Successful Retreatment With Venetoclax in a Patient With Chronic Lymphocytic Leukemia

Ross A. Jackson1, Victoria M. Smith1, Sandrine Jayne1, Cosima Drewes2, Susanne Bens2, Reiner Siebert2, Martin J. S. Dyer1, Harriet S. Walter1

Correspondence: Martin J. S. Dyer (mjsd1@leicester.ac.uk).

The B-cell lymphoma 2 (BCL2) specific inhibitor, venetoclax, has demonstrated remarkable clinical activity in the treatment of chronic lymphocytic leukemia (CLL), alone or in combination with CD20 antibodies, and is now standard of care for frontline and relapsed/refractory disease.1,2 Venetoclax promotes rapid CLL cell death in vitro in a tumor protein 53 (TP53) independent manner and in vivo with comparable overall response irrespective of TP53 mutational status.3,4 However, recent data suggest that loss of TP53 may impair venetoclax efficacy with suboptimal doses.5 Nevertheless, some patients relapse and optimal treatment strategies after venetoclax progression remain unclear, particularly if they have also relapsed on tyrosine kinase inhibitors. Acquired resistance mechanisms to venetoclax include mutations to BCL26 in CLL and the upregulation of alternative anti-apoptotic proteins myeloid leukemia 1 (MCL1) and/or B-cell lymphoma-extra large (BCLXL)7 in models of lymphoma.7 Venetoclax retreatment has been successful in CLL where patients have taken “treatment holidays” prior to disease progression.3 However, the outcome of retreatment following acquired resistance is unknown. Recent data have shown that for some CLL patients, mechanisms of resistance to multiple inhibitors may occur independently in mutually exclusive cells,9 suggesting retreatment with previously efficacious therapies may be effective in these cases. Here, we report the case of a CLL patient successfully retreated with venetoclax following prior venetoclax relapse and intermediate therapy with a combination of 2 B-cell receptor (BCR) inhibitors (BCRi). To our knowledge, this is the first time such a case has been reported.

This study was approved by local Research Ethics Committee and the University Hospitals of Leicester Nation Health Service Trust (06/Q2501/122). Samples were obtained after written informed consent. Mononuclear cells were separated from whole blood or bone marrow using Ficoll histopaque (10771, Sigma-Aldrich). The proportion of CD19-positive B-cells was confirmed by flow cytometry using anti-human CD19-PE (Selleck Chemicals, Houston, TX) was carried out 24 hours before analysis using CellTiter-Glo viability assay.10 Immunoblot was performed as previously described11 using antibodies: mouse anti-BCL-2 (M088701-2, Dako Agilent, Hamburg), rabbit anti-BCL-XL (2762S, Cell Signaling, Beverly, MA), rabbit anti-BCL-2 (Selleck Chemicals, Houston, TX) and the University Hospitals of Leicester Nation Health Service.

Received: April 28, 2022 / Accepted: June 8, 2022

1The Ernest and Helen Scott Haematological Research Institute, Leicester Cancer Research Centre, University of Leicester, United Kingdom
2Institute of Human Genetics, Ulm University and Ulm University Medical Center, Germany

Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the European Hematology Association. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-NID), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal. HemaSphere (2022) 6:9[e752]. http://dx.doi.org/10.1097/HES.0000000000000752. Received: April 28, 2022 / Accepted: June 8, 2022

Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal. HemaSphere (2022) 6:9[e752]. http://dx.doi.org/10.1097/HES.0000000000000752. Received: April 28, 2022 / Accepted: June 8, 2022

http://dx.doi.org/10.1097/HES.0000000000000752.

