during treatment, one due to COVID-19 while in complete remission after treatment completion, ten due to disease progression, and one after treatment discontinuation following an investigator decision). At 12 months, duration of response assessment showed that 11 (57·9%) of 19 of patients were still responding; 95% CI 33·2–76·3; figure 3C). The only baseline characteristics associated with longer duration of response were male sex and PD-1 expression on T cells (appendix p 12). In a post-hoc analysis, quality of response significantly influenced both progression-free survival and duration of response but not overall survival (appendix p 13). Progression-free survival and duration of response at 12 months were both 87·5% (95% CI 38·7–98·1) in patients with complete remission and 36·4% (11·2–62·7) in patients with partial remission. 11 patients who had disease progression on study treatment received subsequent salvage therapy with R-CHOP or R-CHOP-like regimens (figure 2B). Overall eight (42%) of 19 patients in remission at day 21 of cycle 6 were alive in and in continous response at 24 months (figure 2B).

Among the four patients with clonally unrelated disease, three patients had disease progression and were alive at data cutoff after chemoimmunotherapy salvage treatment. One patient had a partial remission at day 21 of cycle 6 but discontinued treatment due to myelodysplasia development.

The only patient categorised as having accelerated chronic lymphocytic leukaemia after centralised histological evaluation discontinued treatment at cycle 2 due to logistical difficulties during the COVID-19 pandemic and was treated with R-CHOP chemoimmunotherapy. The patient was still alive in disease remission at last follow-up.

The median number of cycles administered was 11 (range 1–35; IQR 5–21). All patients developed at least one adverse event (table 2). 17 (61%; 95% CI 40·6–78·5) of 28 patients had grade 3 or worse adverse events, most commonly neutropenia (11 [39%; 21·5–59·4]) of 28 patients). Overall, five grade 3 or worse infections occurred during treatment (grade 5: *E coli* sepsis and *Aspergillus* pneumonia; grade 3: non-COVID-19-related pneumonia, COVID-19-related pneumonia, and COVID-19 gastroenteritis). Seven (25%; 10·7–44·9) of 28 patients developed adverse events of special interest

(table 2). Six (21·4%) patients had immune-related adverse events. All immune-related adverse events were effectively managed with steroids and did not require permanent discontinuation from treatment. No immune-related complications arose in the three patients who proceeded to allogeneic stem-cell transplantation. In addition, no graft-versus-host disease was reported at the data cutoff. Myelodysplasia developed after six cycles of study treatment in a patient who was previously exposed to fludarabine, cyclophosphamide, and rituximab, leading to definitive discontinuation. Serious treatment-emergent adverse events were reported in eight (29%; 14·2–48·7) of 28 patients (appendix p 13), which were most commonly infections (in five [18%; 6·1–36·9] of 28 patients). In the overall cohort, there were two deaths (7%) attributable to adverse events during the study (one death from sepsis and one from fungal pneumonia). A further patient died due to COVID-19-related interstitial pneumonia 7 months after treatment completion. Despite an accelerated venetoclax ramp-up in three patients (the number of days to reach full dosage was 11 days, 16 days, and 18 days), no clinical or laboratory tumour lysis syndrome was recorded.

Overall, two (7%) of 28 patients required temporary venetoclax dose reduction due to neutropenia. Treatment was postponed due to adverse events (two immune-related disorders, one aminotransferase concentration increase, and two COVID-19 infections) in five (18%) of 28 patients. Three patients definitively discontinued treatment due to adverse events (two grade 5 infections and one myelodysplasia).

At day 21 of cycle 6, MRD in the chronic lymphocytic leukaemia cell fraction was unmeasurable in peripheral blood in 13 (93%) of 14 of patients by flow cytometry and in nine (64%) of 14 patients by Ig-HTS. In plasma cfDNA, MRD was unmeasurable in ten (53%) of 19 patients by CAPP-seq (figure 4). In post-hoc analyses, MRD status did not affect survival outcomes or duration of response (appendix p 14). Among the 14 patients with available results for Ig-HTS on peripheral blood and CAPP-seq on plasma cell-free DNA, 11 patients were concordantly identified as having unmeasurable MRD in both peripheral blood and plasma cell-free DNA (appendix p 15), whereas three patients had discordant results. The correlation of flow cytometry-based MRD and clonoSEQ results is shown in the appendix (p 16).

