LYMPHOID NEOPLASIA

BTK inhibitor therapy is effective in patients with CLL resistant to venetoclax

Victor S. Lin,[1-3,]* Thomas E. Lew,[1,3,]* Sasanka M. Handunnetti,[1] Piers Blombery,[1,2,4] Tamia Nguyen,[4] David A. Westerman,[1,2,4] Bryone J. Kuss,[5] Constantine S. Tam,[1,2,6] Andrew W. Roberts,[1-3] John F. Seymour,[1,2] and Mary Ann Anderson[1,3]

[1]Department of Clinical Haematology, Royal Melbourne Hospital and Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; [2]Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, VIC, Australia; [3]Blood Cells and Blood Cancer Division, Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia; [4]Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; [5]Department of Haematology and Genetic Pathology, Flinders University, Adelaide, SA, Australia; and [6]Department of Haematology, St Vincent's Hospital Melbourne, Fitzroy, VIC, Australia

KEY POINTS

- BTKi therapy achieves durable disease control for patients with CLL after progression on venetoclax.

- Prior remission duration ≥24 months and deep response on venetoclax are associated with longer PFS on subsequent BTKi therapy.

Highly active BTK inhibitors (BTKis) and the BCL2 inhibitor venetoclax have transformed the therapeutic landscape for chronic lymphocytic leukemia (CLL). Results of prospective clinical trials demonstrate the efficacy of venetoclax to salvage patients with disease progression on BTKis, but data on BTKi therapy after disease progression on venetoclax are limited, especially regarding durability of benefit. We retrospectively evaluated the records of 23 consecutive patients with relapsed/refractory CLL who received a BTKi (ibrutinib, n = 21; zanubrutinib, n = 2) after stopping venetoclax because of progressive disease. Median progression-free survival (PFS) and median overall survival after BTKi initiation were 34 months (range, <1 to 49) and 42 months (range, 2-49), respectively. Prior remission duration ≥24 months and attainment of complete remission or undetectable measurable residual disease on venetoclax were associated with longer PFS after BTKi salvage ($P = .044$ and $P = .029$, respectively). BTKi therapy achieved durable benefit for patients with the _BCL2_ Gly101Val venetoclax resistance mutation (estimated 24-month PFS, 69%). At a median survivor follow-up of 33 months (range, 2-53), 11 patients remained on BTKi and 12 had stopped therapy because of disease progression (n = 8) or toxicity (n = 4). Our findings indicate that BTKi therapy can provide durable CLL control after disease progression on venetoclax. (_Blood_. 2020;135(25):2266-2270)

Introduction

The BCL2 inhibitor venetoclax[1] is effective in treating relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL), including disease harboring traditional adverse prognostic factors.[2-4] Venetoclax-based therapy frequently induces deep remissions, including undetectable measurable residual disease (uMRD),[5] but progressive disease (PD) is common with longer-term follow-up.[6] Mechanisms of venetoclax resistance include acquisition of _BCL2_ mutations (eg, Gly101Val[7]) and upregulation of other prosurvival proteins through microenvironment signaling, which may be interrupted by B-cell receptor inhibitors.[8]

The BTK inhibitor (BTKi) ibrutinib achieves durable disease control in treatment-naïve and R/R CLL, including in high-risk subgroups.[9,10] Acalabrutinib (ACP-196) and zanubrutinib (BGB-3111) are next-generation BTKis with greater selectivity for BTK and similarly high response rates.[11,12] Although prospective studies have demonstrated the efficacy of venetoclax postibrutinib,[13] limited data exist to inform choice of therapy postvenetoclax.[14-18] Here, we report the characteristics and outcomes of patients with R/R CLL who received BTKi after venetoclax discontinuation for PD.

