Mutations and translocations associated with venetoclax resistance in chronic lymphocytic leukemia (CLL)

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Introduction

The B-cell lymphoma-2 (BCL-2) protein inhibitor venetoclax is a pivotal therapeutic agent in the treatment of chronic lymphocytic leukemia (CLL).
The analysis of the CLL13 study demonstrated that complex karyotype with 5 or more abnormalities independently predicted worse progression-free survival following venetoclax-based treatment. Ours and others' recent work showed that the BCL2 G101V mutation, loss of 8p, gain of 1q21.2-21.3 or overexpression of other BCL-2 family members are all associated with resistance to venetoclax. However, mechanisms of acquired resistance remain unclear. We report the results of clinical sequencing and BCL2 G101V mutation analysis of CLL patents (pts) treated with venetoxlax.

Method

We conducted a retrospective analysis of venetoclax treated patients at the Dana-Farber Cancer Institute.

Patient Selection Criteria

CLL patients who were treated with venetoclax/ABT-199 (VEN) containing regimens in DFCI database;
a total of **265** treatment periods of **236** CLL patients

83 clinical sequencing reports from **41 patients** within 3 months before, during or at next office visit within 3 months after the period of VEN

Excluded **8** patients who had no sample during VEN regimen

69 clinical sequencing reports and **85 BCL2 G101V** results from **33 patients**

Group Progressive Disease (PD):
19 pts with NGS at disease progression
Group Non-Progressors (NP):
14 pts with NGS during response

Samples and examinations:
A total of **69** sequencing results;
41 in Group PD and **28** in Group NP

BCL2 G101V mutation levels from **85** peripheral blood mononuclear cells (MNCs) or bone marrow MNCs samples detected by Droplet digital PCR (ddPCR);
45 in Group PD and **40** in Group NP

Results

We conducted a retrospective analysis of 31 VEN-treated patients at the Dana-Farber Cancer Institute and evaluated **19** patients with NGS data at disease progression, assigned as **Group PD**, and **14** patients during treatment without progression, assigned as **Group NP.**

Table.1 Characteristics of the patients

Factors		Groups PD	NP	p value
Number		19	14	
Gender (%)	Male	12 (63.2)	10 (71.4)	0.719
	Female	7 (36.8)	4 (28.6)	
Age at diagnosis	(years old)	60 [44, 79]	62 [35, 74]	0.827
IGHV status (%)	unmutated	16 (88.9)	9 (81.8)	0.622
	mutated	2 (11.1)	2 (18.2)	
del(17p) (%)		9 (50.0)	1 (7.1)	**0.019**
del(11q) (%)		4 (22.2)	2 (14.3)	0.672
del(13q) (%)		10 (55.6)	7 (50.0)	1
Trisomy12 (%)		9 (50.0)	1 (7.1)	1
Complex karyotype (%)	no complex	8 (47.1)	9 (75.0)	0.132
	3≤, ≤4	4 (23.5)	2 (16.7)	
	5≤	5 (29.4)	1 (8.3)	
ZAP-70 (%)		11 (61.1)	4 (44.4)	0.448
TP53 mutation (%)		15 (78.9)	4 (30.8)	**0.011**
NOTCH1 mutation (%)		5 (26.3)	1 (7.7)	0.361
Treatment before VEN (%)	No	0 (0.0)	1 (7.1)	0.527
	1	4 (21.1)	4 (28.6)	
	2 or more	15 (78.9)	9 (64.3)	
Treatment (%)				
VEN mono		10 (52.6)	8 (57.1)	
IBR-VEN		1 (5.3)	1 (7.1)	
VO/VR		3 (15.8)	1 (7.1)	
IBR-VO		1 (5.3)	0 (0.0)	
Duv-VEN		2 (10.5)	3 (21.4)	
Multiple		2 (10.5)	1 (7.1)	
Time to first treatment	(months)	20.2 [0.89, 136.0]	13.6 [0.7, 83.7]	0.763
Time to VEN (days)	(months)	70.4 [1.8, 192.0]	56.0 [0.0, 159.7]	0.578
Observation period	(months)	17.6 [0.5, 48.6]	19.4 [4.7, 85.4]	0.543

Mutations and karyotype results at the time points of venetoclax response evaluation are shown in **Figure 1a**. Patients with a complex karyotype (CK) (PD vs NP, 63.2% vs 21.4%, P=0.033), deletion 17p (PD vs NP, 42.1% vs 7.14%, P=0.026), and deletion 13q (PD vs NP, 47.4% vs 14.3%, P=0.046) were significantly enriched in the PD group. All patients with del(17p) had CK, and 5 of the 10 patients with deletion 13q co-occurred with both a CK and del(17p), reflecting karyotypic complexity in the PD group. A total of 42% of ↗ the PD group and 14% of the NP group were positive for the *BCL2* G101V mutation. NGS data also showed that *ASXL1* and *NOTCH1* mutations (both P=0.016) were detected only in the PD group.

We recently demonstrated that copy number loss of 8p from whole-exome sequencing data was associated with venetoclax resistance (Blood 2023). In this analysis, the most common chromosomal change detected by karyotype was the loss of chromosome 8 (chr 8) (PD vs NP 25% vs 0%, P=0.0228) (**Figure 1b**). Moreover, unbalanced translocations caused the loss of chr 8p in 4 of 5 cases during VEN. They have recurrent breakpoints at 8q10 and 8p23, all of which cause short arm loss of chr 8. These findings confirm our recent report in a largely independent cohort that loss of chr 8p is associated with VEN resistance. In addition, all of those with unbalanced translocations with loss of 8p at venetoclax progression had complex karyotype with 5 or more abnormalities.

