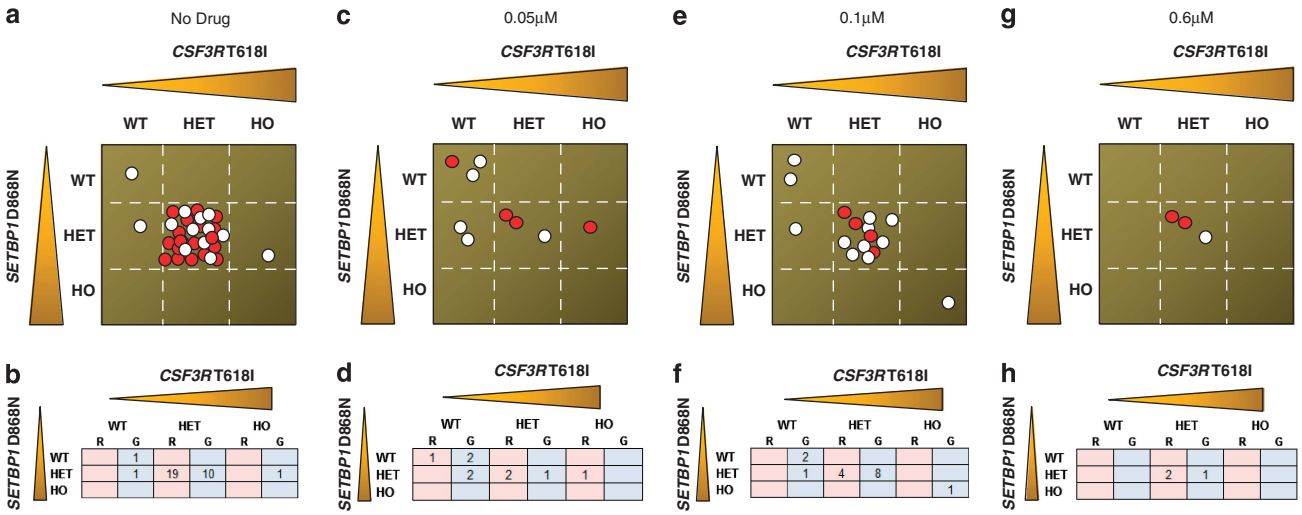

Figure 4. Summary of clonal distribution of *CSF3R* and *SETBP1* mutations in both BFU-E (R) and CFU-GM (G) and mutational allele contribution of both *CSF3R*T618I and *SETBP1*D868N mutations (**a**, **b**: no drug; **c**, **d**: 0.05 μM; **e**, **f**: 0.1 μM; **g**, **h**: 0.6 μM).

Most recent blood count showed a WBC of 107.8×10^{-9}/l and platelet count of 88×10^{-9}/l.

In the current report, we describe a double-mutated CNL patient (*CSF3R*T618I and *SETBP1*D868N) who was refractory to the treatment with both ruxolitinib and hydroxyurea. It is currently unknown if the coexpression of *SETBP1* in this *CSF3R*T618I-mutated patient with CNL contributed to the ineffectiveness of JAK inhibitor therapy both *in vivo* and *in vitro*. At least in this particular patient, we observed myeloid cell restriction of the clone and coexpression of both *CSF3R*T618I and *SETBP1*D868N mutations in erythroid and granulocytic cells; the latter antedated the former in order of acquisition, but it would be inappropriate to make any conclusions based on a single patient study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

TL Lasho[1], A Mims[2], MA Elliott[1], C Finke[1], A Pardanani[1] and A Tefferi[1]
[1]*Division of Hematology, Department of Internal Medicine, Mayo Clinic, Rochester, MN, USA and*
[2]*Division of Hematology and Oncology, Medical University of South Carolina, Charleston, SC, USA*
E-mail: tefferi.ayalew@mayo.edu

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Impact of targeted therapy on outcome of chronic lymphocytic leukemia patients with relapsed del(17p13.1) karyotype at a single center

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Chronic lymphocytic leukemia (CLL) patients with del(17p13.1) exhibit short survival once disease progression necessitates therapy and respond poorly to traditional treatments compared with other cytogenetic subgroups.[1] Poor outcome associated with del(17p13.1) is at least partially linked to malfunction of the tumor suppressor gene TP53 (located on 17p[ref. 2]), which repairs apoptosis induced by chemotherapy.[3–5] Therefore, despite general advances in CLL therapy with chemoimmunotherapy, progress in this subgroup has been limited. Current guidelines suggest allogeneic stem cell transplant as part of initial therapy,[6] as salvage treatment for del(17p13.1) portends an even graver

