



Bendamustine followed by obinutuzumab and venetoclax in chronic lymphocytic leukaemia (CLL2-BAG): primary endpoint analysis of a multicentre, open-label, phase 2 trial

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Summary

Background Targeted agents such as the type II anti-CD20 antibody obinutuzumab and the B-cell lymphoma-2 antagonist venetoclax have shown impressive therapeutic activity in chronic lymphocytic leukaemia. The CLL2-BAG trial was initiated to investigate the combination of these two agents in patients with chronic lymphocytic leukaemia.

Methods In this ongoing multicentre, open-label, investigator-initiated phase 2 trial, patients (aged ≥ 18 years) with chronic lymphocytic leukaemia requiring treatment according to the 2008 International Workshop on Chronic Lymphocytic Leukemia (IWCLL) criteria and an Eastern Cooperative Oncology Group performance status of 0–2 were enrolled at 16 sites in Germany. Patients with a relevant tumour load (absolute lymphocyte count $\geq 25\,000$ cells per μL or lymph nodes with a diameter of ≥ 5 cm) received sequential treatment of debulking with two cycles of bendamustine (70 mg/m^2 intravenously on days 1 and 2 of each of the two 28-day cycles), followed by induction and maintenance with obinutuzumab (1000 mg intravenously on days 1–2, 8, and 15 of the first induction cycle, every 4 weeks in induction cycles 2–6, and every 12 weeks in the maintenance phase) and oral venetoclax (starting in induction cycle 2 with 20 mg/day , with a weekly dose escalation over 5 weeks to the target dose of 400 mg/day). The primary endpoint was the proportion of patients achieving an overall response by investigator assessment at the end of induction treatment. All patients who received at least two induction cycles were included in the efficacy analyses and all patients who received at least one dose of study drug were included in the safety analyses. This study is registered with ClinicalTrials.gov, number NCT02401503.

Findings Between May 6, 2015, and Jan 4, 2016, 66 patients were enrolled (35 treatment naive and 31 with relapsed or refractory disease), three of whom were excluded from the efficacy analysis because they received fewer than two induction cycles. Of the remaining 63 patients in the efficacy-evaluable population, 34 patients (54%) were treatment naive and 29 (46%) had relapsed or refractory disease. At data cutoff (Feb 28, 2017), all patients had completed induction treatment. At the end of the induction, 60 (95%) of 63 patients (95% CI 87–99) had responded, including all 34 patients in the treatment-naive cohort and 26 [90%] of 29 relapsed or refractory patients. The most common grade 3–4 adverse events during debulking were neutropenia and anaemia (five [11%] of 47 patients each), and thrombocytopenia and infection (three [6%] each). The most common grade 3–4 adverse events during induction were neutropenia (29 [44%] of 66 patients), infection (nine [14%]), thrombocytopenia (eight [12%]), infusion-related reactions (five [8%]), and secondary primary malignancy (four [6%]). 89 serious adverse events, including 69 related to study treatment, were reported. These serious adverse events were also mainly infections (four cases in four patients during debulking and 18 cases in 11 patients during induction) and cytopenia (four cases in four patients during debulking and ten cases in seven patients in induction). Five relapsed or refractory patients died: three cases of sepsis were deemed related to study treatment, whereas two deaths from Richter's transformation were not.

Interpretation The sequential application of bendamustine and obinutuzumab combined with venetoclax caused no unexpected or cumulative toxicities. The high proportion of patients who achieved overall responses, both treatment-naive and relapsed or refractory patients irrespective of physical fitness and genetic risk factors, compare favourably to established chronic lymphocytic leukaemia therapies. Further follow-up will help to define whether the remissions with eradication of minimal residual disease achieved with this combination are durable after treatment discontinuation.

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Introduction

Several targeted agents for chronic lymphocytic leukaemia with impressive activity have become available in recent

years, leading to profound changes in the treatment of this disease. Venetoclax induces programmed cell death through selective inhibition of the antiapoptotic B-cell

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Research in context

Evidence before this study

At the time of planning this trial, the targeted agents ibrutinib, idelalisib, and venetoclax were available for the treatment of chronic lymphocytic leukaemia in clinical trials only. Only preliminary results from phase 1/2 trials were available for the BCL-2 antagonist venetoclax, but initial observation of cases with severe—even fatal—tumour lysis syndromes suggested that venetoclax had an unusual clinical efficacy. To achieve an efficacious treatment with a shorter treatment duration, combining venetoclax with the potent anti-CD20 antibody obinutuzumab seemed like a potentially advantageous approach. We searched PubMed with no language restrictions with the terms “venetoclax” OR “ABT-199” AND “chronic lymphocytic leukaemia” for clinical trial reports published between Jan 1, 1980, and Jan 4, 2018. We identified three clinical trials assessing venetoclax in chronic lymphocytic leukaemia. These trials document the high activity of venetoclax as a single agent and even greater activity when combined with the anti-CD20 antibody rituximab. Regarding the combination of venetoclax with the more active anti-CD20 antibody obinutuzumab, only preliminary reports are available including the safety run-in of a phase 3 trial (CLL14, NCT02242942) assessing this combination in the first-line treatment of patients with chronic lymphocytic leukaemia with relevant comorbidities.

Added value of this study

To our knowledge, this phase 2 study is the first to report a primary endpoint analysis on venetoclax combined with

obinutuzumab. The sequential combination of bendamustine, followed by venetoclax and obinutuzumab was an active therapy with a favourable safety profile and no unexpected increase of cytopenia or infections and only three tumour lysis syndromes.

Implications of all the available evidence

Cumulative evidence from two trials—66 patients in this phase 2 study and the 13 patients from the safety run-in of the phase 3 CLL14 trial—suggests that the combination of venetoclax and obinutuzumab might be one of the most efficacious treatment options reported so far for chronic lymphocytic leukaemia. The proportion of patients with minimal residual disease negativity is impressive and compares favourably with the combination of fludarabine, cyclophosphamide, and rituximab, the gold standard for treatment-naïve patients without high-risk genetic parameters. Ongoing phase 3 trials are assessing the combination of venetoclax with obinutuzumab in different patient populations and challenge the role of chemoimmunotherapy in first-line treatment of both physically unfit and fit patients (eg, the CLL14 trial [NCT02242942] and the CLL13 trial [NCT02950051] of venetoclax plus obinutuzumab vs standard chemoimmunotherapy vs venetoclax plus rituximab vs the triple combination of venetoclax, obinutuzumab, and ibrutinib). Until data from these trials with higher patient numbers are available, the use of venetoclax combined with obinutuzumab in routine practice cannot be recommended, especially in the first-line setting.

lymphoma-2 (BCL-2) protein,¹ which is upregulated in chronic lymphocytic leukaemia cells.² It is effective as monotherapy in patients with relapsed or refractory chronic lymphocytic leukaemia, including those with high-risk disease harbouring a 17p deletion (del[17p]).^{3,4} Preclinical evidence of a synergy of venetoclax and the anti-CD20 antibody rituximab¹ was confirmed in a phase 1b trial⁵ in relapsed or refractory chronic lymphocytic leukaemia, which showed deep minimal residual disease (MRD) negative responses in the bone marrow in 28 of 49 patients, indicating disease eradication below the detection limit. Treatment was stopped in 16 patients achieving deep remissions and none of the patients with MRD-negative remissions has relapsed so far.⁶

We hypothesised that combining venetoclax with the more potent type II anti-CD20 antibody obinutuzumab^{7–10} would further increase the depth of response. Preliminary results from an ongoing phase 1b trial¹¹ showed that both agents can be safely combined sequentially starting with either venetoclax or obinutuzumab.

Methods

Study design and participants

The design of the CLL2-BAG trial is based on our so-called sequential triple-T concept of a tailored and targeted treatment aiming for total eradication of

MRD.^{12,13} The trial is a prospective, open-label, multi-centre phase 2 trial investigating sequential treatment consisting of two cycles of bendamustine debulking followed by six induction cycles with obinutuzumab and venetoclax, and up to 24 months of maintenance treatment with obinutuzumab and venetoclax (appendix p 3). The study was an investigator-initiated trial by the German CLL Study Group, with the University of Cologne (Cologne, Germany) being the legal sponsor. 20 German sites (mainly tertiary or university hospitals) participated in the trial, 16 of which recruited patients (appendix p 1).

Treatment-naïve patients and those with relapsed or refractory chronic lymphocytic leukaemia requiring treatment according to International Workshop on Chronic Lymphocytic Leukemia (IWCLL) 2008 criteria,¹⁴ who were at least 18 years of age, with an estimated life expectancy of at least 6 months, and an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 were eligible for inclusion. Patients with an ECOG performance status of 3 deemed related to chronic lymphocytic leukaemia (eg, due to anaemia or severe constitutional symptoms) were also eligible.