22 patients were assessed for mutations in chronic lymphocytic leukaemia cells purified from peripheral blood, 16 patients in DLBCL-RT biopsies, and 24 patients in ctDNA of plasma. At the time of transformation, 11 (50%) of 22 patients had *TP53* mutations in chronic lymphocytic leukaemia cells of the peripheral blood, seven (44%) of 16 in the DLBCL-RT biopsy, and 12 (50%) of 24 in ctDNA of plasma. *TP53* mutations were identified in 15 (63%) of 24 patients in chronic lymphocytic leukaemia cells of peripheral blood, Richter transformation biopsy, or plasma ctDNA specimens. Mutations in *BTK* or *PLCG2* were identified in six (25%) of 24 patients in chronic lymphocytic leukaemia cells of peripheral blood, DLBCL-RT biopsy, or plasma ctDNA specimens (appendix p 17). In patients with all three compartments analysed, only 22·6% (116 of 514) of mutations were consistently identified across all compartments (ie, purified chronic lymphocytic leukaemia cells from peripheral blood, DLBCL-RT biopsy, and plasma ctDNA; appendix p 18).

During treatment, we observed a significant reduction in $CD3^+$, $CD4^+$, and $CD8^+$ T-cell counts (figure 5A–C) and an early and persistent decrease in natural killer cell counts (figure 5D). In terms of the distribution of T-cell differentiation subsets, combination therapy only induced an early

	Any grade	Grade 1–2	Grade 3	Grade 4	Grade 5
Treatment-emergent adverse events					
Neutropenia	12 (43%)	1 (4%)	6 (21%)	5 (18%)	0
Thrombocytopenia	4 (14%)	1 (4%)	2 (7%)	1 (4%)	0
Anaemia	1 (4%)	0	0	1 (4%)	0
Neutropenic fever	5 (18%)	4 (14%)	1 (4%)	0	0
Sepsis	1 (4%)	0	0	0	1 (4%)
COVID-19 infection	5 (18%)	3 (11%)	2 (7%)	0	0
Pneumonia (non-COVID-19 related)	4 (14%)	2 (7%)	1 (4%)	0	1 (4%)
Increase in aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, bilirubin, and alkaline phosphatase concentration	4 (14%)	3 (11%)	1 (4%)	0	0
Infusion-related reactions	3 (11%)	3 (11%)	0	0	0
Hypercalcaemia	1 (4%)	0	1 (4%)	0	0
Purpura	1 (4%)	0	1 (4%)	0	0
Adverse events of special interest					
Increase in amylase and lipase concentration	2 (7%)	2 (7%)	0	0	0
Immune-related myositis	1 (4%)	1 (4%)	0	0	0
Immune-related pancreatitis	1 (4%)	0	1 (4%)	0	0
Immune-related encephalitis	1 (4%)	0	1 (4%)	0	0
Immune-related neuropathy	1 (4%)	1 (4%)	0	0	0
Myelodysplasia	1 (4%)	0	0	1 (4%)	0

Data are n (%). The safety population included 28 patients. All adverse events recorded in the study are shown.

Table 2: **Treatment-emergent adverse events and adverse events of special interest during treatment**

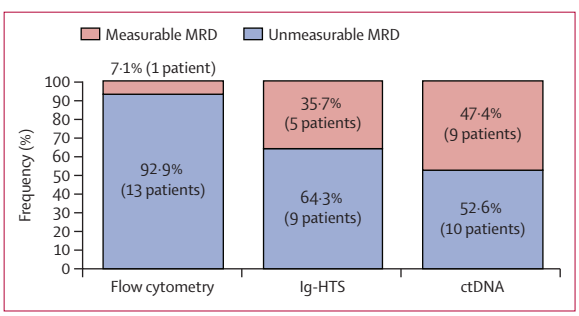

Figure 4: **Minimal residual disease results**
Frequency of measurable and unmeasurable MRD in chronic lymphocytic leukaemia fraction by flow cytometry, Ig-HTS on B cells, and CAPP-seq on plasma cell-free DNA at day 21 of cycle 6. CAPP-seq=cancer personalised profiling by deep sequencing. Ig-HTS=immunoglobulin high-throughput sequencing. MRD=minimal residual disease.

