Table I. Haematological data of the patients at baseline and after treatment with sirolimus or hydroxycarbamide, alone or in combination.

| Patient 1 | Patient 2 |
|-----------|-----------|
|           |           |
| Hb (g/l)  | MCV (fl)  | Hb (g/l)  | MCV (fl)  |
| Baseline  | 73        | 79.0      | 90        | 89.8      |
| Post SIR | 69        | 76.9      |           |           |
| Post HC   | –         | –         | 94        | 118.5     |
| Post HC + SIR | 88      | 86.0      | 108       | 111.9     |

SIR caused a decrease in Hb in one patient and MCV in both patients, while HC increased the Hb and MCV in both patients. Changes were statistically significant (data not shown). Patient 1 was never treated with HC alone, and patient 2 was never treated with SIR alone.

Hb, haemoglobin concentration; HC, hydroxycarbamide; MCV, mean corpuscular volume; SIR, sirolimus.

HbF further in response to HC therapy (Adekile et al, 2015).

The additive effect of HC to other agents was first reported in an animal model treated with HC and recombinant erythropoietin (Al-Khatti et al, 1988) and a similar observation was made in SCD patients (Rodgers et al, 1993).

Our observation makes the case to incorporate sirolimus, and probably other mTOR inhibitors, in post-transplantation immunosuppressive regimens to prevent rejection in patients with SCD. However, more studies are needed before sirolimus and other mTOR inhibitors can be recommended for SCD patients.

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Venetoclax for chronic lymphocytic leukaemia patients who progress after more than one B-cell receptor pathway inhibitor

B-cell receptor signalling pathway inhibitors (BCRi) have changed the treatment paradigm for chronic lymphocytic leukaemia (CLL), with durable responses achieved with ibrutinib monotherapy (Bruton tyrosine kinase inhibitor [BTKi]) (https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/205552s002lbl.pdf; Burger et al, 2015) and idelalisib (phosphatidylinositol-3-kinase inhibitor [PI3Ki]) with rituximab or ofatumumab (https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/206545lbl.pdf; Byrd et al, 2014;
The oral BCL2 inhibitor venetoclax has shown promising efficacy across clinical studies in relapsed/refractory (R/R) CLL and, based on the critical need for therapies following BCRi discontinuation, we report on a post-hoc subgroup analysis of high-risk patients who previously received BTKi and PI3Ki (>1 prior BCRi) from a phase 2 study of venetoclax in patients with CLL R/R to ibrutinib and/or idelalisib (Coutre et al, 2018; Jones et al, 2018).

This phase 2, open-label trial (NCT02141282) enrolled patients with R/R CLL to ibrutinib and/or idelalisib, although prior investigational BTKi or PI3Ki were also allowed (Coutre et al, 2018; Jones et al, 2018). Patients in this post-hoc analysis received >1 prior BCRi, including 16 who received BTKi then PI3Ki, nine who received PI3Ki then BTKi, two who received two BTKi, and one who received PI3Ki, then BTKi, then another PI3Ki (Table 1). Efficacy is also reported for 99 patients who only received one prior BCRi. The institutional review board of each study site approved the study protocol and amendments. Study activities were conducted in accordance with ethical principles of the Declaration of Helsinki and International Conference on Harmonization Guideline for Good Clinical Practice. All patients provided written informed consent.

Patients received venetoclax with weekly ramp-up to 400 mg/day dose to mitigate the risk of tumour lysis syndrome (TLS) (https://www.accessdata.fda.gov/drugsatfda_doc s/label/2016/208573s000lbl.pdf).

Responses were assessed by investigators based on 2008 International Workshop on Chronic Lymphocytic Leukaemia criteria (Hallek et al, 2008) and confirmed with an assessment ≥2 months later. Confirmationary bone marrow aspiration and biopsy were performed at screening and ≤2 months after meeting other criteria for complete remission (CR). Safety was assessed up to 30 days post-treatment. Minimal residual disease (MRD) was evaluated by flow cytometry in central laboratory. Data cut-off for this analysis was 30 June 2017. Analyses were 2-sided with \( P \leq 0.05 \) being considered significant.