Patients who had received a recent treatment for chronic lymphocytic leukaemia were required to have recovered from any acute toxicities, and the previous treatment

See Online for appendix

For the trial protocol see
[http://www.dclsg.de/
downloads/CLL2-BAG_Protocol_
v5.0_A3_20170607.pdf](http://www.dclsg.de/downloads/CLL2-BAG_Protocol_v5.0_A3_20170607.pdf)

regimen had to be stopped within the following time periods before the start of study treatment: chemotherapy within at least 28 days; antibody treatment within at least 14 days; and kinase inhibitors, BCL-2 antagonists, or immunomodulatory agents within at least 3 days. Use of corticosteroids was permitted until the start of study treatment, but had to be reduced to an equivalent of 20 mg or less prednisolone during treatment. Patients with comorbid disease were eligible for inclusion, except those with severe comorbidities with a Cumulative Illness Rating Scale score of 4 or any other life-threatening illness, medical condition, or organ system dysfunction that could compromise the patients' safety or interfere with the absorption or metabolism of the study drugs (eg, inability to swallow tablets or impaired resorption in the gastrointestinal tract). Additionally, patients were required to have a creatinine clearance of at least 30 mL/min and adequate liver and haematological function (platelet count $\geq 25\,000$ per μL , neutrophil count ≥ 1000 per μL , and haemoglobin concentration ≥ 8 g/dL, unless directly attributable to the patient's chronic lymphocytic leukaemia). Patients with a Richter's transformation, CNS involvement, secondary malignancies, or uncontrolled infections requiring systemic treatment and those taking strong CYP3A4 inhibitors or inducers or vitamin K antagonists were excluded. All eligibility criteria are listed in the appendix (pp 1–2).

All patients provided written informed consent before enrolment. The study was approved by the health authorities and institutional review boards of each participating site and was done in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice.

Procedures

Debulking with two cycles of bendamustine (70 mg/m² given intravenously on days 1 and 2 of each of the two 28-day cycles) was recommended for patients with an absolute lymphocyte count (ALC) of at least 25 000 cells per μL or lymph nodes with a diameter of at least 5 cm, unless they had contraindications (eg, refractoriness or known hypersensitivity). Patients without these criteria started with induction cycle 1. In the subsequent induction treatment, 1000 mg obinutuzumab was administered intravenously on days 1–2, 8, and 15 of the first cycle and every 4 weeks in cycles 2–6. Obinutuzumab monotherapy was given in the first cycle because this drug effectively clears peripheral lymphocytosis. Daily oral venetoclax was added in the second cycle with a weekly dose escalation over 5 weeks (20 mg, 50 mg, 100 mg, 200 mg, and 400 mg daily). Safety precautions for mitigation and early detection of tumour lysis syndrome (depending on patient's individual risk) were hydration, allopurinol, rasburicase, blood sampling, or hospital admission (appendix p 4). In the maintenance period, venetoclax was continued daily and obinutuzumab (1000 mg) administered every 12 weeks until unacceptable toxicity, disease progression, new

chronic lymphocytic leukaemia treatment, or for up to 24 months. Treatment was stopped in case of a complete remission and MRD negativity in peripheral blood in two assessments with an interval of 3 months. During treatment, administration of a prophylaxis for *Pneumocystis jirovecii* pneumonias and use of granulocyte-colony stimulating factor were recommended; product choice and dosing was done according to institutional practice. Administration of other anti-infective prophylaxis was also at the investigator's discretion.

At baseline, diagnosis of chronic lymphocytic leukaemia was confirmed centrally by immunophenotyping of circulating lymphocytes and all relevant prognostic parameters (including cytogenetic aberrations by fluorescence in-situ hybridisation, mutational analysis of the immunoglobulin heavy-chain variable-region gene [*IGHV*], tumour protein 53 [TP53], and the serum parameters β_2 microglobulin and thymidine kinase) were assessed in the central laboratories of the German CLL Study Group in Cologne and Ulm (Germany). Additionally, all patients were tested serologically for HIV and hepatitis B and C virus. A central screening process prevented inclusion of patients with an incorrect diagnosis or other violations of eligibility criteria.

CT or MRI was mandatory before the start of treatment with venetoclax for assessment of risk for tumour lysis syndrome, at staging at the end of induction treatment, and at the end of maintenance treatment if abnormal at baseline. Laboratory monitoring of haematological parameters and serum chemistry was mandatory at least three to five times at each dose level of venetoclax in the second and third induction cycle and at least weekly during the first induction cycle with obinutuzumab; however, in patients tolerating the treatment well, the intervals could be extended to every 3 months in the maintenance phase.

Treatment responses were assessed by the investigators according to IWCLL criteria¹⁴ and reviewed centrally by three authors (PC, PL, and OA-S). CT scans or MRI and a bone marrow aspirate were mandatory for confirmation of a complete remission or complete remission with incomplete marrow recovery. Patients who fulfilled all IWCLL criteria of a complete remission or complete remission with incomplete marrow recovery (no evidence of lymphadenopathy, hepatomegaly, or splenomegaly on clinical examination and ultrasound or other imaging, no disease-related symptoms, and normalisation of haematological parameters) but who did not have one or both of these diagnostic modalities were defined as having clinical complete remission or complete remission with incomplete marrow recovery, respectively, and graded as a partial response.

MRD samples from peripheral blood and bone marrow were taken from the final restaging after the induction treatment onwards every 3–6 months (based on the IWCLL response achieved). Samples were analysed centrally in the reference laboratory in Kiel (Germany),

using a well established four-colour flow cytometry assay with a reproducible gating strategy and sensitivity of one chronic lymphocytic leukaemia cell per 10 000 leukocytes (ie, $<10^{-4}$).^{15,16} Results were categorised into three different MRD levels: low ($<10^{-4}$), intermediate ($\geq 10^{-4}$ and $<10^{-2}$), and high ($\geq 10^{-2}$),¹⁷ and MRD negativity was defined as less than 10^{-4} .

Patients were monitored continuously for adverse events at each visit. Adverse events were graded according to the National Cancer Institute Common

Terminology Criteria for Adverse Events, version 4.0, and classified using the Medical Dictionary for Regulatory Activities classification system. Tumour lysis syndromes were classified according to the criteria defined by Cairo and Bishop.¹⁸ All three study drugs had to be withheld in case of grade 3 or 4 adverse events (ie, severe, medically significant, or life-threatening) except for those due to underlying chronic lymphocytic leukaemia (eg, cytopenia due to bone marrow involvement of the chronic lymphocytic leukaemia). Bendamustine and obinutuzumab also had to be interrupted in case of febrile infections of any grade. For venetoclax, a dose reduction by one dose level was recommended in case of a re-occurrence of the same adverse event; however, discontinuation of venetoclax was recommended after the fourth occurrence. For obinutuzumab, no dose modifications were permitted, but the antibody had to be discontinued in case of a grade 4 infusion-related reaction (ie, life-threatening) or a grade 3 infusion-related reaction at re-challenge. All study treatment had to be discontinued in case of the following: disease progression with or without histological transformation; start of a new treatment for chronic lymphocytic leukaemia; progressive multifocal leukoencephalopathy; adverse event or intercurrent illness that precluded further study treatment or a protocol violation that ruled out continuation of study drug, both according to the investigator's discretion; pregnancy in a female patient; failure to use two methods of reliable contraception in female patients of childbearing potential and in male patients with female partners of childbearing potential; withdrawal of consent; or refusal to continue treatment.

Outcomes

The primary endpoint was the proportion of patients achieving an overall response by investigator assessment after induction treatment, assessed at the final restaging 12 weeks after the start of the last induction cycle and defined as the proportion of patients having achieved a complete remission or partial response. Secondary endpoints included the proportion of patients achieving an overall response after debulking, after maintenance treatment according to investigator assessment and central review, and in predefined patient subgroups (according to previous treatment, physical fitness, cytogenetic abnormalities, *IGHV* mutational status, TP53 deficiency, and administration of debulking treatment); the proportion of patients who achieved an overall response at the final restaging according to central review; the proportion of patients who achieved complete remission; MRD assessments in peripheral blood and bone marrow; time-to-event analyses for progression-free survival (defined as the time between registration and first disease progression or death) and overall survival (defined as the time between registration and death); and safety. Other secondary endpoints