and time-limited increase in naive T-cell counts and a parallel decline of the effector memory CD4⁺ T-cell subsets (appendix p 19). During treatment, we also observed a significant reduction in the number of CD4⁺ Th17 cells (figure 5E) and an early and persistent decrease in the proportion and absolute number of CD4⁺CD25^{high}CD127^{low} regulatory T cells (Tregs; figure 5F, G). In terms of checkpoint molecules, the number of PD-1⁺CD8⁺ T cells was persistently reduced starting from day 1 of cycle 2 (figure 5H), whereas no changes in the expression of

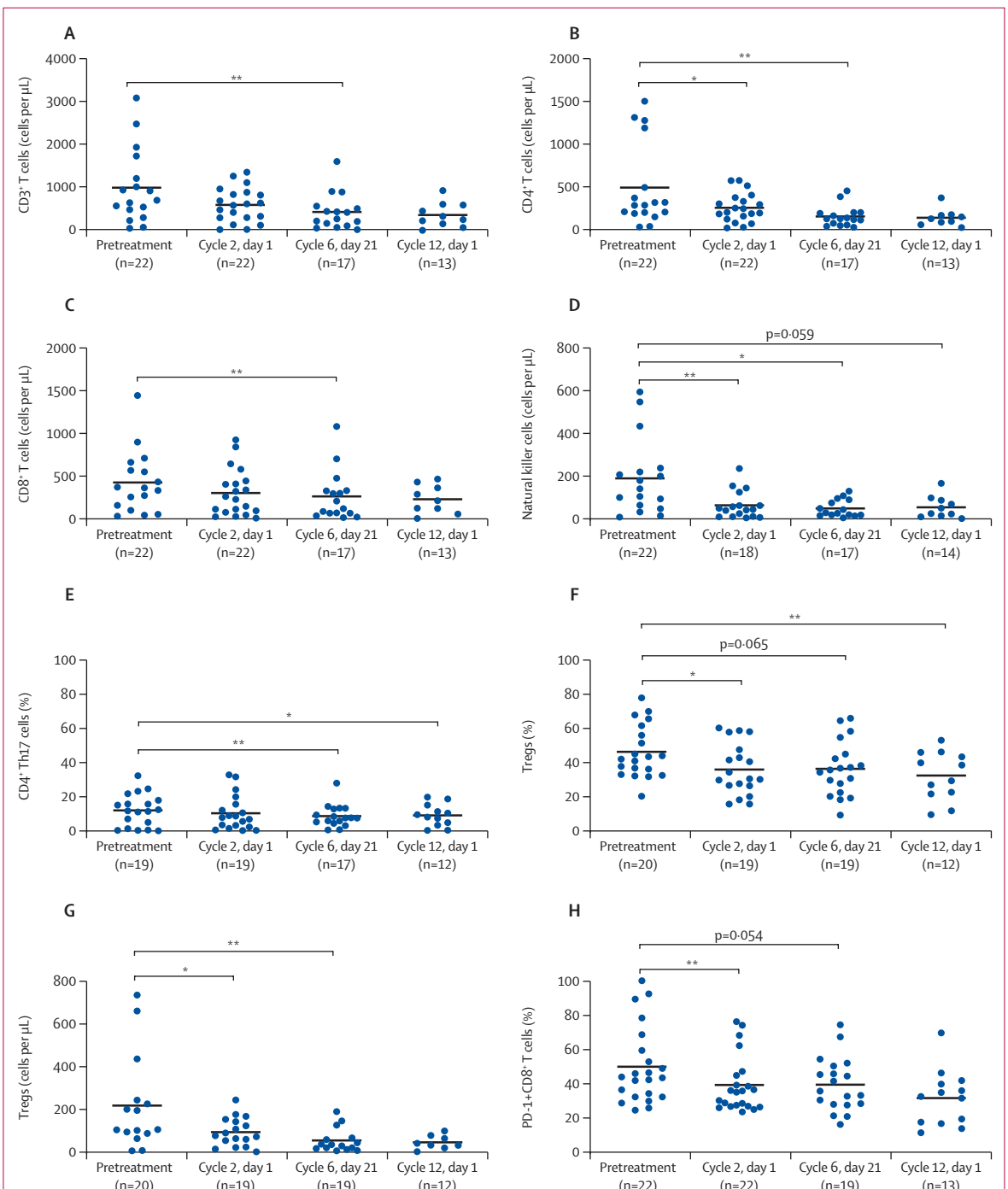

Figure 5: **Immune cell count, CD4⁺ T-cell subsets, and PD-1 expression**
Absolute numbers of CD3⁺ T cells (A), CD4⁺ T cells (B), CD8⁺ T cells (C), and natural killer cells (D) in peripheral blood mononuclear cells. Flow cytometry analysis of CD4⁺CCR6⁺CXC3⁻ Th17 cells (E), the proportion (F) and absolute number (G) of CD4⁺CD25^{high}CD127^{low} Tregs, and the surface expression of PD-1 on CD8⁺ T cells (H). Numbers and asterisks denote p values compared with baseline. *p<0·05. **p<0·01. ***p<0·001. ****p<0·0001. Tregs=regulatory T cells.

CTLA-4 on CD4+ and CD8+ T cells were observed (data not shown). When evaluating the effect of baseline immune parameters on clinical outcome in a post-hoc analysis, the number of PD-1+CD8+ T cells did not influence response rate, duration of response, or survival outcomes (appendix p 19). Instead, in an exploratory post-hoc analysis, we found that patients who were alive and in remission at data cutoff had significantly higher pretreatment proportions of Th1 cells and a higher Th1:Th2 ratio than patients showing disease progression (appendix p 20).

Discussion