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prognosis. Recently, novel therapeutic agents have demonstrated promising clinical activity in del(17p13.1) patients, specifically, cyclin-dependent kinase inbibitors (CDKis; flavopiridol and dinaciclib) and a Bruton's tyrosine kinase inhibitor (BTKi; ibrutinib).[7–9] To elucidate the potential impact of CDKi and BTKi in this population, we examined outcomes of consecutive relapsed/refractory del(17p13.1) patients seen at Ohio State University (OSU), who received salvage therapy with one of these agents or alternative therapy. Our data demonstrate significant impact of both classes of these agents on outcomes of del(17p13.1) patients.

We examined 174 consecutive relapsed/refractory del(17p13.1) CLL patients for outcome following initial salvage treatment given at OSU (OSUTx1) from 2002 to 2013. Patients were treated on previously reported clinical trials or received standard-of-care treatment according to NCCN guidelines. Patients provided informed consent for data collection through the Institutional Review Board-approved study OSU-0025. Stimulated cytogenetic and FISH analyses were performed on blood or bone marrow samples (previously described[10,11]). FISH analyses probed for the chromosome 12 centromere, ATM (11q22.3), D13S319 (13q14.3) and TP53 (17p13.1) (Abbott Molecular, Des Plaines, IL, USA). Karyotypes with $\geqslant 3$ independent aberrations were defined as complex.[12]

Response was assessed by IWCLL 2008 Criteria.[13] Progression-free survival (PFS) was calculated from date of OSUTx1 until progression/death, censoring patients at date of second institutional treatment prior to progression or last contact, if alive and progression-free. Overall survival (OS) was calculated from the date of OSUTx1 until the date of death. Patients who underwent transplant or later ibrutinib (IB) were censored at that time; other patients were censored at last follow up or administratively censored at 48 months as extended follow-up data were not available in those receiving IB and most events occurred in the CDKi and other (O; included both standard and investigational therapies) group within that timeframe. Logistic regression or proportional hazards models evaluated the impact of treatment group on outcome, controlling for other prognostic variables ($P<0.05$), where a multiple imputation technique estimated missing data and combined results for 10 datasets.[14] Variables other than treatment group considered for inclusion in the multivariable models were age, sex, Rai Stage, ECOG performance status (PS), number of prior therapies, white blood cell count (WBC), creatinine, albumin, lactate dehydrogenase levels, percentage of cells with del(17p13.1) and concurrent presence of del(11q22.3), del(13q14.3) or complex karyotype (CK).

At OSUTx1, 16% ($n=27$), 33% ($n=58$) and 51% ($n=89$) of patients received IB-based therapy, CDKi-based therapy or other therapies (O; includes standard and investigational therapies), respectively. Clinical and molecular characteristics were not

Table 1. Pretreatment characteristics of chronic lymphocytic leukemia patients with del(17p) karyotype evaluated at the Ohio State University