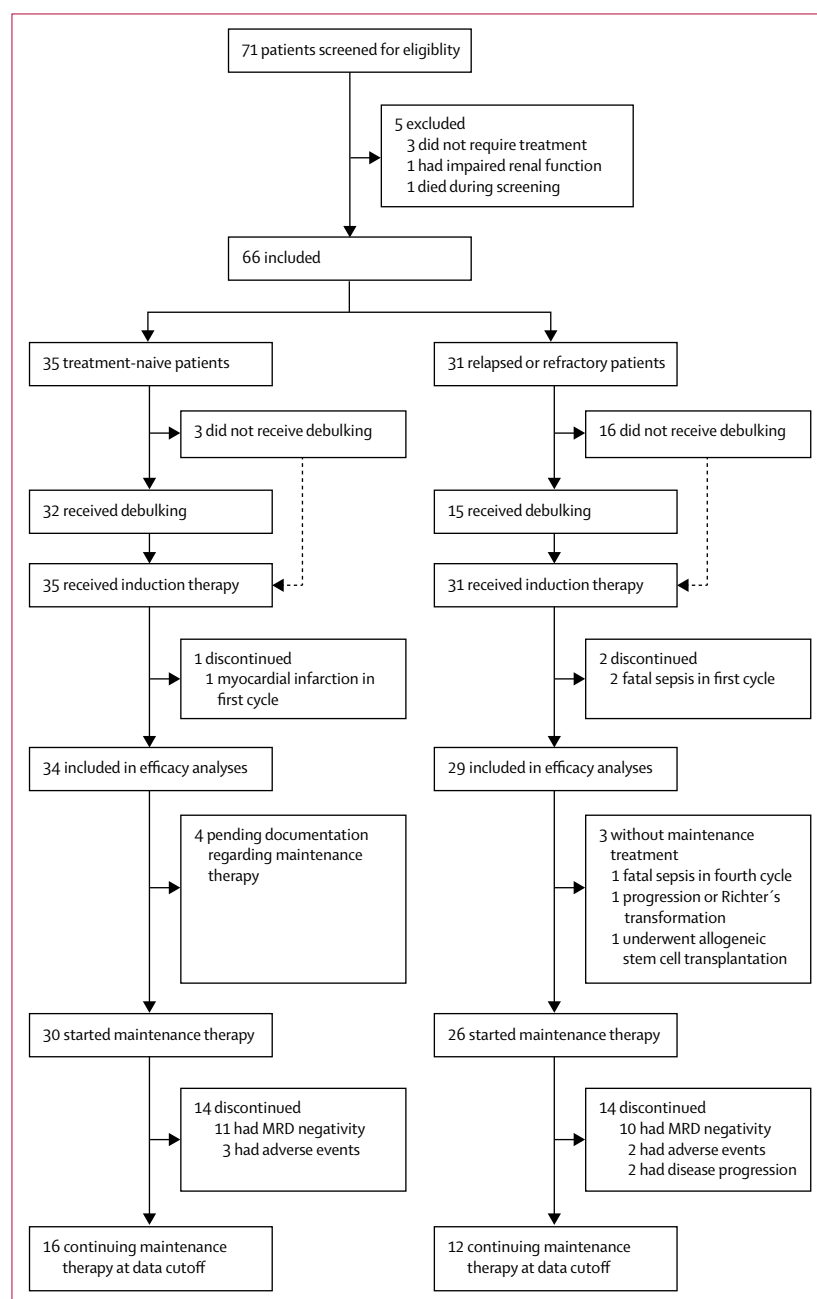


Figure 1: Trial profile
MRD=minimal residual disease.

that were not part of the primary endpoint analysis and will be analysed and reported later with extended follow-up data are the proportion of patients achieving a best response until 6 months after final restaging; event-free survival (defined as the time between registration and first disease progression, initiation of subsequent chronic lymphocytic leukaemia treatment, or death); treatment-free survival (defined as the time between registration and initiation of subsequent chronic lymphocytic leukaemia treatment or death); time to next chronic lymphocytic leukaemia treatment (defined as the time between registration and initiation of subsequent chronic lymphocytic leukaemia treatment); and duration of response (defined as the time between date of first response until disease progression or death). Differences between investigator-assessed and centrally assessed responses are not reported.

Statistical analysis

The primary endpoint (the investigator-assessed proportion of patients achieving an overall response) was tested against the null hypothesis of 75% patients achieving an overall response using a two-sided binomial test. We based this null hypothesis on findings from the CLL2M trial,^{19,20} which assessed six cycles of chemoimmunotherapy with bendamustine and rituximab in treatment-naïve patients with relapsed or refractory disease, and in which 88% of treatment-naïve patients and 59% of relapsed or refractory patients achieved an overall response (ie, in total around 75% patients had an overall response). The study treatment regimen was expected to lead to an improvement in the proportion of patients achieving an overall response to 90%. We calculated that we needed to enrol 54 patients to have 80% power at a 5% significance level. Accounting for an expected 10% dropout, we aimed to recruit 62 evaluable patients, with a fixed lower limit of at least 21 patients in each cohort (ie, at least 21 untreated patients and at least 21 relapsed or refractory patients) needing to be enrolled.

All patients who received at least two complete cycles of induction treatment were included in the efficacy analyses, and all those who received at least one dose of study drug were included in the safety analyses. We analysed the primary and secondary endpoints in both the overall patient population and in the two cohorts of previously untreated and relapsed or refractory patients separately. The 95% CI for the primary endpoint was calculated according to the Clopper-Pearson method. Sensitivity analyses were not done for the primary endpoint analysis. No interim analysis was planned. The Kaplan-Meier method was used for time-to-event analyses. Types of response and other characteristics were summarised by counts and percentages.

Analyses were done using SPSS (version 24) and SAS (version 9.4). This study is registered with ClinicalTrials.gov, number NCT02401503.

Role of the funding source

The funders of the study approved the study design and had the opportunity to review the manuscript, but had no role in data collection, data analysis, or data

	Treatment-naïve patients (n=35)	Relapsed or refractory patients (n=31)	All patients (n=66)
Age, years	58 (53–68)	61 (54–65)	59 (54–67)
Time since initial diagnosis, months	29 (4–73)	106 (45–160)	60 (18–125)
Sex			
Female	7 (20%)	9 (29%)	16 (24%)
Male	28 (80%)	22 (71%)	50 (76%)
Binet stage			
A	7 (20%)	10 (32%)	17 (26%)
B	11 (31%)	7 (23%)	18 (27%)
C	17 (49%)	14 (45%)	31 (47%)
B symptoms present	13 (37%)	11 (35%)	24 (36%)
ECOG performance status			
0	26 (74%)	16 (52%)	42 (64%)
1	9 (26%)	15 (48%)	24 (36%)
CIRS score (comorbidity)			
Median (IQR)	2 (1–4)	2 (1–14)	2 (1–4)
>6	2 (6%)	5 (16%)	7 (11%)
Creatinine clearance, mL/min			
Median (range)	84.7 (76.6–100.0)	81.1 (64.4–97.7)	83.5 (66.2–99.8)
30–69	6 (17%)	11 (35%)	17 (26%)
Tumour lysis syndrome risk*			
High	11 (31%)	7 (23%)	18 (27%)
Intermediate	21 (60%)	18 (58%)	39 (59%)
Low	2 (6%)	5 (16%)	7 (11%)
Assessed after baseline	1 (3%)	1 (3%)	2 (3%)
Absolute lymphocyte count (×10 ⁹ cells per L)	92.3 (48.3–149.5)	16.8 (3.8–55.2)	52.3 (9.8–107.3)
Bulky disease			
Lymph nodes >5 cm	13 (37%)	15 (48%)	28 (42%)
Lymph nodes >10 cm	7 (20%)	6 (19%)	13 (20%)
Splenomegaly >20 cm	4 (11%)	4 (13%)	8 (12%)
Cytogenetics†			
17p deletion	3/34 (9%)	8/29 (28%)	11/63 (18%)
11q deletion	8/34 (24%)	9/29 (31%)	17/63 (27%)
Trisomy 12	4/34 (12%)	2/29 (7%)	6/63 (10%)
None	8/34 (24%)	4/29 (14%)	12/63 (19%)
13q deletion	11/34 (32%)	6/29 (21%)	17/63 (27%)
Molecular genetics			
IGHV unmutated	21/35 (60%)	28/30 (93%)	49/65 (75%)
TP53	6/35 (17%)	12/30 (40%)	18/65 (28%)
NOTCH1	1/35 (3%)	7/30 (23%)	8/65 (12%)
SF3B1	7/35 (20%)	10/30 (33%)	17/65 (26%)

(Table 1 continues on next page)

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	Treatment-naïve patients (n=35)	Relapsed or refractory patients (n=31)	All patients (n=66)
Serum β 2 microglobulin, mg/dL			
Median (IQR)	3.9 (2.7–5.1)	4.9 (2.8–6.7)	4.2 (2.7–5.4)
>3.5	19 (54%)	19 (61%)	38 (58%)
Serum thymidine kinase, U/L			
Median (IQR)	31.3 (19.4–56.1)	50.7 (21.9–88.5)	36.6 (19.6–68.0)
>10	34 (97%)	30 (97%)	64 (97%)
ZAP-70 expression >20%	12 (34%)	18 (58%)	30 (45%)
CD38 expression >30%	12 (34%)	18 (58%)	30 (45%)
CLL-IPI			
Low	3/34 (9%)	0	3/63 (5%)
Intermediate	14/34 (41%)	5/29 (17%)	19/63 (30%)
High	12/34 (35%)	16/29 (55%)	28/63 (44%)
Very high	5/34 (15%)	8/29 (28%)	13/63 (21%)

Data are median (IQR), n (%), or n/N (%). ECOG=Eastern Cooperative Oncology Group. CIRS=cumulative illness rating scale. IGHV=immunoglobulin heavy-chain variable region. TP53= tumour protein 53. ZAP-70=Zeta-chain associated protein kinase 70. IPI=international prognostic index. *High=absolute lymphocyte count (ALC) \geq 25 000 cells per μ L and lymph nodes 5–10 cm or lymph nodes >10 cm; intermediate=ALC \geq 25 000 cells per μ L or lymph nodes 5–10 cm; low=ALC <25 000 cells per μ L and lymph nodes <5 cm. †According to hierarchical model by Döhner and colleagues.²¹

Table 1: Demographics and baseline characteristics

interpretation, and had no influence on the content of the manuscript. PC, JvT, JB, SR, PL, and OA-S had full access to all the data in the study and PC and MH had final responsibility for the decision to submit for publication.