A total of 88.9% of PD cases without Richter's syndrome, except for one, had either *TP53* aberrant diseases and/or *BCL2* mutations, which was significantly higher than in NP cases (P=0.0042) (**Figure 2**) . All cases with positive *BCL2* mutations, except for one in the PD group, required two years of venetoclax treatment to detect the first mutation-positive results. Notably, patients with positive *BCL2* mutations in the NP group were the top three long-term treated patients in our cohort.

Figure 1a. Mutations at evaluation of venetoclax treatment response

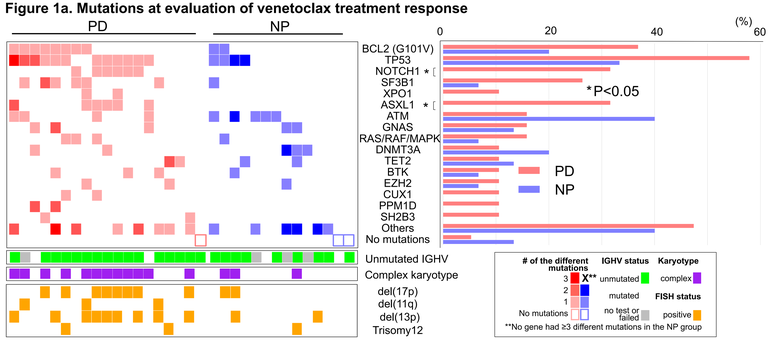


Figure 1b. Karyotype at evaluation of venetoclax treatment response

Groups	Detected unbalanced translocations		
PD	**der 8q10;** 17q10	der 17p11.2	der 10q12
	der 8q10; 21q10	der 17q22	der 14q32
	der 8p23	der 5p15	der 15p11.1
	der 8p23	der 6q21	der 16q11.2
NP	der 4q31		
	der 15q10; 17q10		
	der 20p13		

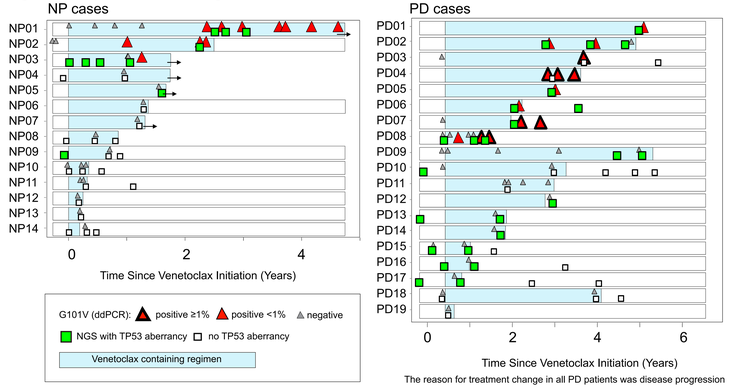
Break points of unbalanced translocations

PD: Chr 8, N=4; Chr 17, N=3; Others, all N=1
NP: All N=1 (Chr 4, 15, 17, 20)

Recurrent break points: 8q10, 8p23

★All unbalanced translocations were with complex karyotype ≥ 5 abnormalities.

Figure 2. Sequential monitoring of mutations

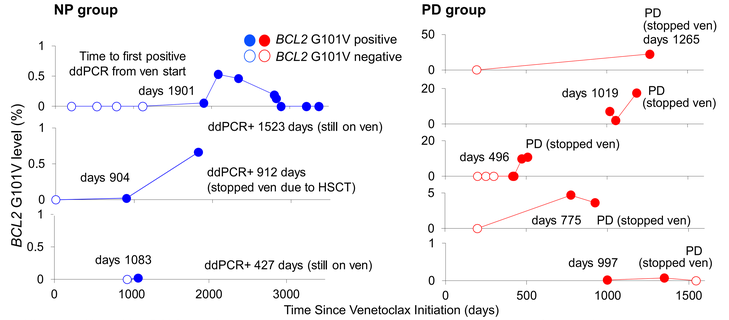


The reason for treatment change in all PD patients was disease progression

Figure 3. *BCL2* G101V levels in ddPCR positive cases with sequential samples

	NP (ddPCR results N=40)	**PD** (ddPCR results N=45)
Positive ddPCR results	11	15 (before PD 6 after PD 9)
Patients with positive ddPCR	3 / 14	8 / 19 *

*All PD patients have ddPCR results before or up to a month after clinical progression.



All cases with positive *BCL2* mutations, except for one in the PD group, required two years of venetoclax treatment to detect the first mutation-positive results (**Figure 3**). Patients with positive *BCL2* mutations in the NP group were the top three long-term treated patients in our cohort. Four out of five patients in the PD group displayed more than 5% *BCL2* mutations at the time of progression, whereas they remained within 1% in the entire NP group during venetoclax treatment.

Summary

- Resistance to venetoclax is significantly associated with a complex karyotype (PD vs NP: 68.4% vs 25.0%, P=0.018), particularly unbalanced translocations at chromosome 8 leading to 8p deletions, all found in highly complex karyotypes with five or more abnormalities.
- 88.9% of PD cases had TP53 and/or BCL2 G101V abnormalities, compared to 33.3% of non-progressing cases (p<0.05).
- Long-term treatment with venetoclax (1 – 2 years) may contribute to the emergence of the BCL2 G101V mutation.
- In patients with the BCL2 G101V mutation, levels stayed below 1% in non-progressing cases but exceeded 5% in 80% of PD cases.

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