Phase I study of single-agent CC-292, a highly selective Bruton's tyrosine kinase inhibitor, in relapsed/refractory chronic lymphocytic leukemia

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Supplemental Material

Supplementary Methods

Supplementary Tables S1–8.

Supplementary Figure Legends

Supplementary Figures S1-2.

Supplementary Methods

Patient eligibility

Eligible patients were aged ≥ 18 years with a documented diagnosis of: CLL/SLL requiring therapy according to the International Workshop on Chronic Lymphocytic Leukemia (iwCLL) guidelines,[1] B-NHL according to the International Working Group Response Criteria for Malignant Lymphoma,[2] or WM according to the International Prognostic Scoring System for Waldenström macroglobulinemia (IPSSWM).[3] Patients had to have received ≥1 therapy and have relapsed after or been refractory to their last prior treatment. Additional eligibility criteria were:

- Eastern Cooperative Oncology Group (EGOG) performance status of ≤ 2
- Life expectancy of ≥ 3 months
- Adequate renal (creatinine clearance ≥ 30 mL/min) and hepatic (total bilirubin ≤ 1.5 × and transaminases ≤ 3 × upper limit of normal) function
- Hematologic eligibility criteria
  - absolute neutrophil count of ≥ 1,000/mm^3
  - hemoglobin level of ≥ 8 g/dL
  - platelet count of ≥ 30,000/mm^3 for CLL/SLL patients or ≥ 50,000/mm^3 for B-NHL/WM patients

The study included a heterogeneous B-NHL population (including follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, and WM) of 29 patients total. The median age of B-NHL/WM patients was 67 years (range 29–80 years); 51.7% were male (**Supplementary Table S2**). The B-NHL/WM patients had received a median of 3 prior therapies (range 1–10) at study initiation and more than half (72.4%) of the patients had clinical stage 3–4 disease.

The study was conducted according to good clinical practice and the ethical principles outlined in the Declaration of Helsinki. All patients provided written informed consent. The Institutional Investigational Review Board of each participating site approved this study.

Study design and treatment

The trial was an open-label, multicenter, 3+3 dose-escalation design. Eligible patients were treated with monotherapy CC-292 at doses ranging from 125 mg to 1,000 mg once daily, and 375 mg and 500 mg twice daily (**Supplementary Table S1**). A total of 6 CLL/SLL patients and 6 B-NHL/WM patients each were planned for enrolment at the 1,000 mg once-daily and 375 mg and 500 mg twice-daily dose levels. Intra-patient dose escalation was permitted up to the preliminary RP2D. Prophylaxis for tumor lysis syndrome was not mandated.

Study endpoints and assessments

The primary endpoint of the trial was to determine safety, DLTs, and the RP2D of CC-292. Secondary objectives of the trial included characterizing the preliminary antitumor efficacy of CC-292, and evaluating its PK and PD profiles. Exploratory endpoints included assessing the effects of CC-292 on BTK signaling in tumor tissue and peripheral blood B-cells. There is no detailed efficacy analysis of the B-NHL and WM patient populations in this manuscript due to the small number of patients and the heterogeneous nature of B-NHL and WM.

For patients with B-NHL/WM, AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.[4] For patients with CLL/SLL, hematologic AEs were graded according to the iwCLL guidelines.[1] Ann Arbor staging, International Prognostic Scoring System for Waldenström macroglobulinemia (IPSSWM), and the Rai staging system were used to report B-NHL, WM, and CLL/SLL staging, respectively.

Safety was monitored continuously throughout the trial and is reported for all patients included in the safety population. This included recording the type, frequency, and severity of AEs along with their relationship to CC-292.

ORR was evaluated according to iwCLL guidelines[1] as well as the updated guidelines modified to account for the early lymphocytosis seen with BCR pathway inhibitors.[5] An efficacy analysis was performed for all efficacy evaluable patients enrolled with a diagnosis of CLL/SLL. Responses are reported as PR or PR-L according to iwCLL criteria.[1]

Statistical analysis

The safety population comprised all patients receiving ≥ 1 dose of CC-292. The efficacy-evaluable population comprised all CLL/SLL patients. In addition to standard iwCLL criteria, the lymph node responses of patients with CLL/SLL were reported independently of increases in blood lymphocyte counts. This is because previous studies of kinase inhibitors in CLL/SLL patients have demonstrated lymph node shrinkage but with increased blood lymphocyte counts (PR-L); this is believed to represent a drug-mediated redistribution (lymphocyte migration and trafficking) phenomenon. The number of patients with CLL/SLL experiencing PR-L converting to standard iwCLL responses, and the observed time-course for these conversions, is reported.