Patients with DLBCL-RT had a response rate of 67·9% (95% CI 47·6–84·1) when treated with the combination of atezolizumab, venetoclax, and obinutuzumab, surpassing the historically best overall response rate seen with chemoimmunotherapy,[3] with a complete response recorded in 28·6% (95% CI 11·8–45·3) patients. Duration of response and overall survival at 12 months was higher than in historical reports.[2–4] Moreover, eight (42%) of 19 patients were alive and in continuous remission at 24 months or more from treatment initiation. These findings, coupled with the high rates of unmeasurable MRD in peripheral blood and clearance of ctDNA in plasma, support the activity of this treatment regimen in addressing both the chronic lymphocytic leukaemia and DLBCL components of the disease, which typically coexist in Richter transformation.

The MOLTO trial's study population is reflective of real-world clinical practices, encompassing various factors such as age, performance status, previous chronic lymphocytic leukaemia therapies, and pathobiological features of the transformed disease. The analysis of mutations performed for this study on chronic lymphocytic leukaemia cells of peripheral blood, DLBCL-RT biopsy, or plasma ctDNA specimens, suggests a complex clonal architecture of DLBCL-RT and a substantial variation in genetic makeup based on the anatomical compartment. These features also include a high prevalence of *TP53* abnormalities, complex karyotype, and unmutated *IGHV* status. Additionally, the rate of clonally related diseases in our series (20 [83%] of 24 evaluable patients) and results regarding cell-of-origin classification and *c-MYC* and *BCL2* double expressor status were consistent with previously published data.[2,7,27,28]

Although not significant considering the small sample size, the triplet combination therapy exhibited little activity in three of the four patients with clonally unrelated disease, who survived after second-line chemoimmunotherapy. Combined with the evidence of more favourable outcomes in clonally unrelated diseases,[7] this observation supports that the clonal relationship between DLBCL and chronic lymphocytic leukaemia might serve as a predictive biomarker for directing patients with clonally unrelated DLBCL-RT to chemoimmunotherapy.