Of 127 patients enrolled (Coutre et al, 2018; Jones et al, 2018), 28 received >1 prior BCRi (Table 1), with a median follow-up on venetoclax of 11-8 months (range: 0–1-30-6) for 28 patients who received >1 prior BCRi; this was 13.9 months (0–2–30-5) for 99 patients who received 1 prior BCRi. Investigator-assessed objective response rate (ORR) for patients who received >1 vs. 1 prior BCRi was 43% (12/28: 1 CR, 11 partial remission [PR]) vs. 75% (74/99: 10 CR/CRi, 64 PR; Fig 1A). Median time to first response was 2-5 months, regardless of number of prior BCRi received. There was no difference in number/types of prior therapies received or disease burden for responders versus non-responders. Median progression-free survival (PFS) for patients who received >1 prior BCRi was 16-4 months (95% confidence interval [CI]: 8, -) and was not reached for patients who had received 1 prior BCRi; 12-month Kaplan–Meier estimates for these subgroups were 58% (95% CI: 37%, 75%) and 82% (95% CI: 72%, 88%), respectively (Fig 1B). Median overall survival was not reached for all patients, and 12-month estimates for patients who received >1 vs. 1 prior BCRi were 89% (95% CI: 70%, 96%) and 93% (95% CI: 86%, 97%), respectively (Fig 1C). Median duration of response for patients who received >1 prior BCRi was 16-6 months (95% CI: 14-5, -) and was not reached for patients who received 1 prior BCRi; 12-month estimates for these patient subgroups were 92% (95% CI: 54%, 99%) and 88% (95% CI: 77%, 94%), respectively (Fig 1D).

Based on sequence of treatment, ORR on venetoclax was 50% (8/16) for patients who received BTKi then PI3Ki and 30% (3/9) for those who received PI3Ki then BTKi. Two patients received two prior BTKi, one achieved PR and one had stable disease on venetoclax. Two patients received a PI3Ki, then BTKi, then another PI3Ki and progressed on venetoclax. When assessed by discontinuation of last BCRi for adverse events (AEs) versus PD, ORR on venetoclax was 50% (3/6) and 38% (8/21), respectively. Though based on these small numbers, venetoclax was active in patients who discontinued the most recent BCRi for either AEs or PD, with higher response rates for those who stopped therapy due to AEs on prior BCRi.

Six of 14 patients who had received >1 prior BCRi assessed for MRD had undetectable MRD in blood (<10^-4 CLL cells), and one of two patients assessed had confirmed MRD-negativity in bone marrow. All achieved PR and continue on study.

Eighteen of 28 patients who received >1 prior BCRi discontinued venetoclax: 11 due to CLL progression [median time to PD, 8-1 months (0–1–22-5)], two had Richter transformation at 4-4 and 16-3 months, two due to AEs, one per investigator request, one for non-compliance, and one proceeded to stem cell transplant (SCT) in PR. The median time to next CLL therapy from venetoclax discontinuation was 19-5 days (0–175); including commercially-available venetoclax \( n = 2 \), ibrutinib \( n = 2 \), idelalisib + rituximab \( n = 1 \), allogeneic SCT \( n = 2 \), and chimeric antigen receptor T cell (CAR-T) therapy \( n = 3 \).

All 28 patients experienced at least one AE, and safety profile was consistent with prior reports of venetoclax monotherapy in R/R CLL (Coutre et al, 2018; Jones et al, 2018). Common AEs included grade 1/2 gastrointestinal toxicities and grade 3/4 cytopenias. Two patients died, one due to PD and one due to Corynebacterium sepsis. No patients had TLS per Howard criteria (Howard et al, 2011).

This represents the first retrospective analysis of this high-risk patient population, who were uniformly evaluated as part of a prospective trial with an effective therapy. With higher ORR and longer PFS for patients who only received 1 vs. >1 prior BCRi, these data support the earlier use of venetoclax in the treatment paradigm to optimize...
Table I. Patient demographics and baseline characteristics.