Results

Between May 6, 2015, and Jan 4, 2016, 71 patients were screened and 66 patients were enrolled. The unintended over-recruitment of four patients happened because of the popularity and the rapid recruitment of the trial (8 months instead of 1 year as planned). When the sites were informed about the upcoming end of recruitment, the number of patients already consented for the trial was higher than the number needed. Because venetoclax was not available at that time and some patients had very few or no other therapeutic options, we decided to include these patients. Three patients with fewer than two induction cycles (including two early deaths due to sepsis in the first induction cycle) were excluded from the efficacy analysis, but remained in the safety population (figure 1). At data cutoff (Feb 28, 2017), median follow-up was 16 months (IQR 15–18).

35 (53%) of the 66 enrolled patients were treatment naïve and 31 (47%) had relapsed or refractory chronic lymphocytic leukaemia. In the efficacy-evaluable population, 34 (54%) of 63 patients were treatment naïve and 29 (46%) had relapsed or refractory disease, with a median of two previous therapies (range 1–9; IQR 1–3). The most common previous therapies in all patients were bendamustine plus rituximab (20 times in 17 patients) or fludarabine, cyclophosphamide, and rituximab (13 times

in 13 patients; appendix p 4). Few patients had received novel agents (five patients received ibrutinib and one patient ibrutinib and idelalisib); four patients had undergone stem cell transplantations (two allogenic and two autologous). 18 (29%) of 63 patients for whom data were available had a del(17p) or TP53 mutation and 49 (75%) of 65 for whom data were available had an unmutated *IGHV* status (table 1).

47 (71%) of all 66 enrolled patients (45 [71%] of 63 efficacy-evaluable patients) received bendamustine debulking and 37 of all 66 enrolled patients and 36 efficacy-evaluable patients completed the planned two cycles of debulking (appendix p 5). 19 (29%) of 66 patients immediately started with the induction therapy, owing to a low tumour burden in four, contraindications for bendamustine in four, physician's decision because of a del(17p) or TP53 mutation in five, and physician's decision without further information in five. 60 (95%) of 63 patients in the efficacy population received all six induction cycles; one relapsed or refractory patient died of sepsis during the fourth cycle and two relapsed or refractory patients discontinued treatment owing to non-response in the fifth and sixth cycle and later died of a Richter's transformation. 56 (89%) of 63 patients in the efficacy population received all eight obinutuzumab infusions as scheduled and 49 (78%) completed the ramp up of venetoclax as planned. 61 (97%) of 63 patients reached the target dose of venetoclax (400 mg); the other two patients had early neutropenia despite growth factor support (both in the relapsed or refractory group). Although 38 (60%) of 63 patients had venetoclax dose modifications, 33 (87%) of which were owing to adverse events, the median dose intensity of venetoclax in the induction treatment was 100% (IQR 98–100) in the treatment-naïve cohort and 97% (78–102) in the relapsed or refractory cohort (appendix p 5). During venetoclax ramp up, all patients received laboratory monitoring and hydration and 45 (71%) of 63 were admitted to hospital for monitoring during at least one dose escalation. 14 (22%) of 63 patients received prophylactic rasburicase in addition to allopurinol, which was received by 61 (97%) patients.

As of data cutoff, maintenance treatment had started in at least 56 patients (30 [88%] of 34 treatment naïve and 26 [90%] of 29 relapsed or refractory; documentation pending in four patients), of whom 43 had already completed the second cycle (24 treatment naïve and 19 relapsed or refractory) and 28 (14 and 14) had stopped maintenance treatment.

At the final restaging after the end of induction treatment, in the efficacy-evaluable population, all 34 treatment-naïve patients and 26 (90%) of 29 relapsed or refractory patients achieved an overall response according to investigator assessment (table 2), with 60 of 63 patients overall (95%, 95% CI 87–99) achieving an overall response ($p=0.0002$ when tested against the null hypothesis of 75% overall responses). According to investigator assessment,

five patients had a complete remission and 55 had a partial response, of whom 20 (14 treatment naive and six relapsed or refractory) did not have a bone marrow biopsy and one (treatment naive) did not have a CT scan, but fulfilled all other criteria for a complete remission.

MRD negativity in peripheral blood was achieved in 55 (87%) of 63 patients: 31 (91%) of 34 treatment-naive patients and 24 (83%) of 29 relapsed or refractory patients. MRD assessments from bone marrow were available in eight patients (four each with a complete remission [three treatment naive and one relapsed or refractory] and partial response [one and three, respectively]) and were all negative. 16 (94%) of 17 patients with a del(17p) or TP53 mutation responded (six of six treatment naive and ten of 11 relapsed or refractory) and 13 (76%) were MRD negative (five of six and eight of 11, respectively). Responses did not vary markedly by the presence of other cytogenetic abnormalities, *IGHV* status, patient's physical fitness, or administration of debulking (appendix p 5).

After the initial debulking with bendamustine, 24 (53%) of 45 patients (19 [61%] of 31 treatment naive and five [36%] of 14 relapsed or refractory) had achieved a response. 13 patients (29%; seven treatment naive and six relapsed or refractory) had stable disease and four (9%; two in each group) progressed but proceeded with induction treatment on study. Eight of the 17 patients with a del(17p) or TP53 mutation received a debulking (five treatment naive and three relapsed or refractory), and after up to two cycles of bendamustine, three of these patients had achieved an overall response (two and one, respectively), three had stable disease (one and two, respectively), one had progressed (treatment naive), and one was not assessable for response (treatment naive). Six of the 15 patients with previous treatment with bendamustine plus rituximab (all in the relapsed or refractory cohort) were re-exposed to bendamustine in the trial: one responded to the debulking, two had stable disease, two progressed, and one was not assessable for response. At baseline, 42 (63%) of the 63 patients in the efficacy-evaluable population had an ALC greater than 25 000 cells per μL (figure 2A), suggesting an intermediate or high risk of a tumour lysis syndrome. However, the ALC was normalised in 31 and reduced by 50% or more in 37 of 44 patients after bendamustine debulking, which also affected the patient's risk for tumour lysis syndrome (appendix p 6). As of data cutoff, 62 (98%) of 63 evaluable patients had a normalisation of their lymphocyte counts (figure 2A).

At baseline, 12 patients had lymph nodes larger than 10 cm, which defines a high risk of tumour lysis syndrome, and a further 13 had lymph nodes of at least 5 cm (figure 2B). The largest diameter of the enlarged lymph nodes at baseline was normalised to 1.5 cm at data cutoff in all but 11 patients (17%).

Consequently, investigator-assessed patient responses improved over time (figure 2C), and by data cutoff 21 patients had stopped maintenance treatment because

	Treatment-naive patients (n=34)	Relapsed or refractory patients (n=29)	All patients (n=63)
Patients achieving an overall response	34 (100%, 90–100)	26 (90%, 73–98)	60 (95%, 87–99)
Response			
Complete remission	3 (9%)	2 (7%)	5 (8%)
Clinical complete remission or complete remission with incomplete recovery of bone marrow	14 (41%)	6 (21%)	20 (32%)
Partial response	17 (50%)	18 (62%)	35 (56%)
Stable disease	0	0	0
Progressive disease	0	3 (10%)	3 (5%)
MRD in peripheral blood			
Negative ($<10^{-4}$)	31 (91%)	24 (83%)	55 (87%)
Intermediate ($\geq 10^{-4}$ to $<10^{-3}$)	3 (9%)	2 (7%)	5 (8%)
Positive ($\geq 10^{-3}$)	0	1 (3%)	1 (2%)
Missing	0	2 (7%)	2 (3%)
MRD in bone marrow			
Negative ($<10^{-4}$)	4 (12%)	4 (14%)	8 (13%)
Intermediate ($\geq 10^{-4}$ to $<10^{-3}$)	0	0	0
Positive ($\geq 10^{-3}$)	0	0	0
Missing	30 (88%)	25 (86%)	55 (87%)
Data are number (%; 95% CI) or number (%). MRD=minimal residual disease.			

Table 2: Response at the end of induction therapy in the efficacy population

they had achieved a MRD-negative response (11 treatment naive and ten relapsed or refractory; figure 2D): four had a complete remission (one treatment naive and two relapsed or refractory), three had a complete remission with incomplete recovery of bone marrow (one and two, respectively), and 14 had a partial response (eight and six, respectively; of these patients, ten did not have a bone marrow aspirate and one did not have a CT scan for confirmation of complete remission). Thus far, these patients have no or short follow-up after treatment discontinuation (median 0.1 months, range 0–6, IQR 0–3). Additionally, two patients stopped maintenance treatment because of disease progression (both in the relapsed or refractory cohort) and five because of adverse events (three treatment naive and two relapsed or refractory; all five were MRD negative in peripheral blood).