Methods

Study design

We retrospectively reviewed data from 23 consecutive patients with R/R CLL, treated at the Royal Melbourne Hospital and Peter MacCallum Cancer Centre from September 2011 to September 2019, who received BTKi therapy for CLL after disease progression on venetoclax within 1 of 4 trials (registered at www.clinicaltrials.gov as #NCT01328626,[2] #NCT01682616,[4] #NCT01889186,[3] and #NCT02980731[19]; supplemental Table 1, available on the _Blood_ Web site). These patients had been treated with venetoclax at 100 to 600 mg per day (majority, 400 mg per day), with 7 receiving concurrent rituximab. Disease progression occurred during continuous venetoclax therapy in 22 patients and during venetoclax retreatment in 1 patient who had relapsed after interrupting therapy in complete remission (CR). Progressive CLL after venetoclax was treated

Table 1. Clinicopathological variables associated with PFS after BTKi initiation

Pre-BTKi variable	No. of patients	Association with PFS after BTKi initiation	
		HR (95% CI)	Log-rank *P*
Age ≥65 y		2.03 (0.23-17.7)	.515
Y	20		
N	3		
≥4 prior lines of therapy		0.38 (0.11-1.40)	.132
Y	17		
N	6		
Fluradabine refractory		0.60 (0.18-2.04)	.416
Y	15		
N	8		
Bulky adenopathy (>5 cm)		NA	NA
Y	1		
N	19		
***NOTCH1* mutation**		0.67 (0.08-5.52)	.698
Y	3		
N	16		
IgHV unmutated		NA	NA
Y	13		
N	1		
del(17p) and/or *TP53* mutation		2.24 (0.42-11.98)	.332
Y	16		
N	5		
CK		0.69 (0.17-2.81)	.600
Y	13		
N	6		
BTKi post-RT*		0.92 (0.25-3.43)	.898
Y	5		
N	18		
CR or uMRD to venetoclax		0.15 (0.02-1.08)	**.029**
Y	8		
N	15		
≥24 mo on venetoclax		0.31 (0.09-1.03)	**.044**
Y	14		
N	9		
***BCL2* G101V**		0.24 (0.05-1.16)	.054
Y	8		
N	11		

Bold font indicates significance.

CI, confidence interval; CR, complete response; HR, hazard ratio; IgHV, immunoglobulin heavy chain variable region; NA, not applicable; RT, Richter transformation.

*Patients receiving BTKi therapy for progressive CLL after treatment for RT on venetoclax with salvage chemotherapy ± autologous SCT.

with ibrutinib at 420 mg per day (n = 21) or zanubrutinib at 160 mg twice per day (n = 2).

Study patients

Pre-BTKi patient characteristics were recorded, including age, prior therapies, fludarabine refractoriness (failure to respond or PD ≤6 months after fludarabine-based therapy[20]), bulky adenopathy (nodes >5 cm on computed tomography[21]), del(17p), *TP53* mutation, and complex karyotype (CK; ≥3 clonal chromosomal aberrations on conventional metaphase karyotype[22,23]). *TP53* variants were detected with a sensitivity of 5% variant allele frequency using targeted next-generation sequencing of all coding exons of *TP53* (NM_000546.5) and assessed for pathogenicity using curation sources including the Genome Aggregation Database, Catalogue of Somatic Mutations in Cancer, and the International Agency for Research on Cancer (IARC) *TP53* Database. *BCL2* Gly101Val mutation was detected using droplet digital polymerase chain reaction.[7] uMRD was defined as detection of <1 CLL cell per 10^4 leukocytes in peripheral blood or bone marrow using multiparameter flow cytometry, analyzing >200 000 leukocytes.[24]

Statistical analysis

Kaplan-Meier method was used to estimate progression-free survival (PFS) and overall survival from BTKi initiation. Patients with planned interruption of BTKi treatment preceding stem cell transplantation (SCT) without prior PD were censored at BTKi cessation. Patients without an event were censored at date of last follow-up or data cutoff (30 September 2019) when last follow-up occurred later. Associations between clinicopathological variables and outcomes were analyzed using the Cox proportional hazards model to calculate hazard ratios with α = 0.05. All data were analyzed using STATA software (verison 14.1 for Mac) and GraphPad Prism (version 7.0b for Mac).

Results and discussion

Pre-BTKi cohort characteristics are summarized in Table 1. Median age at BTKi initiation was 72 years (range, 50-89). Median time from CLL diagnosis to initiation of venetoclax and BTKi among the 19 patients for whom data were available was 8 years (range, 3-19) and 11 years (range, 5-22), respectively. Median number of prior therapies was 4 (range, 2-9); 21 patients (91%) had received fludarabine plus cyclophosphamide plus rituximab. No patient had been previously treated with B-cell receptor inhibitors. A majority of patients had disease harboring *TP53* abnormalities (76%) or CK (68%) pre-BTKi. All lesions had been documented prevenetoclax except 2 new cases of *TP53* abnormalities and 4 cases of CK (previously noncomplex, n = 2; previously untested, n = 2) detected after progression on venetoclax (Figure 1A).