Characteristic	*Overall,* N = *174*	*Ibrutinib,* N = *27*	*CDKi,* N = *58*	*Other,* N = *89*	P[a]
Median age (years)	63	64	63	63	0.60
Range	39–83	50–77	39–81	42–83	
Female sex, no. (%)	60 (34)	8 (30)	16 (28)	36 (40)	0.25
White race, no. (%)	160 (93)	25 (93)	55 (95)	80 (92)	0.85
Unknown	2	0	0	2	
Rai stage, no. (%)					1.00
0/I/II	49 (29)	8 (30)	17 (29)	24 (28)	
III/IV	121 (71)	19 (70)	41 (71)	61 (72)	
Unknown	4	0	0	4	
ECOG PS, no. (%)					0.12
0/1	150 (89)	26 (96)	53 (93)	71 (84)	
2/3	19 (11)	1 (4)	4 (7)	14 (16)	
Unknown	5	0	1	4	
Prior therapies $\geqslant$ 3, no. (%)	93 (53)	19 (70)	37 (64)	37 (42)	0.005
Median prior treatments, no.	3	4	3	2	0.001
Range	1–10	1–9	1–10	1–7	
Median WBC count, $\times 10^9$/l	17.9	18.8	12.1	25.5	0.04
Range	0.4–289.1	1.3–289.1	1.2–144.5	0.4–287.0	
Unknown	7	0	0	7	
Median creatinine, mg/dl	0.95	1.06	0.94	0.96	0.09
Range	0.47–2.70	0.47–2.09	0.47–1.76	0.50–2.70	
Unknown	13	0	0	13	
Median albumin, g/dl	3.8	3.8	3.9	3.8	0.80
Range	1.9–5.0	2.6–4.8	2.7–4.5	1.9–5.0	
Unknown	18	0	1	17	
Median LDH[b], U/l	221	200	221	235	0.11
Range	78–1091	120–1091	97–687	78–1007	
Unknown	17	0	0	17	
Median 17p, %	74	74	70	77	0.44
Range	6–100	7–99	6–97	6–100	
FISH[c], no. (%)					
Del(11q)	36 (21)	8 (30)	15 (26)	13 (15)	0.10
Del(13q)	100 (57)	16 (59)	34 (59)	50 (56)	0.93
Trisomy 12	28 (16)	6 (22)	9 (16)	13 (15)	0.66
CK, no. (%)	121 (70)	22 (81)	38 (66)	61 (69)	0.32

Abbreviations: CK, complex karyotype; LDH, lactate dehydrogenase; PS, performance status; WBC, white blood cell. [a]*P*-values result from testing the association between treatment group and categoric or continuous variables, respectively, using Fisher's exact or the non-parametric Kruskal–Wallis test. [b]Upper limit of normal LDH is 190 U/l. [c]Aberrations are not mutually exclusive.

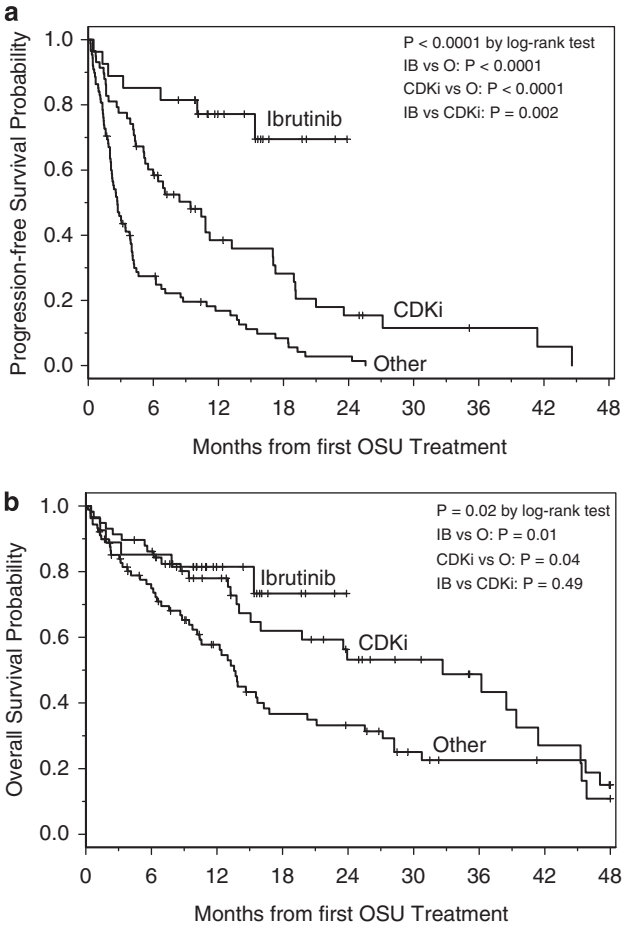

Figure 1. (**a**) Kaplan–Meier curves of PFS and (**b**) OS for relapsed/refractory CLL patients with del(17p) karyotype treated with either ibrutinib-based regimens, CDKi-based regimens or all other regimens. CDKi, cyclin-dependent kinase inhibitor; IB, ibrutinib; O, other therapies; OS, overall survival; OSU, Ohio State University; PFS, progression-free survival.