After a median follow-up of 16 months (range 13–22, IQR 15–18), four progressions (including three with Richter's transformation) and three deaths were reported in relapsed or refractory patients. Thus, median progression-free and overall survival were not reached in either cohort (figure 3). Progression-free survival at 15 months in the efficacy-evaluable population was 100% (95% CI not evaluable) in treatment-naive patients and 83% (69–97) in relapsed or refractory patients; estimated 15-month progression-free survival was 92% (85–99). Overall survival at 15 months in the efficacy-evaluable population was 100% (95% CI not evaluable) in

treatment-naïve patients and 90% (79–100) in relapsed or refractory patients; estimated 15-month overall survival was 95% (90–100).

In a post-hoc analysis, 42 (67%) of 63 patients in the efficacy population had low serum immunoglobulin G concentrations at baseline; no meaningful changes were noted during treatment (appendix p 7).

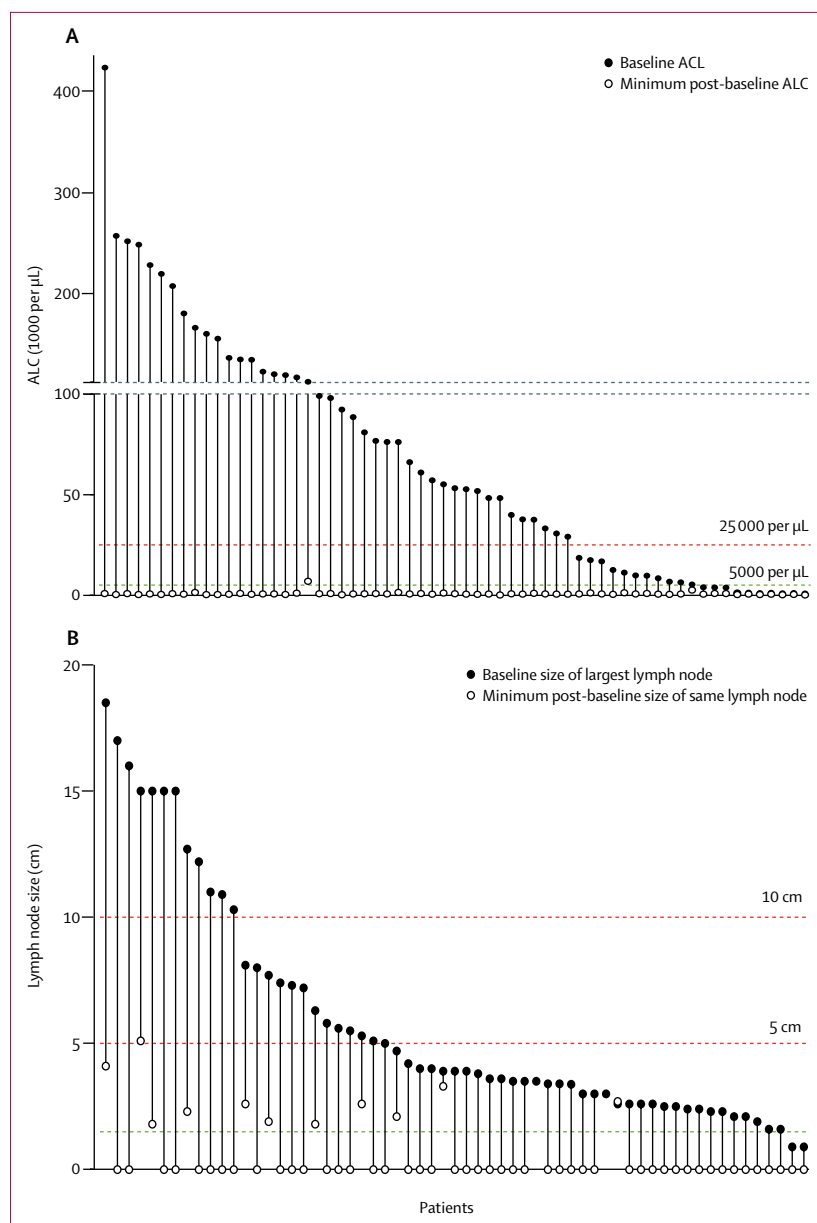
As of data cutoff, 677 adverse events were reported in all 66 enrolled patients (the safety population): 309 in the treatment-naïve cohort and 368 in the relapsed or refractory cohort (appendix p 7). 100 (15%) adverse events occurred during debulking, 439 (65%) during induction, and 138 (20%) in maintenance or follow-up (appendix pp 7–11).

According to investigator assessment, 427 (63%) of 677 adverse events were deemed related to study treatment. 551 (81%) of all reported adverse events resolved (291 needed treatment) and 539 (80%) did not require an adjustment of study drug. 264 (39%) adverse events were grade 1 and 203 (30%) grade 2; 172 (25%) were severe or clinically significant (grade 3), 34 (5%) were deemed life-threatening (grade 4), and three (<1%) had a fatal outcome; the grade was missing for one adverse event. Until data cutoff, five patients have died, all in the relapsed or refractory group: three deaths were due to sepsis that was deemed related to study treatment while two deaths of a Richter's transformation were not (appendix p 18).

89 (13%) of the 677 adverse events were serious, including 69 related to study drug. 12 patients experienced study-drug-related serious adverse events during debulking and 23 during induction. The most common serious adverse events were infections (four cases in four patients during debulking and 18 cases in 11 patients during induction), especially pneumonia or bronchopneumonia (one during debulking and six in three patients during induction), sepsis (four cases during induction), and cytomegalovirus infections (two cases in one patient during induction), as well as cytopenia (four cases in four patients during debulking and ten cases in seven patients in induction), especially neutropenia (two cases in two patients during debulking and six cases in five patients during induction) and thrombocytopenia (four cases in three patients during induction). Other study-drug-related serious adverse events reported in more than one patient were infusion-related reaction (six cases in six patients during induction), coronary artery disorder (one case during debulking and three cases in three patients during induction), tumour lysis syndrome (one case in one patient and two cases in two patients, respectively), neoplasms (two squamous cell carcinomas and one malignant melanoma in three patients, all during induction), and increased creatinine (two cases in two patients during debulking). A full list of serious adverse events is provided in the appendix (pp 13–16).

During debulking, 34 (72%) of 47 patients experienced adverse events of any grade, including 16 (34%) with grade 3–4 events (table 3). The most common grade 3–4 adverse events during debulking were neutropenia and anaemia (five 11%) of 47 patients each), and thrombocytopenia and infection (three [6%] each). Debulking did not lead to an increased incidence of cytopenia or infection in the induction phase (appendix p 17).

During induction, 63 (96%) of 66 patients had adverse events, of whom 41 (62%) had grade 3–4 events and three died (table 3). Most adverse events, especially grade 3–4 cytopenia and infection, were more frequent in the relapsed or refractory patients than in the treatment-naïve patients (table 3). The most common adverse events during induction were infections, especially upper respiratory tract infections and haematological toxicities, especially neutropenia and thrombocytopenia. These



(Figure 2 continues on next page)

cytopenia events required an adjustment of study drug in 46 (53%) of 86 of cases, but all patients were able to complete the six induction cycles. The most common grade 3–4 adverse events during induction were neutropenia (29 [44%] of 66 patients), infection (nine [14%]), thrombocytopenia (eight [12%]), infusion-related reactions (five [8%]), and secondary primary malignancy (four [6%]). Seven of 30 patients with neutropenia experienced infections (grade 1–2 in three patients and 3–4 in four patients) in the context of neutropenia (appendix p 18).

Three laboratory and no clinical tumour lysis syndromes were reported and only one of the laboratory syndromes occurred with venetoclax treatment. Additionally, four patients had relevant increases of either lactate dehydrogenase or potassium during venetoclax ramp up, which did not fulfil the Cairo-Bishop definition of a tumour lysis syndrome (appendix p 18).

Discussion

In this study, at the end of induction, a 95% overall response and an 87% MRD negativity in peripheral blood was achieved in a mixed population of treatment naive and relapsed or refractory patients irrespective of past treatment, physical fitness, and genetic factors. In addition to these encouraging efficacy results, the trial did not reveal unexpected or cumulative toxicities.