| Patients who received | n = 28 |
|-----------------------|--------|
| Age, years: median (range) | 65 (53–80) |
| ECOG performance status, n (%) | |
| 0 | 6 (21) |
| 1 | 16 (57) |
| 2 | 6 (21) |
| Number of prior therapies, median (range) | 6.5 (2–15) |
| Prior BCRi therapies, n (%) | |
| Ibrutinib* | 27 (96) |
| Idelalisib† | 25 (89) |
| Ibrutinib and idelalisib | 24 (86) |
| Investigational agents | 11 (39) |
| Acalabrutinib (ACP-196)‡ (BTKi) | 6 (21) |
| AVL292 (BTKi) | 2 (7) |
| INCB040093 (PI3Ki) | 2 (7) |
| Duvelisib (PI3Ki) | 1 (4) |
| Laboratory values,$§ median (range) | |
| Beta-2 microglobulin, mg/l | 2.9 (2–5.7) |
| Creatinine clearance, ml/min | 72 (44–140) |
| Lymphocyte count, × 10^9/l | 13.5 (5–407) |
| 25 × 10^9/l or more, n (%) | 11 (39) |
| Haemoglobin, g/l | 119 (82–152) |
| Platelet count, × 10^9/l | 123 (12–452) |
| Neutrophil count, × 10^9/l | 3.9 (1.1–8.4) |
| Bulky nodal disease, n (%) | |
| 5 cm or more | 15 (54) |
| 10 cm or more | 5 (18) |
| Tumour lysis syndrome risk category,¶ n (%) | |
| High | 10 (36) |
| Medium | 11 (39) |
| Low | 7 (25) |
| Prognostic factors,** n/N (%) | |
| Unmutated IGHV | 16/23 (70) |
| 17p deletion | 10/26 (36) |
| 11q deletion | 15/27 (54) |
| 13q deletion | 13/28 (46) |
| 12q trisomy | 4/25 (14) |
| TP53 mutation | 6/27 (22) |
| CD38 positive | 14/27 (52) |
| ZAP70 positive | 4/23 (17) |
| 17p deletion and/or TP53 mutation | 12/28 (43) |

BCRi, B-cell receptor signalling pathway inhibitor; ECOG, Eastern Cooperative Oncology Group (performance status ranges from 0 to 5, where higher numbers indicate greater disability).

*Patients had received prior ibrutinib for a median of 17 months (range: 2–53). Best response on ibrutinib for 22 patients with available data: 1 complete remission, 11 partial remission and 10 did not respond to treatment. Of 16 patients who received ibrutinib as their most recent BCRi, one discontinued due to adverse events (AEs) and progressed off therapy and 15 discontinued with progressive disease (PD).

†Patients had received prior idelalisib for a median of 4 months (range: 1–33). Of 20 patients with available data, eight had a best response of partial remission (PR) and 12 did not respond to idelalisib. Of 12 patients who received idelalisib as their most recent BCRi, five discontinued due to AEs and progressed off therapy, six with PD, and one had refractory disease.

‡Six patients had received the investigational agent acalabrutinib (ACP-196) for a median of 9.4 months (range: 1–23). Two patients had a best response of PR and 4 did not respond to therapy. Patients discontinued acalabrutinib due to PD (n = 4), AEs (n = 1), or stopped the clinical trial due to Bruton tyrosine kinase (BTK) resistance (n = 1).

§Values were assessed after screening but before the first venetoclax dose.

¶Low – all lymph nodes ≤5 cm with an absolute lymphocyte count (ALC) <25 × 10^9/l; Medium – any lymph node ≥5 cm to <10 cm or an ALC ≥25 × 10^9/l; High – any lymph node ≥10 cm or lymph node ≥5 cm and ALC ≥25 × 10^9/l.

**Site-reported data. Data are presented for all patients with available data.
response, though venetoclax was still active in this poor-risk group of patients who had received >1 prior BCRi. Given the relatively small number of patients treated with venetoclax after discontinuing multiple BCRi who had lower responses and remission durations than those treated earlier in their disease, consideration of transitioning such patients after disease control is obtained to either CAR-T therapy trials or allogeneic SCT could be pursued if patients are eligible.

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Data sharing statement: AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymized, individual and trial-level data (analysis data sets), as well as other information (e.g., protocols and Clinical Study Reports), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications. This clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement (DSA). Data requests can be submitted at any time and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.
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Author contributions

WW, JB, MD, RF, BC, HE, LH, MC: collected data, analysed and interpreted data, wrote the manuscript. LZ, MV: analysed and interpreted data, wrote the manuscript. JP: designed study, analysed and interpreted data, wrote the manuscript.