statistically different across groups except the IB and CDKi groups that had more patients with $\geqslant 3$ prior therapies (70% in IB versus 64% in CDKi versus 42% in O), and as expected, the median WBC was the lowest in the CDKi group as many CDKi clinical trials prohibited WBC $>200\,K/\mu l$ to prevent tumor lysis syndrome[15] (Table 1). Overall response to OSUTx1 significantly differed among groups ($P<0.01$), where 56%, 45% and 24% of patients in the IB, CDKi and O groups, respectively, achieved at least a partial response (PR), and 85%, 64% and 53% achieved at least stable disease (SD). Likewise, PFS was significantly extended with IB and CDKi compared with O ($P<0.0001$), and PFS was longer with IB compared with CDKi ($P=0.002$); 12-month estimates were 77% (95% confidence interval (CI) = 0.56–0.89), 38% (95% CI = 0.25–0.52), and 17% (95% CI = 0.10–0.26) in the IB, CDKi and O groups, respectively (Figure 1a). OS was also significantly extended with IB and CDKi compared with O ($P=0.01$ and $P=0.04$, respectively), having 12-month estimates of 81% (95% CI = 0.61–0.92), 78% (95% CI = 0.64–0.87) and 58% (95%CI = 0.46–0.68), although by 48 months, estimates for CDKi and O were similarly low (Figure 1b). With a 12-month median follow-up, no large differences in OS between IB and CDKi were yet observed ($P=0.49$).

In a multivariable analysis for response, treatment group was significantly associated with achieving at least PR ($P=0.0005$) independent of the number of prior therapies ($\geqslant 3$ versus <3 prior therapies: odds ratio (OR) = 0.47 (95% CI = 0.23–0.96; $P=0.04$)), and presence of del(13q) (yes versus no: OR = 0.45 (95%CI = 0.23–0.87; $P=0.02$)). The odds of response were higher with IB and CDKi compared with O, but not significantly different between IB and CDKi (all pairwise OR: IB versus O = 5.66 (95% CI = 2.12–15.08); CDKi versus O = 3.38 (95% CI = 1.57–7.31); IB versus CDKi = 1.67 (95% CI = 0.65–4.32)). In a multivariable analysis for PFS, treatment group was significantly associated with PFS ($P<0.0001$) independent of the number of prior therapies ($\geqslant 3$ versus <3 prior therapies: hazard ratio (HR) = 1.55 (95%CI = 1.08–2.24; $P=0.02$)), albumin (each 1 g/dl increase: HR = 0.56 (95%CI = 0.37–0.85; $P=0.007$)), and CK (yes versus no: HR = 1.65 (95%CI = 1.12–2.43; $P=0.01$)). The risk of progression/death decreased by 90% and 60%, respectively, with IB or CDKi compared with O, with a significantly larger decrease in risk with IB compared with CDKi (HR: IB versus O = 0.10 (95%CI = 0.04–0.22); CDKi versus O = 0.40 (95%CI = 0.27–0.60); IB versus CDKi = 0.24 (95%CI = 0.11–0.54)). In a multivariable analysis for OS, treatment group was significantly associated with OS ($P=0.003$) independent of the number of prior therapies ($\geqslant 3$ versus <3 prior therapies: HR = 1.73 (95%CI = 1.06–2.82; $P=0.03$)), albumin (each 1 g/dl increase: HR = 0.33 (95%CI = 0.19–0.56; $P<0.0001$)), CK (yes versus no: HR = 1.94 (95%CI = 1.18–3.19; $P=0.01$)), ECOG PS ($\geqslant 1$ versus 0: HR = 1.93 (95%CI–1.07-3.48; $P=0.03$)), and WBC (each 50×10^9/l increase: HR = 0.82 (95%CI = 0.68–0.99; $P=0.04$)). Risk of death decreased by 73% and 47%, respectively, with IB and CDKi compared with O, with no significant difference between IB and CDKi (HR: IB versus O = 0.27 (95%CI = 0.11–0.66); CDKi versus O = 0.53 (95%CI = 0.31–0.89); IB versus CDKi = 0.52 (95%CI = 0.21-1.29)). Notably, age did not correlate with response or PFS/OS.