Pharmacokinetics/Pharmacodynamics

Serial blood samples were collected at the Cycle (C) 1 Day (D) 1 and C1D15 time points; few samples were collected on C1D2, C1D8, C1D16, C1D22, C2D1, and C3D1 for PK characterization of CC-292. Plasma samples were analyzed for CC-292 concentrations by a validated liquid chromatography/tandem mass spectrometry method (Tandem Labs, West Trenton, NJ, USA).

CC-292 covalently occupies the BTK adenosine triphosphate (ATP)-binding site. The extent of inhibition of BTK by CC-292 was determined by a quantitative assay utilizing a covalent probe that can react with free BTK, but is blocked from reaction if CC-292 is already present at the BTK ATP-binding site.[6] This assay was used to directly determine the residual amount of free BTK in peripheral blood mononuclear cell lysates (Cambridge Biomedical, Boston, MA, USA). Peripheral blood mononuclear cell occupancy was determined by comparison of on-treatment versus pre-treatment levels. In a subset of patients who volunteered to provide samples (n = 11), on-treatment BTK receptor occupancy in lymph node tissue was estimated in 10 evaluable lymph node tissue lysates acquired 4-hours post-dose at the C1D15 time point. This assessment was qualitative due to the absence of an untreated control lysate, but the presence of BTK was tested by Western blot and for 1 patient, by comparison with a novel enzyme-linked immunosorbent assay for total BTK.

Plasma exposure levels of CC-292 were analyzed following treatment with 125 mg, 250 mg, 400 mg, 625 mg, 750 mg, or 1,000 mg once-daily doses. The geometric mean area under the curve 0–24 hours ($AUC_{0–24}$) are 1,675, 2,155, 5,242, 3,636, 7,972, and 16,230 ng*hr/mL, respectively, and the geometric mean C_{max} values are 695, 653, 1,593, 1,054, 1,851, and 3,047 ng/mL, respectively (**Supplementary Table S6**). Following treatment with 375 mg and 500 mg twice-daily doses, the geometric mean $AUC_{0–24}$ were 9,729 and 14,697 ng*hr/mL, respectively, and the geometric mean C_{max} were 1,534 and 1,958 ng/mL, respectively (**Supplementary Table S6).** A moderate accumulation for $AUC_{0–24}$ (i.e., an increase of 35–53% in geometric mean) and C_{max} values (i.e., an increase of 23% in geometric mean) was observed on Day 15 for patients in the 500 mg twice daily group compared with Day 1 for patients in the 1,000 mg once daily group.

References

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Supplementary Table S1. Trial design (N = 113).

Cohort	Dose (mg)	Number of patients		
		CLL (n = 84)	B-NHL (n = 23)	WM (n = 6)
Once daily				
DL 1	125	3	0	0
DL 2	250	4	2	0
DL 3	400	1	2	0
DL 4	625	1	5	0
DL 5	750	29	2	0
DL 6a	1,000	7	5	1
Twice daily				
DL 6b	375	6	3	3
DL 7	500	33	4	2

B-NHL, B-cell non-Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; DL, dose level; SLL, small lymphocytic leukemia; WM, Waldenström macroglobulinemia.

Supplementary Table S2. Baseline B-cell NHL/WM patient characteristics.

Baseline patient characteristics	**n = 29**
Age, median (range), years	67 (29–80)
Male sex, %	51.7
Clinical stage 3–4,[a] %	72.4
Time from diagnosis, median (range), months	60 (7.2–204)
Prior therapies, median (range)	3 (1–10)
Time from last prior therapy, median (range), months	4.8 (0–96)
Refractory to last prior regimen, %	55.1

[a] Ann Arbor staging was used for B-cell NHL patients and IPSSWM for WM patients. IPSSWM, International Prognostic Scoring System for Waldenström macroglobulinemia; NHL, non-Hodgkin lymphoma; WM, Waldenström macroglobulinemia.