Median duration of remission to venetoclax pre-BTKi was 29 months (range, 1-59). Twenty-one patients (91%) had attained objective responses to venetoclax (PR, n = 15; CR, n = 6), with 4 (17%) achieving uMRD. All patients had ceased venetoclax because of PD (progressive CLL, n = 18; RT, n = 5 [Hodgkin variant, n = 2; diffuse large B-cell lymphoma, n = 3]). Each patient with RT was first treated with salvage chemotherapy (PR, n = 1; CR, n = 4), including autologous SCT (n = 2), and achieved durable control of transformed disease, with
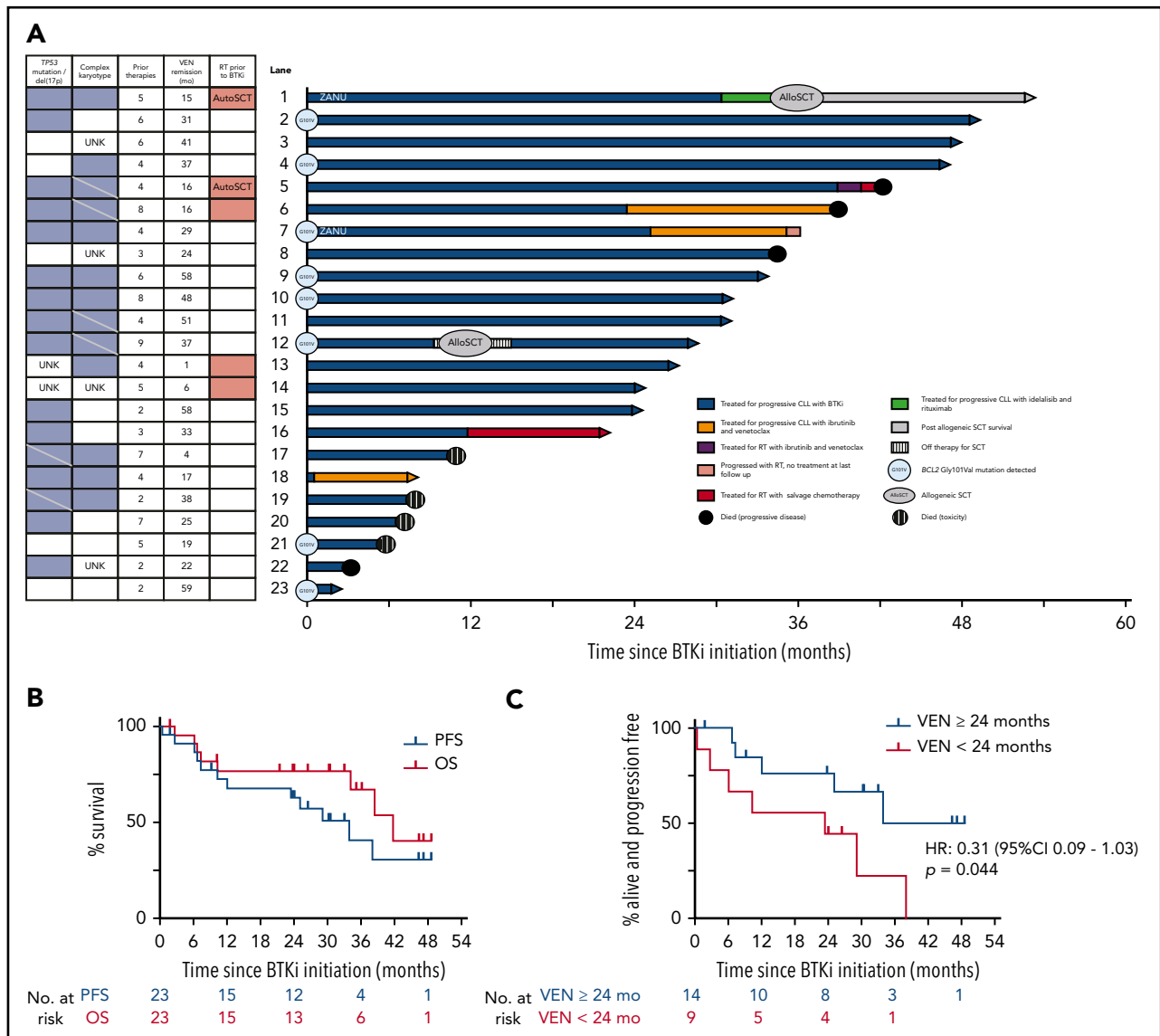
Figure 1. Outcomes of BTKi therapy for CLL after progression on venetoclax (VEN). (A) Individual patient characteristics and timelines of outcomes and treatments after BTKi initiation. Arrows at the ends of lanes indicate ongoing response to treatment at last follow-up, circles indicate death, and other treatments have specific symbols as indicated. The columns on the left indicate relevant pre-BTKi features. Gray fill indicates presence of a variable; white fill indicates absence. Where a *TP53* abnormality or CK was first detected after progression on VEN, the cells have a diagonal line. In all other cases, the CLL patient had these genetic features before treatment with VEN. Patients who were treated for RT before BTKi are denoted by a red fill. Ibrutinib was the BTKi used in all patients except where zanubrutinib (ZANU) is indicated. Histology, treatment, and response of RT were as follows: lanes 1 and 5: diffuse large B-cell lymphoma (DLBCL): rituximab, ifosfamide, carboplatin, and etoposide followed by autologous (auto) SCT (CR in both cases); lane 6: DLBCL: rituximab, methotrexate, vincristine, and procarbazine followed by radiotherapy (partial remission [PR]); lane 13: Hodgkin variant: doxorubicin, bleomycin, vinblastine, and dacarbazine (CR); and lane 14: Hodgkin variant: cyclophosphamide, doxorubicin, etoposide, and prednisolone followed by radiotherapy (CR). (B) PFS (blue) and overall survival (OS) on BTKi (red) from initiation of BTKi after disease progression on VEN. In both, patient data were censored at allogeneic (allo) SCT if this occurred in first response to BTKi. (C) PFS on BTKi stratified by prior remission to VEN ≥24 (blue) or <24 months (red). HR, hazard ratio; UNK, unknown.