Herein, we describe the largest cohort of relapsed/refractory del(17p13.1) CLL patients treated at a single institution with specific attention to two promising new classes of agents that have demonstrated activity in this cohort. These data demonstrate that del(17p13.1) patients treated with IB- or CDKi-based regimens have improved response, PFS and OS, compared with similar patients receiving alternative investigational or traditional therapies. Further, ibrutinib significantly increased response rates and extended PFS compared with treatment with CDKi. Difference in response rates between these two treatment groups became more pronounced when patients who achieved at least SD were included, as standard response criteria[13] do not fully capture the clinical benefit achieved by patients receiving ibrutinib, which can cause persistent lymphocytosis.[7]

Notably, a higher proportion of patients with $\geqslant 3$ prior therapies were treated in the IB and CDKi groups. Historically, heavily pre-treated patients are less likely to respond to therapy and may be excluded from clinical trials. In multivariable models, it was also noted that these patients were less likely to respond and had higher risk of progression/death; however, the relative benefit of treatment with IB and CDKi was irrespective of the number of prior therapies. For example, in patients who had received <3 prior therapies, response rates (at least PR) were 75%, 52% and 29% with IB, CDKi and O, respectively, while response rates in patients with $\geqslant 3$ prior treatments were 47%, 41% and 16%.

Although multivariable analyses were performed to adjust for potentially important variables, our analyses are limited by the retrospective nature of the study and could be confounded by unmeasured baseline differences in the patients who enrolled on a clinical trial versus those who were given standard therapy and differences in the enrollment criteria between trials. Factors previously associated with poor CLL patient survival, such as un-mutated IgVH mutational status and elevated β2-microglobulin levels, were not included in our analyses as these were only available for a small proportion of the patients. However, an over-representation of patients with these risk factors in one of the groups could contribute to decreased survival in that group. As the treatments were given over a period of 10 years, an improvement in supportive care may contribute somewhat to improved outcomes in patients on the most recent clinical trials.

Additionally, our OS analysis is limited by a shorter follow-up period in the IB group (median: 12.5 months). Regardless, OS is currently significantly longer with IB compared with O, and if the trend continues, may show a significant improvement in OS with IB compared with CDKi once follow-up matures. OS estimates in the CDKi and O groups become similar at ~4 years following therapy despite significant improvement in response rate with CDKi, indicating better initial clearance of the disease and short-term survival but not necessarily long-term survival.

In summary, treatment of relapsed del(17p13.1) CLL patients with IB and CDKi at OSU have demonstrated improved response, PFS and OS when compared with patients treated with other therapies. Future efforts should focus on continued prospective investigation of these effective agents in attempt to further improve the outcome of these patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors critically revised the manuscript and approved the final submitted version. DS and AR acquired, analyzed and interpreted data and drafted the manuscript. JJ, JW, KM, SJ, LA, JF, MG, GL, AJ, NM and NH contributed to acquisition of data. NH acquired and analyzed cytogenetic data. DS and JB developed the concept for the study.

DM Stephens[1,2], AS Ruppert[2], JA Jones[1,2], J Woyach[1,2], K Maddocks[1,2], SM Jaglowski[1,2], LA Andritsos[1,2], JM Flynn[1,2], MR Grever[1,2], G Lozanski[4], AJ Johnson[1,2,5], N Muthusamy[1,2], NA Heerema[2,3] and JC Byrd[1,2,5]

[1]*Division of Hematology, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA;*
[2]*Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA;*
[3]*Division of Cytogenetics, Department of Pathology, The Ohio State University, Columbus, OH, USA;*
[4]*Division of Hematopathology, Department of Pathology, The Ohio State University, Columbus, OH, USA and*
[5]*Division of Medicinal Chemistry, College of Pharmacy, The Ohio State University, Columbus, OH, USA*
E-mail: john.byrd@osumc.edu

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Colocalization of BCL2-positive and -negative follicular lymphoma

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Composite lymphomas are regularly reported and several combinations of lymphoma cell proliferations can be observed. Follicular lymphoma *in situ* (FLIS) was first recognized by Elaine Jaffe and her group in 2002.[1] In their report, they identified 23 lymph node biopsies with focal germinal centres containing centrocytes staining strongly for BCL2, whereas most of the remaining lymph nodes showed BCL2-negative follicular hyperplasia.[1] Of interest, in the series, two cases of FLIS were associated with other low-grade B-cell lymphomas.[1] In a series of paired FLIS and manifest follicular lymphoma (FL), Schmidt *et al.*[2] demonstrated that FLIS and manifest FL cases were clonally

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