HemaSphere (2022) 6:9[e752].
Figure 1. Clinical counts and in vitro analysis. (A), CT images taken before initial venetoclax treatment (i) and 14 wk later (ii) demonstrating clearance of CLL cells observed by reduction in lymphadenopathy. (B), WBC over the course of treatment. Samples used for in vitro studies are indicated with sample numbers (1–5) and times taken are indicated by dashed lines. Venetoclax treatment periods indicated in blue and BCRi indicated in green. (C), CLL cells collected at samples 1–5 (and a bone marrow sample taken at the same time as sample 4) were incubated with increasing concentrations of venetoclax for 24 h before analysis of cell viability by CellTiter-Glo assay (n = 3). (D), EC_{50} values of venetoclax treatment in samples 1–5, calculated using GraphPad Prism. (E), Rate of decrease of WBC over period of venetoclax dose escalation for both initial treatment and retreatment. (F), CLL cells collected at samples 1, 2, and 4 were incubated with increasing concentrations of A1331852 or S63845 for 24 h before analysis of cell viability by CellTiter-Glo assay (n = 2). (G), Immunoblot analysis of lysates prepared from PBMCs of samples 1, 2, and 4. BCL2 = B-cell lymphoma 2; BCLxL = B-cell lymphoma-extra large; BCRi = B-cell receptor inhibitor; BM = bone marrow; CLL = chronic lymphocytic leukemia; CT = computerised tomography; CTG = CellTiter-Glo; EC_{50} = half maximal effective concentration; MCL1 = myeloid leukemia 1; MW = molecular weight; PBMC = peripheral blood mononuclear cells; WBC = white cell count.
Figure 2. Genomic analysis of PBMC samples. (A), WES was carried out on sequential PBMC samples, with VAFs of driver mutations plotted for each sample. (B), Whole Genome View generated from CytoScan HD Array CNV analysis of sample 1 (i) and sample 2 (ii), with regions of gain indicated by upward deflections and loss by downward deflections in the weighted Log2 ratio. LOH is presented by deviations from the 3 standard lines (0, 0.5, and 1) in the BAF. (C), CNV analysis of chromosome 17 generated from WES data of sample 1 (i) sample 2 (ii) with regions of gain indicated by upward deflections and loss by downward deflections presented as a Log R ratio (a normalized measure of the total signal intensity for 2 alleles), BAF = B-allele frequency; CNV = copy number variation; LOH = loss of heterozygosity; PBMC = peripheral blood mononuclear cells; VAF = variant allele frequency; WES = whole exome sequencing.
Table S2) with rapid resolution of lymphadenopathy (Figure 1A–ii). Venetoclax sensitivity was confirmed in vitro (sample 1, half maximal effective concentration 1.4 nM) (Figure 1C and D).

The patient was treated with venetoclax for 21 months, with low-level (0.01%) disease remaining in the bone marrow, as assessed by multicolor flow cytometry. However, she subsequently progressed very rapidly in August 2015 while still on drug. Disease progression was associated with an increase in lymphocyte count from 17.7 x 10^9/L to 196.6 x 10^9/L in just 8 weeks. Clinical resistance was mirrored by an almost 1000-fold increased resistance to venetoclax in vitro (sample 2, EC_{50} 9445 nM).

She next received a combination of tirabrutinib (20 mg twice daily [BD]) and idelalisib (50 mg BD) (NCT02968563). She remained well without significant toxicity for 47 months, attaining stable partial remission. However, she developed pancytopenia due to progressive bone marrow infiltration in November 2019. At relapse to BCRi, there was no recurrence of the bulky lymphadenopathy observed prior to initial venetoclax therapy.

In vitro analysis on sample 3 taken 11 months after starting BCRi treatment, while the patient was still responding, showed a partial regain of sensitivity to venetoclax (EC_{50} 227.9 nM). Furthermore, blood and bone marrow samples taken at BCRi progression showed comparable venetoclax sensitivity as sample 1 (sample 4, EC_{50} 1.3 nM) (Figure 1C and D).

Based on these results, she was retreated with venetoclax in December 2019. Within 4 weeks, her WBC recovered with rapid clearance of CLL cells at a comparable rate to that observed initially (Figure 1E). There was a recovery of normal hematological indices and resolution of splenomegaly. Unfortunately, she died in April 2020 due to coronavirus disease 2019-associated pneumonia. A timeline of WBC count with duration of treatments is presented (Figure 1B).

To understand the causes of variable venetoclax sensitivities, WES was performed on the same CLL samples as in vitro venetoclax sensitivity analyses.

A productive unmutated IGHV rearrangement (1–69), possible driver mutations in SEF3B1, KMT2C, FANCA, and SPEN, and copy number alterations to chromosomes 4p16.3-p15.1, 5q34-q35.3, 7q21.11-q32.3, and Xq28-q28 were detected at comparable frequencies across all samples (Figure 2A and B, Suppl. Figures S1 and S2, Suppl. Tables S3 and S4).