subsequent CLL progression triggering BTKi initiation. *BCL2* Gly101Val mutation was detected at progression in 8 (42%) of 19 patients tested (Figure 1A).

Survival and subsequent treatments after BTKi initiation are shown in Figure 1A. Median interval between venetoclax cessation and BTKi initiation was 1 month (range, <1 to 11) for patients with progressive CLL and 25 months (range, 18-39) for patients initially treated for RT. All 20 patients for whom treatment indication was known met International Workshop on Chronic Lymphocytic Leukemia criteria.[25] Twenty patients (91%) achieved objective responses to BTKi (PR/PR except for

lymphocytosis, n = 16; CR, n = 4). All CRs met clinical International Workshop on Chronic Lymphocytic Leukemia criteria[25]; 3 of 4 were confirmed with computed tomography and bone marrow assessment. One patient achieved PR after early addition of concurrent venetoclax with insufficient time on BTKi monotherapy to evaluate response. Median PFS after BTKi initiation was 34 months (95% CI, 10.4 to undefined; range, <1 to 49; Figure 1B). At a median survivor follow-up of 33 months (range, 2-53), 11 patients (48%) remained on BTKis, including 1 who interrupted ibrutinib therapy for allogeneic SCT (Figure 1A lane 12). Twelve (52%) had stopped BTKis because of PD (n = 8 [RT, n = 2 (recurrent, n = 1; de novo, n = 1); CLL, n = 6]) or

toxicity (n = 4 [sepsis, n = 1; pneumonia, n = 1; intracranial hemorrhage, n = 2 (occurring in context of myelodysplasia and severe thrombocytopenia treated with azacitidine, n = 1; acute hemorrhage into newly identified cerebral tumors of undetermined histology while on ibrutinib, n = 1)]). Median overall survival after BTKi initiation was 42 months (95% CI, 34.2 to undefined; Figure 1B). There were 8 deaths in total resulting from PD (n = 4) or toxicity (n = 4). Two patients derived clinical benefit from venetoclax-ibrutinib combination after sequentially progressing on single-agent venetoclax and then single-agent ibrutinib for 15 and 10 months, respectively (Figure 1A, lanes 6 and 7).