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CXCR4 mutations in lymphoplasmacytic lymphoma lead to altered CXCR4 expression

In addition to the MYD88 p.(L265P) mutation, approximately 30% of patients with lymphoplasmacytic lymphoma (LPL) carry a mutation of the CXCR4 gene (Treon et al, 2014), resulting in diminished response to therapy with Bruton tyrosine kinase (BTK) inhibitors (Cao et al, 2015; Treon et al, 2015) as well as more aggressive disease (Treon et al, 2014). The mechanism responsible for this resistance has not yet been elucidated and protein expression of CXCR4 in LPL has not been investigated.

CXCR4 leads to activation of the nuclear factor (NF)κB pathway (Han et al, 2001; Hideshima et al, 2002; Chatterjee et al, 2014) whereas BTK inhibitors eventually target the NFκB pathway, thereby decreasing cell survival in B-cell lymphomas (Figure S1). As these inhibitors show less efficacy in CXCR4-mutated LPL patients, we hypothesized that CXCR4 mutations influence CXCR4 as well as NFκB protein expression. Therefore, we investigated CXCR4 expression and mutation status in LPL patients, as well as (classical) NFκB p105/p50 expression, in order to further decipher the mechanism responsible for BTK inhibitor resistance.

A total of 30 LPL bone marrow biopsies obtained between 2004 and 2017 were selected from the archives of the University Medical Centre Utrecht, the Netherlands. Slides of the bone marrow biopsies were stained for CXCR4 (Biolegend, San Diego, CA, USA; 12G5, 1:800) and NFκB p105/p50 (Abcam, Cambridge, UK; E381, 1:500), using a Ventana BenchMark Ultra (Roche, Almere, the Netherlands). A total of 3 normal bone marrow biopsies were included to analyse protein expression of CXCR4 and NFκB in normal haematopoiesis. After staining, slides were scored independently by two investigators. CXCR4 expression was scored as being primarily cytoplasmic or nuclear. For NFκB, staining pattern (heterogeneous versus diffuse) was combined with strength of staining (weak versus strong staining), with a cut-off of 70%: if ≥70% of the lymphoma cells expressed diffuse and strong staining of NFκB, the case was described as “diffuse and strong”. In addition, next generation sequencing (NGS) was performed with the Ion Chef™ on the PGM™ and S5™ Systems (Thermo Fisher Scientific, Waltham, MA, USA) using an in-house NGS haematology panel, custom-designed for leukaemias and lymphomas (Table S1). The following genes were reported in this study and their variants annotated according to these transcripts: MYD88 (NM_002468), CXCR4 (NM_001008540) and CD79B (NM_001039933).

In normal bone marrow biopsies, CXCR4 showed weak nuclear staining of erythropoiesis and megakaryopoiesis (Fig 1A). No staining was seen in granulopoiesis. In contrast, NFκB showed intense cytoplasmic staining of granulopoiesis, while erythropoiesis was negative and megakaryopoiesis was either negative or showed weak cytoplasmic staining (Fig 1B).

Of the 30 LPL bone marrow cases, 29 (97%) had a MYD88 p.(L265P) mutation, 9 (30%) of which also carried a CXCR4 mutation (Tables SII and SIII). In addition, one CXCR4 mutated case had a CD79B mutation.

Seven out of 8 (88%) CXCR4-mutated cases showed a CXCR4 cytoplasmic staining pattern (P = 0.001; Fig 1C); CXCR4 staining was not interpretable in the remaining case. In contrast, the vast majority of cases without a CXCR4 mutation showed a nuclear staining pattern of CXCR4 (17 out of 21 cases: 81%; Fig 1D; Table I). Furthermore, cytoplasmic CXCR4 expression was associated with a characteristic heterogeneous, weak staining pattern of NFκB, occurring in 7 out of 10 cases with cytoplasmic CXCR4 staining (70%; P = 0.001; Fig 1E; Table I). In contrast, nuclear CXCR4 expression was associated with a diffuse and strong staining pattern of NFκB in 15 out of 16 cases with nuclear CXCR4 staining (94%; Fig 1F; Table I).

No significant correlation was detected when comparing CXCR4 mutation status with NFκB staining (P = 0.206; Table SIV).

As LPL patients with a CXCR4 mutation are less responsive to targeted treatment with BTK inhibitors, it is important to decipher the mechanism underlying this resistance in order to develop and improve (targeted) treatment options. Currently, the mechanism of this diminished response is poorly understood.

In our study, immunohistochemical expression of CXCR4 in LPL showed two dominant patterns: cytoplasmic and...