Differences observed between samples included the variant allele frequency (VAF) of the loss-of-function TP53 R110L mutation and the size of the chromosome 17p deletion. Prior to initial venetoclax treatment (sample 1), TP53 R110L had a VAF of 42.7%. CNV analysis demonstrated a heterozygous chromosome 17p13 deletion extending across the centromere (Figure 2C-i). At venetoclax relapse, TP53 R110L was not detected by WES (sample 2). CNV analysis at this time showed a TP53 focal copy number loss, and not the previously observed arm loss, extending from base pairs (bp) 7,168,109-7,593,927 (425,818bp, hg19), which was detected in all subsequent samples (Figure 2C-ii). This likely represents the emergence of a new subclone selected for during venetoclax exposure. This was not detected by FISH as the commercial probes used (TP53/ MPO and locus specific identifier TP53) extended beyond the focal loss. Venetoclax resistance was not associated with detectable mutations in BCL2 or genes of BCL2 interacting proteins. Similar findings have been observed in the MURANO (venetoclax and rituximab in relapsed/refractory CLL, NCT02005471) trial, where they observed a lack of acquired BCL2 mutations. In addition, there were no changes in protein expression of BCL2 or other anti-apoptotic proteins (Figure 1G), nor altered sensitivity to BCLX, or MCL1 specific inhibitors (Figure 1F).

After 11 months of BCRi therapy during clinical response (sample 3), TP53 R110L became detectable again at low level (VAF of 4.2%). This coincided with the increased in vitro venetoclax sensitivity observed. These changes in VAF occurred despite observing no altered VAFs in other driver mutations, allowing a distinction between the subclonal TP53 R110L mutation and other likely clonal driver mutations (Figure 2A, Suppl. Table S3). Unexpectedly the reemergence of TP53 R110L was not accompanied by the reemergence of the 17p13 deletion. Instead, the TP53 focal copy number loss remained, suggesting greater subclonal complexity than just 2 competing subclonal populations.

At this point, no BTK inhibitor (BTKi)-associated BTK or PLCG2 mutations were detected. After another 36 months of therapy at BCRi disease progression (sample 4), BTK T474I (associated with BTKi resistance) was detectable at a VAF of 16.9%. The VAF of TP53 R110L increased to 35.0% and remained stable until time of death.

In summary, we have demonstrated that clonal evolution and genetic complexity in a patient with CLL allowed for sensitivity to venetoclax to be regained after initial venetoclax progression and subsequent prolonged BCRi therapy. The venetoclax-sensitive subclone could be characterized by WES, and its reemergence tracked throughout BCRi treatment. Retreatment with venetoclax was informed by in vitro drug testing, which may therefore have clinical utility to monitor and predict in vivo responses. We note that these observations were made during intermediate combination therapy of 2 BCRis, and it is unclear if similar clonal selection would occur with single-agent BCRi.

This study is limited to observations in just 1 case of venetoclax progression. Retrospective in vitro analysis of CLL cases treated with sequential targeted therapies may help to provide further evidence of treatment resensitization in a larger patient cohort. In addition, there was only a short follow-up of 4 months after commencing venetoclax retreatment, so it remains unclear how durable and comparable to the initial venetoclax treatment the response would have been. These data suggest that consecutive exposure to different classes of targeted agents may allow the reemergence of drug-sensitive subclones, providing rationale for retreatment with previously efficacious therapies, or the use of combination therapies early to target multiple subclones with distinct sensitivities.

ACKNOWLEDGMENTS

We would like to thank the research nurses at the Hope Clinical Trial Unit, Leicester Royal Infirmary, as well as the staff of the tumor genetics laboratories of the Universities Kiel and Ulm. This research used the ALICE High Performance Computing Facility at the University of Leicester.

AUTHOR CONTRIBUTIONS

RAJ and VMS performed research. All authors involved in data analysis and interpretation. RAJ and VMS drafted the article that was revised and approved by all authors (article writing). MJSD and HSW designed and supervised the study.

DISCLOSURES

MJSD has received research funding from Gilead Sciences, and honorarium and travel grants from AbbVie. HSW has received research funding from Gilead Sciences, and honorarium and travel grants from AbbVie. All the other authors have no conflicts of interest to disclose.