Univariate analyses were performed to explore putative clinicopathological variables associated with PFS after BTKi initiation (Table 1). Patients achieving remission with venetoclax ≥24 months had longer PFS on subsequent BTKi of borderline significance (median PFS for remission ≥24 vs <24 months, 34 vs 23 months; P = .044; hazard ratio, 0.31; 95% CI, 0.09 -1.03; Figure 1C). All 8 patients who achieved CR/uMRD with venetoclax were in the subgroup achieving prolonged remission with venetoclax, and a similar association with prolonged PFS on BTKi was seen (P = .029). All 4 patients with prior uMRD on venetoclax remained on BTKi at a median of 21 months (range, 2-46). These univariate associations require cautious interpretation, given the modest patient numbers, and require confirmation in future analyses of larger cohorts. Among the 8 patients in whom the BCL2 Gly101Val mutation was identified, median PFS has not been reached at a median survivor follow-up of 33 months (range, 2-49; estimated 24-month PFS, 69%; supplemental Figure 1); median duration of remission with venetoclax was 37 months (range, 19-59).

In summary, these data indicate that BTKi therapy is an effective therapeutic option for patients with heavily pretreated, high-risk CLL after progression on venetoclax, including patients with prior RT in remission or CLL harboring the BCL2 Gly101Val mutation. We await further prospective trial data to validate therapeutic sequencing in the novel agent era.

Acknowledgment

Authorship

Contribution: J.F.S., A.W.R., C.S.T., and M.A.A. conceived of the project and designed the study; J.F.S., A.W.R., C.S.T., M.A.A., S.M.H., and B.J.K. were responsible for patient care; D.A.W. contributed flow cytometry and molecular data; V.S.L., T.E.L., and S.M.H. collected the data; V.S.L. and T.E.L. analyzed the data; T.N. and P.B. performed and analyzed genomic data; V.S.L. and T.E.L. wrote the first version of the manuscript; and all authors reviewed the data and contributed to critical revision of the manuscript.

Conflict-of-interest disclosure: A.W.R., M.A.A., T.E.L., and V.S.L. are employees of the Walter and Eliza Hall Institute of Medical Research, which receives milestone and royalty payments related to venetoclax. A.W.R., M.A.A., and T.E.L. are recipients of a share in royalty payments paid to the Walter and Eliza Hall Institute of Medical Research. S.M.H. has received honoraria from Gilead and nonfinancial assistance from AbbVie. C.S.T. has received honoraria and research funding from AbbVie and Janssen and honoraria from BeiGene. A.W.R. has received research funding from AbbVie, Genentech, Servier, Janssen, and BeiGene. J.F.S. receives research funding from AbbVie, Genentech, Celgene, and Janssen and is an advisory board member for and has received honoraria from AbbVie, Acerta, Celgene, Genentech, Janssen, Roche, Sunesis, and Takeda. M.A.A. has received honoraria from AbbVie and CSL Behring. T.E.L. has received honoraria from AbbVie. The remaining authors declare no competing financial interests.

ORCID profiles: V.S.L., 0000-0003-3432-0785; T.E.L., 0000-0001-6804-2432; S.M.H., 0000-0001-8790-4675; P.B., 0000-0002-9902-0022; B.J.K., 0000-0002-5811-1331; C.S.T., 0000-0002-9759-5017; A.W.R., 0000-0002-7341-5720; J.F.S., 0000-0003-2188-6835; M.A.A., 0000-0002-3515-0215.

Correspondence: Mary Ann Anderson, Department of Clinical Haematology, Royal Melbourne Hospital and Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; e-mail: manderson@wehi.edu.au.

Footnotes

REFERENCES

1. Souers AJ, Leverson JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013;19(2):202-208.

2. Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. N Engl J Med. 2016;374(4):311-322.

3. 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(6):768-778.

4. 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(2):230-240.