The MOLTO trial regimen was safe, with a low treatment-related mortality and a very low number of immune-related adverse events. Moreover, none of the three patients who received stem-cell transplantation developed graft-versus-host disease. Given the absence of data on this triple combination in DLBCL-RT, we chose to employ the same dosage and ramp-up schedule used for venetoclax in chronic lymphocytic leukaemia. However, accelerated dose escalation was allowed at investigators' discretion in cases of inadequate disease control. Even in patients who received accelerated dose escalation, no tumour lysis syndrome events were recorded.

The landscape of first-line treatment for Richter transformation has evolved with the introduction of BTK and BCL2 pathway inhibitors, and ongoing research is continuing to shape the field. Incorporating venetoclax into the etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (R-EPOCH) regimen resulted in an overall response rate of 62% and a complete response rate of 50%, along with a median progression-free survival of 10·1 months and a median overall survival of 19·6 months.[9] However, haematological toxicity, febrile neutropenia, and an elevated infection rate were observed, posing potential limitations to the application of this combination. Venetoclax is being assessed together with R-CHOP to reduce the toxicity rate.[10] The same chemoimmunotherapy backbone was chosen in a prospective, randomised trial evaluating R-CHOP with or without acalabrutinib in patients with newly diagnosed DLBCL-RT.[29]

In contrast to a previous study in which increased expression of PD-L1 and PD-1 was reported among patients who responded to checkpoint inhibitors,[15] we did not find a correlation between PD-1 or PD-L1 expression on tumour cells or cells in the tumour microenvironment and outcomes.

The dual interactions occurring between malignant cells and the immune system have a pivotal role in the pathobiology of Richter transformation and make immunotherapeutic strategies a particularly appealing approach. In line with previous data,[14,30] immunohistochemical staining done on lymph nodes at Richter transformation diagnosis showed that, in most cases, tumour cells overexpressed PD-1. It has been recently suggested that Richter transformation cells can act as PD-1+ regulatory B cells, promoting T-cell exhaustion and Treg expansion, thereby favouring tumour progression.[30] The immunological monitoring of patients enrolled in the MOLTO trial showed that the combined administration of atezolizumab, venetoclax, and obinutuzumab exerted several effects on the immune system, as shown by the decline in T-cell and natural killer-cell counts and by the decreased frequency of potentially tumour-supportive T-cell subsets, such as Tregs and PD-1+CD8+ T cells. Therefore, the activity of this combination treatment is most likely to be not only due to a direct cytotoxic effect on tumour cells but also due to an effective interruption of the interactions occurring

between Richter transformation cells and the supportive immune environment.

Multiple trials have explored the synergy of checkpoint inhibitors alongside BTK inhibitors. Two separate trials have investigated the combination of nivolumab and ibrutinib in Richter transformation, whether as first-line or salvage therapy.[17,18] In a small single-centre trial, the overall response rate was 42%, and the complete response rate was 33%.[17] The median duration of response was 15 months and the median overall survival was 13 months.[17] A multicentre study reported an overall response rate of 65%, a complete response rate of 10%, and a median progression-free survival of 5 months.[18] The RT1 trial assessed tislelizumab in combination with zanubrutinib and reported an overall response rate of 47·5%, along with a median progression-free survival of 6·7 months, as per the intention-to-treat analysis.[19] In general, combinations of BTK inhibitors with checkpoint inhibitors have proved to be relatively safe and few immune-related adverse event were observed.

Our study broadens the understanding of the synergistic effects of checkpoint inhibitors in DLBCL-RT. Our findings add valuable insights beyond existing evidence, which has primarily focused on BTK inhibitors as the companion drug. Most patients with DLBCL-RT are expected to have undergone a series of continuous treatments with covalent and non-covalent BTK inhibitors for their previous chronic lymphocytic leukaemia. This trajectory might lead to acquired clinical resistance to this class of agents and the emergence of BTK mutations, rendering the use of BTK inhibitors challenging during Richter transformation treatment.

A potential limitation of our study might stem from the inclusion of patients who were naive to venetoclax considering that, in the near future, DLBCL-RT populations will already be exposed to venetoclax. Nevertheless, venetoclax is employed in a time-limited manner in chronic lymphocytic leukaemia, and existing data have not indicated the development of resistance with fixed-duration schedules.[31] Another limitation of this study is the low statistical power for the subgroup analyses due to the small sample size.