As expected, haematological toxicities and infections were the most common adverse events during bendamustine debulking and also during induction with obinutuzumab and venetoclax. With three deaths from sepsis in 66 enrolled patients, the treatment-related mortality seems high; however, in cases of low patient numbers, a few patients can have a substantial effect on the overall results. Furthermore, the fact that an all-comer population, including patients with few or no other therapeutic options, was included into this trial needs to be taken into account. Except for the three fatal sepsis cases, adverse events were generally manageable. Although more than half of the haematological toxicities led to dose modifications, mostly of venetoclax, few patients discontinued treatment owing to adverse events. Obinutuzumab and venetoclax seemed to cause fewer cytopenia events in patients without past therapy compared with those with previous exposure to chemotherapy. The incidences of neutropenia, thrombocytopenia, and infections in the

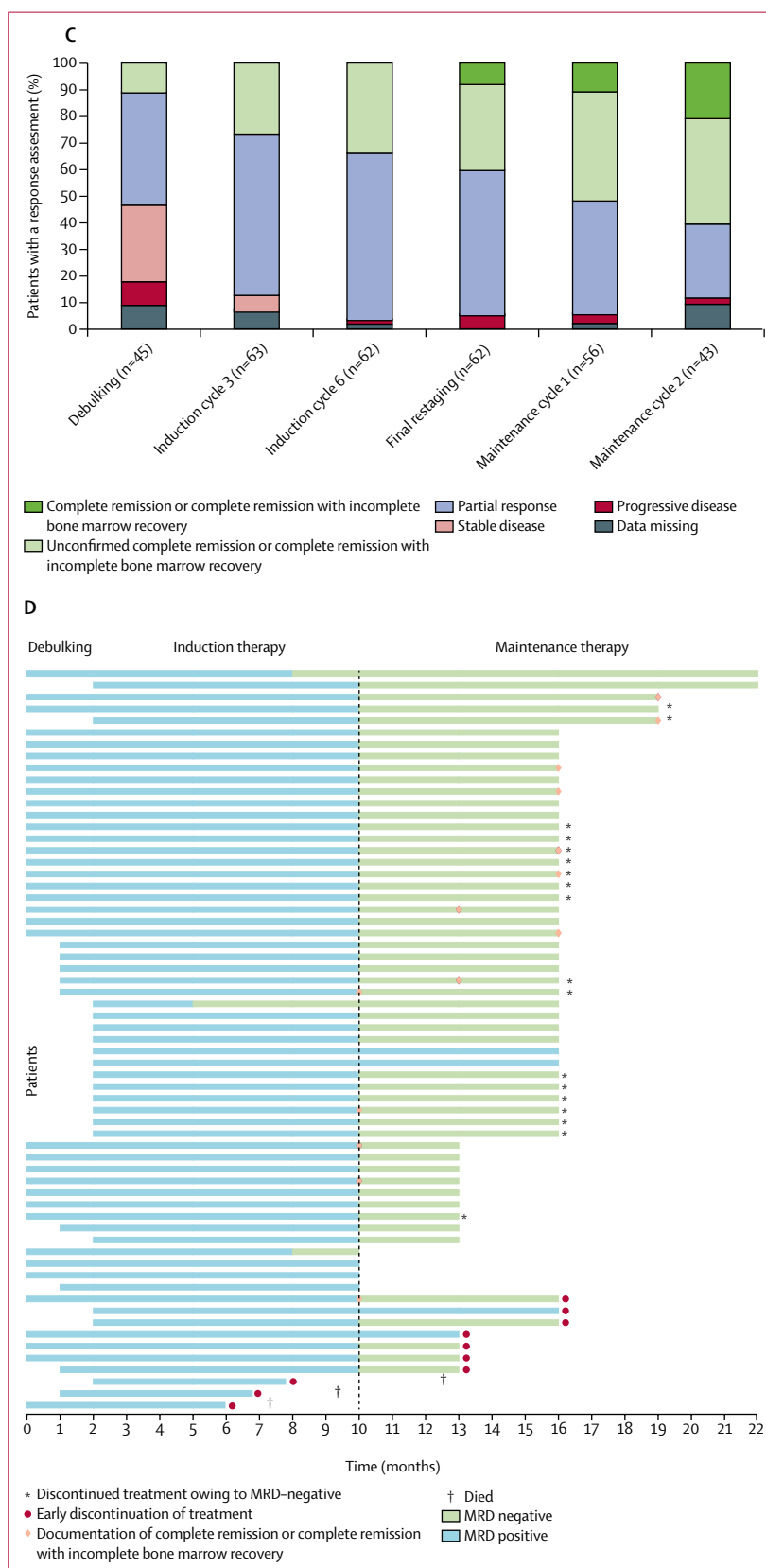


Figure 2: Response, time to first MRD negativity, and discontinuation of treatment in the efficacy population
Change from baseline (A) to minimum post-baseline value in ALC. (B) Change from baseline to minimum post-baseline size of largest lymph node (unidimensional measurement by CT or MRI scan). Two patients with CT or MRI done after the baseline assessment are not represented on the figure because neither baseline nor post-baseline size was available, and two patients did not have a CT or MRI after baseline. (C) Change in response over time with continued treatment. (D) Time to first documentation of MRD negativity and treatment discontinuation. MRD=minimal residual disease. ALC=absolute lymphocyte count.

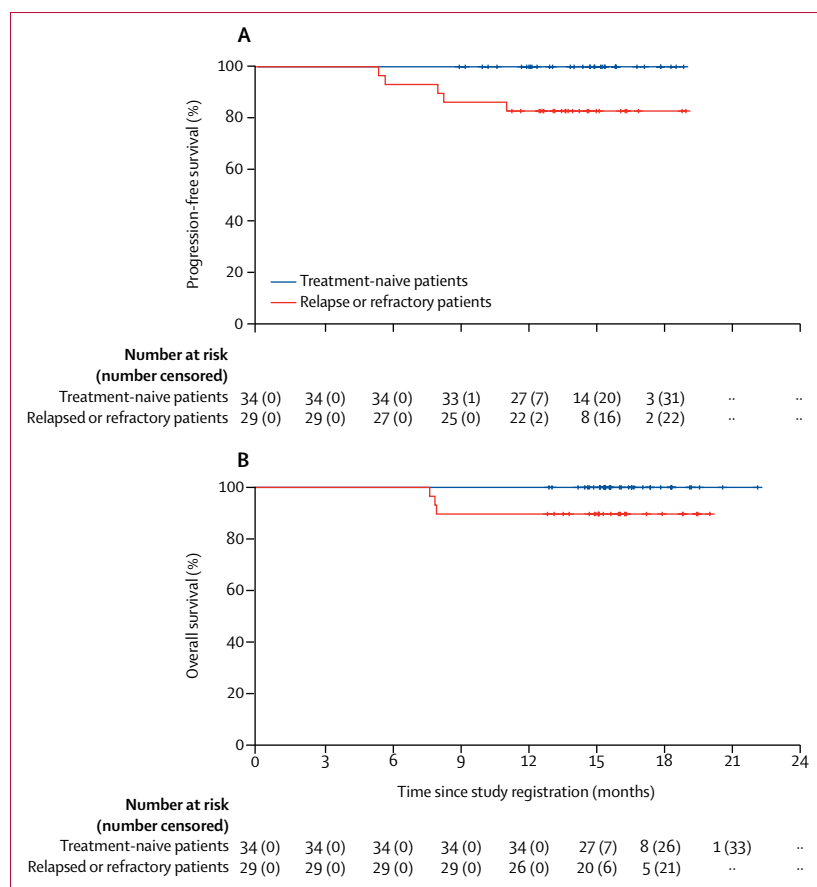


Figure 3: Progression-free (A) and overall survival (B) in the efficacy population

treatment-naïve cohort compared favourably with those reported for standard first-line chemoimmunotherapies (appendix p 19).^{10,22–24} In the relapsed or refractory cohort, the incidence of grade 3–4 neutropenia and thrombocytopenia was higher than that previously reported with ibrutinib^{25–27} or idelalisib plus rituximab,²⁸ but similar to that recorded with venetoclax either as a single agent or combined with rituximab.^{3–5} Although tumour lysis syndrome is a complication of venetoclax^{3–5} and combining venetoclax with other drugs might further increase the risk of its occurrence, only one laboratory case of tumour lysis syndrome related to venetoclax was noted in this study. Thus, the established safety precautions that were taken, such as a stepwise dose escalation and prophylactic treatment according to the individual risk category, were successful. Additionally, the initial debulking in patients with a higher tumour load and the sequential administration of obinutuzumab and venetoclax seemed to contribute to the safety of this regimen.

The primary goal of debulking with bendamustine was to reduce the tumour load before initiation of treatment with obinutuzumab and venetoclax to improve treatment tolerability. Up to two cycles with a bendamustine dose of

70 mg/m² (as established for relapsed patients) were used for all patients with debulking. This mild chemotherapy regimen was chosen instead of a more intensive chemoimmunotherapy to avoid long-lasting neutropenia and infectious complications, which could delay the start of induction treatment with the two more efficacious agents. Nevertheless, half of patients with debulking achieved a response according to IWCLL criteria, among the patients with a del(17p) or TP53 mutation or with past exposure to bendamustine and rituximab only a few achieved an IWCLL response after debulking. However, bendamustine contributed to normalisation of the lymphocyte count so that the risk category for development of tumour lysis syndrome could be downgraded in a relevant proportion of patients with debulking. Although more than two-thirds of patients experienced adverse events during debulking, mostly cytopenia and infections, this proportion is in the expected range and—most importantly—debulking did not seem to make patients more prone to cytopenia and infections in the induction phase. Owing to low patient numbers, to ascertain whether or not bendamustine debulking is beneficial for all patients or for particular subgroups is difficult. Additional analyses including two other phase 2 trials with a similar trial design also assessing bendamustine debulking followed by ibrutinib with either obinutuzumab or ofatumumab are underway (NCT02345863 and NCT02689141).