5. Kater AP, Seymour JF, Hillmen P, et al. Fixed duration of venetoclax-rituximab in relapsed/refractory chronic lymphocytic leukemia eradicates minimal residual disease and prolongs survival: post-treatment follow-up of the MURANO phase III study. J Clin Oncol. 2019;37(4):269-277.

6. Roberts AW, Ma S, Kipps TJ, et al. Efficacy of venetoclax in relapsed chronic lymphocytic leukemia is influenced by disease and response variables. Blood. 2019;134(2):111-122.

7. 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(3):342-353.

8. Bojarczuk K, Sasi BK, Gobessi S, et al. BCR signaling inhibitors differ in their ability to overcome Mcl-1-mediated resistance of CLL B cells to ABT-199. Blood. 2016;127(25):3192-3201.

9. Woyach JA, Ruppert AS, Heerema NA, et al. Ibrutinib regimens versus chemoimmunotherapy in older patients with untreated CLL. N Engl J Med. 2018;379(26):2517-2528.

10. O'Brien S, Furman RR, Coutre S, et al. Single-agent ibrutinib in treatment-naïve and relapsed/refractory chronic lymphocytic leukemia: a 5-year experience. Blood. 2018; 131(17):1910-1919.

11. Tam CS, Trotman J, Opat S, et al. Phase 1 study of the selective BTK inhibitor zanubrutinib in B-cell malignancies and safety and efficacy evaluation in CLL. *Blood*. 2019; 134(11):851-859.

12. Byrd JC, Harrington B, O'Brien S, et al. Acalabrutinib (ACP-196) in relapsed chronic lymphocytic leukemia. *N Engl J Med*. 2016; 374(4):323-332.

13. Jones JA, Mato AR, Wierda WG, et al. Venetoclax for chronic lymphocytic leukaemia progressing after ibrutinib: an interim analysis of a multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2018;19(1):65-75.

14. Seymour JF, Kipps TJ, Eichhorst BF, et al. Four-year analysis of Murano study confirms sustained benefit of time-limited venetoclax-rituximab (VenR) in relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL) [abstract]. *Blood*. 2019;134(suppl 1). Abstract 355.

15. Anderson MA, Tam C, Lew TE, et al. Clinicopathological features and outcomes of progression of CLL on the BCL2 inhibitor venetoclax. *Blood*. 2017;129(25):3362-3370.

16. Mato AR, Thompson M, Allan JN, et al. Real-world outcomes and management strategies for venetoclax-treated chronic lymphocytic leukemia patients in the United States. *Haematologica*. 2018;103(9):1511-1517.

17. Mato AR, Roeker LE, Eyre TA, et al. Efficacy of therapies following venetoclax discontinuation in CLL: focus on B-cell receptor signal transduction inhibitors and cellular therapies [abstract]. *Blood*. 2019;134(suppl 1). Abstract 502.

18. Brown JR, Davids MS, Chang JE, et al. Outcomes of ibrutinib (Ibr) therapy in ibr-naïve patients (pts) with chronic lymphocytic leukemia (CLL) progressing after venetoclax (Ven) [abstract]. *Blood*. 2019;134(suppl 1). Abstract 4320.

19. Cochrane T, Chagorova T, Robak T, et al. Venetoclax improves quality of life for patients with relapsed/refractory chronic lymphocytic leukemia [abstract]. *Blood*. 2018;132(suppl 1). Abstract 4858.

20. Keating MJ, Flinn I, Jain V, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. *Blood*. 2002;99(10): 3554-3561.

21. Tam CS, O'Brien S, Wierda W, et al. Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. *Blood*. 2008; 112(4):975-980.

22. Mayr C, Speicher MR, Kofler DM, et al. Chromosomal translocations are associated with poor prognosis in chronic lymphocytic leukemia. *Blood*. 2006;107(2):742-751.

23. Haferlach C, Dicker F, Schnittger S, Kern W, Haferlach T. Comprehensive genetic characterization of CLL: a study on 506 cases analysed with chromosome banding analysis, interphase FISH, IgV(H) status and immunophenotyping. *Leukemia*. 2007;21(12): 2442-2451.

24. Rawstron AC, Villamor N, Ritgen M, et al. International standardized approach for flow cytometric residual disease monitoring in chronic lymphocytic leukaemia. *Leukemia*. 2007;21(5):956-964.

25. Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*. 2018; 131(25):2745-2760.