As a whole, the range of therapeutic options for patients with DLBCL-RT is expanding. The triplet combination therapy of atezolizumab, venetoclax, and obinutuzumab, and zanubrutinib plus tislelizumab could be complementary choices with similar activity and safety profiles, and currently available results warrant validation in phase 3 trials. These combinations could offer effective treatment options for patients who exhibit resistance to BTK inhibitors (atezolizumab venetoclax, and obinutuzumab) or venetoclax (zanubrutinib and tislelizumab) when Richter transformation has occurred.

Contributors

MMon, AT, DR: study concept and design. AMF, AT, MC, PLZ, MMot, GG, GQ, LS, GC, MD, RCa, TZ, MMon: provision of study materials or patients. ACo, KP, DR, GS, EZ: molecular analysis and minimal residual disease analysis. RCh, VT, ACa: histological revision and immunohistochemistry evaluations. MC, RJ, VG: dynamics of immune cell population analysis. AS: statistical analysis. AMF, AT, MMon: clinical data collection. AMF, AT, and MMon had access to and verified the raw data. All authors participated in the data analysis, the interpretation of results, and manuscript preparation. All authors had access to relevant data and had final responsibility for the decision to submit for publication.

Declaration of interests

AT reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Janssen, Beigene, and Abbvie; support for attending meetings or travel from Abbvie, Janssen, and Beigene; and participation on a data safety monitoring board or advisory board from Janssen, Abbvie, Beigene, Lilly, and AstraZeneca. AMF reports consulting fees from Janssen; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Janssen, Beigene, and AstraZeneca; support for attending meetings or travel from Abbvie, Janssen, Beigene, and AstraZeneca; and participation on a data safety monitoring board or advisory board from Janssen, Beigene, and AstraZeneca. ACo reports grants or contracts from Gilead, BMS, Beigene, Abbvie, Janssen-Cilag, and AstraZeneca; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from AstraZeneca, Abbvie, and Janssen-Cilag; support for attending meetings or travel from Janssen; and participation on a data safety monitoring board or advisory board from BMS. MC reports grants or contracts from Johnson & Johnson and Abbvie; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Abbvie and AstraZeneca; support for attending meetings or travel from Johnson & Johnson, Abbvie, and AstraZeneca; and participation on a data safety monitoring board or advisory board from Johnson & Johnson, Abbvie, AstraZeneca, Beigene, and GSK. RC reports support for the present manuscript from the US National Institutes of Health and National Cancer Institute (R01CA196703–01). PLZ reports consulting fees and payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Takeda, BMS, MSD, Roche, Gilead, Novartis, Abbvie, Beigene, Kyowa Kirin, and Janssen. MMot reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events; support for attending meetings or travel; and participation on a data safety monitoring board or advisory board from Abbvie, Janssen, and AstraZeneca. GG reports grants from Associazione Italiana per la Ricerca sul Cancro and Piano Nazionale di Ripresa e Resilienza; consulting fees from Abbvie, AstraZeneca, Beiene, Hikma, and Johnson & Johnson; and participation on a data safety monitoring board or advisory board from Abbvie, AstraZeneca, Beigene, Hikma, Johnson & Johnson, and Lilly. LS reports consulting from Abbvie, Beigene, Lilly, AstraZeneca, Janssen, and Merck; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Abbvie, Beigene, Lilly, Octapharma, AstraZeneca, Janssen, and Merck; support for attending meetings or travel from AstraZeneca, Janssen, and Beigene; and participation on a data safety monitoring board or advisory board from Merck. RCa reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Abbvie, Gilead, Gentilipharma, Pierre Fabre, Celgene, and Menarini stemline; support for attending meetings or travel from Pierre Fabre, Beigene, and Servier; and participation on a data safety monitoring board or advisory board from Celgene, Abbvie, Gentilipharma, and Daichi Samkio. TZ report consulting fees and payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Roche, Novartis, Gilead, Janssen, AstraZeneca, Lilly, and Abbvie. EZ reports consulting fees from Abbvie, Beigene, BMS, Cures, Elly and Lilly, Incyte, Ipsen, Merck, Miltenyibiomedicine, and Roche; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Abbvie, AstraZeneca, Beigene, and Gilead; support for attending meetings or travel from Abbvie; and participation on a data safety monitoring board or advisory board from Merck. DR reports support for the present manuscript from Adaptive; grants or contracts, consulting fees, payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Abbvie, AstraZeneca, Beigene, BMS, Janssen,