The efficacy of the study treatment regimen achieved in this mixed population is encouraging, especially since the MRD negativity rate of 91% in the peripheral blood in the treatment-naïve cohort is among the highest reported so far in chronic lymphocytic leukaemia. In fact, it is almost twice as high as reported with fludarabine, cyclophosphamide, and rituximab, the currently most efficacious first-line treatment.^{22,23} Moreover, the MRD negativity rates achieved in the relapsed or refractory patients (83%) and in patients with del(17p) or TP53 mutations (77%) compare favourably with all other established chronic lymphocytic leukaemia treatment regimens, including novel targeted agents.^{25–30} With single-agent venetoclax, MRD negativity was achieved in 35–40% of tested and 5–17% of all patients with relapsed or refractory chronic lymphocytic leukaemia;^{3,4} however, with the addition of rituximab to venetoclax, 67% of tested and 57% of all patients became MRD negative in the bone marrow, mostly after 7 months of treatment.⁵ Regarding the combination of venetoclax with obinutuzumab, so far the only data available are from the safety run-in of the phase 3 CLL14 trial with 13 previously untreated patients; all 13 patients responded, including seven of 12 who achieved a complete remission, 11 of 12 with MRD negativity in peripheral blood, and five of seven with MRD negativity in the bone marrow.³³

However, in the CLL2-BAG trial, the proportions of patients with MRD negativity in the bone marrow (eight of eight tested patients [13% of the 63 patients in the efficacy population]) and of confirmed complete

	Treatment-naïve patients			Relapsed or refractory patients			All patients		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
During debulking									
Number of patients	32	32	32	15	15	15	47	47	47
Patients with an adverse event	14 (44%)	5 (16%)	4 (13%)	4 (27%)	7 (47%)	0	18 (38%)	12 (26%)	4 (9%)
Blood and lymphatic system disorders	2 (6%)	2 (6%)	4 (13%)	0	6 (40%)	0	2 (4%)	8 (17%)	4 (9%)
Anaemia	1 (3%)	2 (6%)	0	0	3 (20%)	0	1 (2%)	5 (11%)	0
Neutropenia (HLT)	1 (3%)	1 (3%)	2 (6%)	0	2 (13%)	0	1 (2%)	3 (6%)	2 (4%)
Thrombocytopenia	1 (3%)	0	2 (6%)	0	1 (7%)	0	1 (2%)	1 (2%)	2 (4%)
Ear and labyrinth disorders	4 (13%)	0	0	1 (7%)	0	0	5 (11%)	0	0
Vertigo	4 (13%)	0	0	1 (7%)	0	0	5 (11%)	0	0
Gastrointestinal disorders	9 (28%)	1 (3%)	0	2 (13%)	0	0	11 (23%)	1 (2%)	0
Constipation	4 (13%)	0	0	0	0	0	4 (9%)	0	0
Diarrhoea	4 (13%)	0	0	1 (7%)	0	0	5 (11%)	0	0
Nausea and vomiting symptoms (HLT)	3 (9%)	1 (3%)	0	1 (7%)	0	0	4 (9%)	1 (2%)	0
General disorders and administration site conditions	9 (28%)	0	0	4 (27%)	1 (7%)	0	13 (28%)	1 (2%)	0
Fatigue	2 (6%)	0	0	3 (20%)	0	0	5 (11%)	0	0
Infections and infestations	6 (19%)	2 (6%)	0	1 (7%)	1 (7%)	0	7 (15%)	3 (6%)	0
Musculoskeletal and connective tissue disorders	2 (6%)	0	0	3 (20%)	0	0	5 (11%)	0	0
Respiratory, thoracic, and mediastinal disorders	2 (6%)	0	0	3 (20%)	0	0	5 (11%)	0	0
Skin and subcutaneous tissue disorders	5 (16%)	1 (3%)	0	1 (7%)	0	0	6 (13%)	1 (2%)	0
Rash	4 (13%)	1 (3%)	0	0	0	0	4 (9%)	1 (2%)	0
During induction									
Number of patients	35	35	35	31	31	31	66	66	66
Patients with adverse events	13 (37%)	10 (29%)	9 (26%)	6 (19%)	14 (45%)	8 (26%)	19 (29%)	24 (36%)	17 (26%)
Blood and lymphatic system disorders	1 (3%)	4 (11%)	8 (23%)	1 (3%)	12 (39%)	7 (23%)	2 (3%)	16 (24%)	15 (23%)
Neutropenia (HLT)	1 (3%)	5 (14%)	7 (20%)	0	13 (42%)	4 (13%)	1 (2%)	18 (27%)	11 (17%)
Thrombocytopenia	0	0	1 (3%)	2 (6%)	5 (16%)	2 (6%)	2 (3%)	5 (8%)	3 (5%)
Cardiac disorders	2 (6%)	1 (3%)	0	2 (6%)	3 (10%)	0	4 (6%)	4 (6%)	0
Eye disorders	2 (6%)	0	0	4 (13%)	0	0	6 (9%)	0	0
Gastrointestinal disorders	19 (54%)	0	0	12 (39%)	0	0	31 (47%)	0	0
Diarrhoea	7 (20%)	0	0	5 (16%)	0	0	12 (18%)	0	0
Flatulence, bloating, and distention (HLT)	5 (14%)	0	0	1 (3%)	0	0	6 (9%)	0	0
Gastrointestinal and abdominal pains (HLT)	3 (9%)	0	0	3 (10%)	0	0	6 (9%)	0	0
Nausea and vomiting symptoms (HLT)	8 (23%)	0	0	6 (19%)	0	0	14 (21%)	0	0
General disorders and administration site conditions	13 (37%)	0	0	9 (29%)	0	0	22 (33%)	0	0
Fatigue	11 (31%)	0	0	5 (16%)	0	0	16 (24%)	0	0
Pyrexia	3 (9%)	0	0	4 (13%)	0	0	7 (11%)	0	0
Infections and infestations	12 (34%)	3 (9%)	0	12 (39%)	6 (19%)	0	24 (36%)	9 (14%)	0
Bronchitis	4 (11%)	0	0	1 (3%)	1 (3%)	0	5 (8%)	1 (2%)	0
Upper respiratory tract infections (HLT)	7 (20%)	1 (3%)	0	9 (29%)	0	0	16 (24%)	1 (2%)	0
Injury, poisoning, and procedural complications	11 (31%)	0	0	8 (26%)	5 (16%)	0	19 (29%)	5 (8%)	0
Infusion-related reaction	9 (26%)	0	0	6 (19%)	5 (16%)	0	15 (23%)	5 (8%)	0
Investigations	3 (9%)	3 (9%)	1 (3%)	4 (13%)	1 (3%)	1 (3%)	7 (11%)	4 (6%)	2 (3%)
Metabolism and nutrition disorders	6 (17%)	0	0	4 (13%)	2 (6%)	0	10 (15%)	2 (3%)	0
Musculoskeletal and connective tissue disorders	4 (11%)	0	0	7 (23%)	3 (10%)	0	11 (17%)	3 (5%)	0
Musculoskeletal and connective tissue disorders (HLGT)	3 (9%)	0	0	4 (13%)	0	0	7 (11%)	0	0

(Table 3 continues on next page)

	Treatment-naïve patients			Relapsed or refractory patients			All patients		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
(Continued from previous page)									
Neoplasms, benign, malignant, and unspecified (including cysts and polyps)	1 (3%)	1 (3%)	0	1 (3%)	3 (10%)	0	2 (3%)	4 (6%)	0
Nervous system disorders	4 (11%)	0	0	2 (6%)	1 (3%)	0	6 (9%)	1 (2%)	0
Psychiatric disorders	3 (9%)	1 (3%)	0	3 (10%)	0	0	6 (9%)	0	0
Respiratory, thoracic, and mediastinal disorders	5 (14%)	0	0	9 (29%)	0	0	14 (21%)	0	0
Skin and subcutaneous tissue disorders	9 (26%)	0	0	3 (10%)	0	0	12 (18%)	0	0
Rash	3 (9%)	0	0	3 (10%)	0	0	6 (9%)	0	0
Vascular disorders	3 (9%)	1 (3%)	0	3 (10%)	2 (6%)	1 (3%)	6 (9%)	3 (5%)	1 (2%)
Hypertension	3 (9%)	1 (3%)	0	0	2 (6%)	0	3 (5%)	3 (5%)	0

Data are number of patients (%). The table shows adverse events that occurred in at least 10% of patients in the safety population. Tables of all grade 3–5 adverse events that occurred are provided in the appendix (pp 8–12). HLT=high level term. HLG=high level group term.