SOURCES OF FUNDING

This work was supported by funds from the Scott Waudby Trust, the Hope Against Cancer charity, the University of Leicester, the Kay Kendall Leukemia Fund, Gilead Sciences, Inc, and the Deutsche Forschungsgemeinschaft through SFB1074 (project B10).

REFERENCES

1. Fischer K, Al-Sawaf O, Bahlo J, et al. Venetoclax and obinutuzumab in patients with CLL and coexisting conditions. N Engl J Med. 2019;380:2225–2236.

2. Seymour JE, Kipp TJ, Eichhorst BF, et al. Four-year analysis of MURANO study confirms sustained benefit of time-limited venetoclax-rituximab
Venetoclax (VenR) in relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL). *Blood*. 2019;134(Supplement 1):355–355.

3. Anderson MA, Deng J, Seymour JF, et al. The BCL2 selective inhibitor venetoclax induces rapid onset apoptosis of CLL cells in patients via a TP53-independent mechanism. *Blood*. 2016;127:3215–3224.

4. Stilgenbauer S, Eichhorst B, Schetelig J, et al. Venetoclax for patients with chronic lymphocytic leukemia with 17p deletion: results from the full population of a phase II pivotal trial. *J Clin Oncol*. 2018;36:1973–1980.

5. Thijssen R, Diepstraten ST, Moujalled D, et al. Intact TP-53 function is essential for sustaining durable responses to BH3-mimetic drugs in leukemias. *Blood*. 2021;137:2721–2735.

6. Blombery P, Anderson MA, Gong JN, et al. Acquisition of the recurrent Gly101Val mutation in BCL2 confers resistance to venetoclax in patients with progressive chronic lymphocytic leukemia. *Cancer Discov*. 2019;9:342–353.

7. Tahir SK, Smith ML, Hessler P, et al. Potential mechanisms of resistance to venetoclax and strategies to circumvent it. *BMC Cancer*. 2017;17:399.

8. Thompson MC, Allan JN, Sail K, et al. Venetoclax re-treatment of chronic lymphocytic leukemia (CLL) patients after a previous venetoclax-based regimen. *Blood*. 2020;136(Supplement 1):39–41.

9. Thompson ER, Nguyen T, Kankanige Y, et al. Single-cell sequencing demonstrates complex resistance landscape in CLL and MCL treated with BTK and BCL2 inhibitors. *Blood Adv*. 2022;6:503–508.

10. Kozaki R, Vogler M, Walter HS, et al. Responses to the selective Bruton’s tyrosine kinase (BTK) inhibitor tirabrutinib (ONO/GS-4059) in diffuse large B-cell lymphoma cell lines. *Cancers (Basel)*. 2018;10:E127.

11. Smith VM, Dietz A, Henz K, et al. Specific interactions of BCL-2 family proteins mediate sensitivity to BH3-mimetics in diffuse large B-cell lymphoma. *Haematologica*. 2020;105:2150–2163.

12. Martin-Subero JJ, Harder L, Geisk S, et al. Interphase FISH assays for the detection of translocations with breakpoints in immunoglobulin light chain loci. *Int J Cancer*. 2002;98:470–474.

13. Wang B, Niu D, Lam TH, et al. Mapping the p53 transcriptome universe using p53 natural polymorphs. *Cell Death Differ*. 2014;21:521–532.

14. Seymour JF, Wu JQ, Popovic R, et al. Assessment of the clonal dynamics of acquired mutations in patients (Pts) with relapsed/refractory chronic lymphocytic leukemia (R/R CLL) treated in the randomized phase 3 Murano trial supports venetoclax-rituximab (VenR) fixed-duration combination treatment (Tx). *Blood*. 2021;138(Supplement 1):1548–1548.

15. Maddocks KJ, Ruppert AS, Lozanski G, et al. Etiology of ibrutinib therapy discontinuation and outcomes in patients with chronic lymphocytic leukemia. *JAMA Oncol*. 2015;1:80–87.

16. Letai A, Bhola P, Welm AL. Functional precision oncology: testing tumors with drugs to identify vulnerabilities and novel combinations. *Cancer Cell*. 2022;40:26–35.