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Data sharing

Clinical trial data can be requested by any qualified researchers who engage in rigorous, independent, and scientific research and will be provided following review and approval of a research proposal, statistical analysis plan, and execution of a data sharing agreement. These data will be accessible for 12 months, with possible extensions considered. Data requests can be submitted to alessandra.tedeschi@ospedaleniguarda.it.

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References

1 Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. *Leukemia* 2022; **36:** 1720–48.

2 Al-Sawaf O, Robrecht S, Bahlo J, et al. Richter transformation in chronic lymphocytic leukemia (CLL)—a pooled analysis of German CLL Study Group (GCLLSG) front line treatment trials. *Leukemia* 2021; **35:** 169–76.

3 Langerbeins P, Busch R, Anheier N, et al. Poor efficacy and tolerability of R-CHOP in relapsed/refractory chronic lymphocytic leukemia and Richter transformation. *Am J Hematol* 2014; **89:** e239–43.

4 Durot E, Michallet AS, Leprêtre S, Le QH, Leblond V, Delmer A. Platinum and high-dose cytarabine-based regimens are efficient in ultra high/high-risk chronic lymphocytic leukemia and Richter's syndrome: results of a French retrospective multicenter study. *Eur J Haematol* 2015; **95:** 160–67.

5 Eyre TA, Schuh A, Wierda WG, et al. Acalabrutinib monotherapy for treatment of chronic lymphocytic leukaemia (ACE-CL-001): analysis of the Richter transformation cohort of an open-label, single-arm, phase 1-2 study. *Lancet Haematol* 2021; **8:** e912–21.

6 Wierda WG, Lewis DJ, Ghia P, et al. Efficacy of pirtobrutinib, a highly selective, non-covalent (reversible) BTK inhibitor in richter transformation: results from the phase 1/2 BRUIN study. *Blood* 2022; **140** (suppl 1)**:** 846–49.

7 Rossi D, Spina V, Deambrogi C, et al. The genetics of Richter syndrome reveals disease heterogeneity and predicts survival after transformation. *Blood* 2011; **117:** 3391–401.

8 Davids MS, Roberts AW, Seymour JF, et al. Phase I first-in-human study of venetoclax in patients with relapsed or refractory non-hodgkin lymphoma. *J Clin Oncol* 2017; **35:** 826–33.

9 Davids MS, Rogers KA, Tyekucheva S, et al. Venetoclax plus dose-adjusted R-EPOCH for Richter syndrome. *Blood* 2022; **139:** 686–89.

10 Davids MS, Rogers KA, Jain N, et al. Initial results of a multicenter phase 2 study of venetoclax in combination with R-CHOP (VR-CHOP) for patients with Richter syndrome. International Conference on Malignant Lymphoma; June 13–17, 2023 (abstr 343).

11 Hampel P, Parikh S, Wierda W, et al. Venetoclax-based treatment of patients with Richter Syndrome: outcomes from a multicenter retrospective study. European Hematology Association; June 9–12, 2022 (abstr P651).

12 Nadeu F, Royo R, Massoni-Badosa R, et al. Detection of early seeding of Richter transformation in chronic lymphocytic leukemia. *Nat Med* 2022; **28:** 1662–71.

13 Augé H, Notarantonio AB, Morizot R, et al. Microenvironment remodeling and subsequent clinical implications in diffuse large B-cell histologic variant of Richter syndrome. *Front Immunol* 2020; **11:** 594841.