Table 3: Adverse events

remissions (8% at the end of induction) is low, mainly because a bone marrow aspirate or CT scan had not yet been done. Since most patients continued venetoclax and obinutuzumab as maintenance treatment, the missing examinations are expected to be done later. Our findings suggest that the proportion of patients with a confirmed complete remission and with MRD negativity in bone marrow increases over time. However, a limitation of our study is that bone marrow biopsies for confirmation of complete remission and MRD assessment were not mandatory at a specific timepoint (eg, staging at the end of induction treatment). Instead, these examinations were recommended as soon as the best clinical response was achieved because we wished to avoid repetitive CT scans or bone marrow biopsies.

Another limitation is the exclusion of patients with fewer than two induction cycles from the efficacy analyses; this was predefined in the protocol to avoid a false-negative result because the administration of study drug was less predictable in the all-comer population recruited in our study than in clinical trials with strict eligibility criteria. Of the three patients who were excluded from the analyses, two died from sepsis and one had a myocardial infarction during the first cycle; consequently, they received only obinutuzumab and never started treatment with venetoclax and obinutuzumab. The inclusion of an all-comer population can also be viewed as a limitation because it led to a heterogeneous patient population; however, we think that this fact helps to transfer the results to clinical routine because they are more generalisable to real-world patient populations. Activity of the treatment seemed to be similar among the patient subgroups with different clinical and biological risk factors; only patients with a del(17p) or TP53 mutation had a lower MRD negativity rate than patients without this mutation. Thus, overall, the efficacy of the study treatment regimen was encouraging across all subgroups.

In the aforementioned trial assessing the combination of venetoclax and rituximab,⁶ 16 patients with a deep remission (complete remission irrespective of MRD status or partial response with MRD negativity) stopped treatment after a median of 10 months. 14 patients with MRD negativity remained in remission after a median of 10 months off venetoclax, whereas the two patients with a MRD-positive complete remission experienced an asymptomatic progression. Thus, MRD negativity seems to predict progression-free survival more accurately than the clinical response assessment according to IWCLL guidelines. These findings confirm earlier results from a meta-analysis of patients treated with first-line chemoimmunotherapy.³¹ Further follow-up and research is necessary to define whether response assessment should be based on measurement of MRD in addition to—or even instead of—assessment of IWCLL response and whether treatment can be stopped in individuals with MRD negativity in peripheral blood. Shortened treatment durations could help to avoid problems with patient compliance and would be more cost-effective since novel agents constitute a financial burden for the health-care systems. Furthermore, efficient therapies are needed to avoid resistance due to clonal evolution arising from a therapeutic pressure selecting small subclones with resistance mechanisms.³² The combination of venetoclax and obinutuzumab yields fast responses with MRD-negative remissions in most patients. Based on the experience with venetoclax combined with rituximab in another trial⁵ and with venetoclax and obinutuzumab in this and another study,³³ these deep, MRD-negative remissions seem to last for a substantial time after treatment termination. Thus, the combination of venetoclax with an anti-CD20 antibody might constitute a novel paradigm of treatment and overcome the need for an indefinite treatment, as is the case with the other approved targeted agents to maintain responses in chronic lymphocytic leukaemia.

Contributors

PC, JvT, JB, AE, SB, BE, and MH were responsible for the conception and design of the study. PC, JvT, JB, SR, PL, OA-S, A-MF, and KF were responsible for trial management. PC, JvT, PL, OA-S, AE, ET, TS, LFvW, HH, SB, C-MW, SS, BE, and MH were responsible for the recruitment and treatment of patients. PC, PL, and OA-S did a central review of all clinical data. ET, K-AK, SB, MR, MK, and SS did the laboratory analyses. JB and SR did the statistical analysis. All authors interpreted the data. PC wrote the first draft and all authors reviewed and approved the manuscript.

Declaration of interests

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References

- Souers AJ, Levenson JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med* 2013; **19**: 202–08.
- Hanada M, Delia D, Aiello A, Stadtmauer E, Reed JC. bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia. *Blood* 1993; **82**: 1820–28.
- Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. *N Engl J Med* 2016; **374**: 311–22.
- Stilgenbauer S, Eichhorst B, Schetelig J, et al. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. *Lancet Oncol* 2016; **17**: 768–78.
- Seymour JF, Ma S, Brander DM, et al. Venetoclax plus rituximab in relapsed or refractory chronic lymphocytic leukaemia: a phase 1b study. *Lancet Oncol* 2017; **18**: 230–40.
- Anderson MA, Brander DM, Ma S, et al. Durability of responses on continuous therapy and following drug cessation in deep responders with venetoclax and rituximab. 22nd Congress of the European Hematology Association; Madrid, Spain; June 22–25, 2017. P247.
- Mossner E, Brunker P, Moser S, et al. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. *Blood* 2010; **115**: 4393–402.
- Patz M, Isaeva P, Forcob N, et al. Comparison of the in vitro effects of the anti-CD20 antibodies rituximab and GA101 on chronic lymphocytic leukaemia cells. *Br J Haematol* 2011; **152**: 295–306.
- Herter S, Herting F, Mundigl O, et al. Preclinical activity of the type II CD20 antibody GA101 (obinutuzumab) compared with rituximab and ofatumumab in vitro and in xenograft models. *Mol Cancer Ther* 2013; **12**: 2031–42.
- Goede V, Fischer K, Busch R, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. *N Engl J Med* 2014; **370**: 1101–10.
- Flinn IW, Brunvand M, Choi MY, et al. Safety and efficacy of a combination of venetoclax (GDC-0199/ABT-199) and obinutuzumab in patients with relapsed/refractory or previously untreated chronic lymphocytic leukemia - results from a phase 1b Study (GP28331). *Blood* 2015; **126**: 494.
- Hallek M. Signaling the end of chronic lymphocytic leukemia: new frontline treatment strategies. *Blood* 2013; **122**: 3723–34.
- Cramer P, von Tresckow J, Bahlo J, et al. CLL2-BXX phase-II trials: sequential, targeted treatment for eradication of minimal residual disease in CLL. *Future Oncol* 2018; **14**: 499–513.
- Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood* 2008; **111**: 5446–56.
- Bottcher S, Ritgen M, Kneba M. Flow cytometric MRD detection in selected mature B-cell malignancies. *Methods Mol Biol* 2013; **971**: 149–74.
- Rawstron AC, Villamor N, Ritgen M, et al. International standardized approach for flow cytometric residual disease monitoring in chronic lymphocytic leukaemia. *Leukemia* 2007; **21**: 956–64.
- Bottcher S, Ritgen M, Fischer K, et al. Minimal residual disease quantification is an independent predictor of progression-free and overall survival in chronic lymphocytic leukemia: a multivariate analysis from the randomized GCLLSG CLL8 trial. *J Clin Oncol* 2012; **30**: 980–88.
- Cairo MS, Bishop M. Tumour lysis syndrome: new therapeutic strategies and classification. *Br J Haematol* 2004; **127**: 3–11.
- Fischer K, Cramer P, Busch R, et al. Bendamustine in combination with rituximab for previously untreated patients with chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. *J Clin Oncol* 2012; **30**: 3209–16.
- Fischer K, Cramer P, Busch R, et al. Bendamustine combined with rituximab in patients with relapsed and/or refractory chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. *J Clin Oncol* 2011; **29**: 3559–66.
- Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med* 2000; **343**: 1910–16.

- 22 Hallek M, Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. *Lancet* 2010; **376**: 1164–74.
- 23 Eichhorst B, Fink AM, Bahlo J, et al. First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial. *Lancet Oncol* 2016; **17**: 928–42.
- 24 Keating MJ, O'Brien S, Albitar M, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. *J Clin Oncol* 2005; **23**: 4079–88.
- 25 Byrd JC, Brown JR, O'Brien S, et al. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. *N Engl J Med* 2014; **371**: 213–23.
- 26 Byrd JC, Furman RR, Coutre SE, et al. Three-year follow-up of treatment-naïve and previously treated patients with CLL and SLL receiving single-agent ibrutinib. *Blood* 2015; **125**: 2497–506.
- 27 Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med* 2013; **369**: 32–42.
- 28 Furman RR, Sharman JP, Coutre SE, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med* 2014; **370**: 997–1007.
- 29 Barr PM, Brown JR, Hillmen P, et al. Impact of ibrutinib dose adherence on therapeutic efficacy in patients with previously treated CLL/SLL. *Blood* 2017; **129**: 2612–15.
- 30 Jain P, Thompson PA, Keating M, et al. Long-term outcomes for patients with chronic lymphocytic leukemia who discontinue ibrutinib. *Cancer* 2017; **123**: 2268–73.
- 31 Kovacs G, Robrecht S, Fink AM, et al. Minimal residual disease assessment improves prediction of outcome in patients with chronic lymphocytic leukemia (CLL) who achieve partial response: comprehensive analysis of two phase III studies of the German CLL Study Group. *J Clin Oncol* 2016; **34**: 3758–65.
- 32 Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. *Nature* 2015; **526**: 525–30.
- 33 Fischer K, Al-Sawaf O, Fink AM, et al. Venetoclax and obinutuzumab in chronic lymphocytic leukemia. *Blood* 2017; **129**: 2702–05.