14 He R, Ding W, Viswanatha DS, et al. PD-1 expression in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and large B-cell Richter transformation (DLBCL-RT): a characteristic feature of DLBCL-RT and potential surrogate marker for clonal relatedness. *Am J Surg Pathol* 2018; **42:** 843–54.

15 Ding W, LaPlant BR, Call TG, et al. Pembrolizumab in patients with CLL and Richter transformation or with relapsed CLL. *Blood* 2017; **129:** 3419–27.

16 Armand P, Murawski N, Molin D, et al. Pembrolizumab in relapsed or refractory Richter syndrome. *Br J Haematol* 2020; **190:** e117–20.

17 Jain N, Senapati J, Thakral B, et al. A phase 2 study of nivolumab combined with ibrutinib in patients with diffuse large B-cell Richter transformation of CLL. *Blood Adv* 2023; **7:** 1958–66.

18 Younes A, Brody J, Carpio C, et al. Safety and activity of ibrutinib in combination with nivolumab in patients with relapsed non-Hodgkin lymphoma or chronic lymphocytic leukaemia: a phase 1/2a study. *Lancet Haematol* 2019; **6:** e67–78.

19 Al-Sawaf O, Ligtvoet R, Robrecht S, et al. Tislelizumab plus zanubrutinib for Richter transformation: the phase 2 RT1 trial. *Nat Med* 2024; **30:** 240–48.

20 Vogiatzi F, Heymann J, Müller K, et al. Venetoclax enhances the efficacy of therapeutic antibodies in B-cell malignancies by augmenting tumor cell phagocytosis. *Blood Adv* 2022; **6:** 4847–58.

21 Morschhauser F, Feugier P, Flinn IW, et al. A phase 2 study of venetoclax plus R-CHOP as first-line treatment for patients with diffuse large B-cell lymphoma. *Blood* 2021; **137:** 600–09.

22 Goede V, Fischer K, Dyer MJS, et al. Overall survival benefit of obinutuzumab over rituximab when combined with chlorambucil in patients with chronic lymphocytic leukemia and comorbidities: final survival analysis of the CLL11 study. European Hematology Association; June 14–17, 2018 (abstr S151).

23 Tilly H, Gomes da Silva M, Vitolo U, et al. Diffuse large B-cell lymphoma (DLBCL): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015; **26** (suppl 5)**:** 116–25.

24 Budwit-Novotny DA, McCarty KS, Cox EB, et al. Immunohistochemical analyses of estrogen receptor in endometrial adenocarcinoma using a monoclonal antibody. *Cancer Res* 1986; **46:** 5419–25.

25 Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol* 2014; **32:** 3059–68.

26 Tsimberidou AM, O'Brien S, Khouri I, et al. Clinical outcomes and prognostic factors in patients with Richter's syndrome treated with chemotherapy or chemoimmunotherapy with or without stem-cell transplantation. *J Clin Oncol* 2006; **24:** 2343–51.

27 El Hussein S, Medeiros LJ, Lyapichev KA, et al. Immunophenotypic and genomic landscape of Richter transformation diffuse large B-cell lymphoma. *Pathology* 2023; **55:** 514–24.

28 Behdad A, Griffin B, Chen YH, et al. PD-1 is highly expressed by neoplastic B-cells in Richter transformation. *Br J Haematol* 2019; **185:** 370–73.

29 Appleby N, Eyre TA, Cabes M, et al. The STELLAR trial protocol: a prospective multicentre trial for Richter's syndrome consisting of a randomised trial investigation CHOP-R with or without acalabrutinib for newly diagnosed RS and a single-arm platform study for evaluation of novel agents in relapsed disease. *BMC Cancer* 2019; **19:** 471.

30 Mahmoud AM, Gaidano G, Mouhssine S. Immunological aspects of Richter Syndrome: from immune dysfunction to immunotherapy. *Cancers* 2023; **15:** 1015.

31 Jain N, Croner LJ, Allan JN, et al. Absence of BTK, BCL2, and PLCG2 mutations in chronic lymphocytic leukemia relapsing after first-line treatment with fixed-duration ibrutinib plus venetoclax. *Clin Cancer Res* 2024; **30:** 498–505.