Supplementary Table S3. Baseline patient characteristics for all patients.

Characteristic	N = 113
Age, median (range), years	66 (29–89)
Male sex, %	56.6
Clinical stage 3–4, %[a]	62.8
Time from diagnosis, median (range), years	7.5 (0.3–2.6)
Prior therapies, median (range)	3 (1–12)
Time from last prior therapy, median (range), years	0.7 (0–7.3)
Refractory to last prior regimen, %	40.7

[a] Ann Arbor staging was used for B-NHL patients and IPSSWM for WM patients; and Rai staging system for CLL/SLLpatients. B-NHL, B-cell non-Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; IPSSWM, International Prognostic Scoring System for Waldenström macroglobulinemia; SLL, small lymphocytic leukemia; WM, Waldenström macroglobulinemia.

Supplementary Table S4. Grade 3 or higher and TEAEs occurring in all patients (N = 113).

	CC-292 dosing[a]				
	750 mg once-daily (n = 31)	**1,000 mg once-daily (n = 13)**	**375 mg twice daily (n = 12)**	**500 mg twice daily (n = 39)**	**Total[b] (N = 113)**
Neutropenia	4 (13)	7 (54)	2 (17)	1 (3)	18 (16)
Thrombocytopenia	4(13)	1 (8)	0	2 (5)	9 (8)
Pneumonia	1 (3)	1 (8)	0	2 (5)	4 (4)
Anemia	1 (3)	0	0	2 (5)	3 (3)
Hyperglycemia	1 (3)	0	0	2 (5)	3 (3)
Febrile neutropenia	2 (6)	0	0	0	2 (2)
Sepsis	2 (6)	0	0	0	2 (2)
Abscess limb	0	1 (8)	0	0	1 (1)
Aortitis	0	0	0	1 (3)	1 (1)
Arthritis bacterial	1 (3)	0	0	0	1 (1)
Arthralgia	1 (3)	0	0	0	1 (1)
Cataract	0	0	1 (8)	0	1 (1)
Confused state	1 (3)	0	0	0	1 (1)
Convulsion	0	0	0	1 (3)	1 (1)
Dehydration	0	0	0	1(3)	1 (1)
Diarrhea	1 (3)	0	0	0	1(1)
Fecal incontinence	0	0	0	1 (3)	1 (1)
Fatigue	1 (3)	0	0	0	1 (1)
Hematoma	0	1 (8)	0	0	1 (1)
Hyperkalemia	0	0	0	1 (3)	1 (1)

	CC-292 dosing[a]				
	750 mg once-daily (n = 31)	**1,000 mg once-daily (n = 13)**	**375 mg twice daily (n = 12)**	**500 mg twice daily (n = 39)**	**Total[b] (N = 113)**
Hypotension	0	1 (8)	0	0	1 (1)
Hypoxia	0	1 (8)	0	0	1 (1)
Left bundle branch block	0	0	0	1 (3)	1 (1)
Pneumonitis	0	1 (8)	0	0	1 (1)
Pyrexia	1 (3)	0	0	0	1 (1)

[a] All values n (%). [b] Total includes all dose levels, including 125 mg, 250 mg, 400 mg, and 625 mg once daily. TEAEs, treatment-emergent adverse events.

Supplementary Table S5. TEAEs of any grade occurring in ≥ 10% of all patients (N = 113).

	CC-292 dosing[a]				
	750 mg once daily (n = 31)	**1,000 mg once daily (n = 13)**	**375 mg twice daily (n = 12)**	**500 mg twice daily (n = 39)**	**Total[b] (N = 113)**
Diarrhea	22 (71)	11 (85)	8 (67)	22 (56)	77 (68)
Fatigue	15 (48)	12 (92)	4 (33)	15 (38)	51 (45)
Thrombocytopenia	16 (52)	5 (38)	4 (33)	11 (28)	41 (36)
Nausea	11 (35)	7 (54)	2 (17)	15 (38)	39 (35)
Neutropenia	10 (32)	8 (62)	3 (25)	10 (26)	35 (31)
Pyrexia	11 (35)	6 (46)	2 (17)	10 (26)	31 (27)
Cough	9 (29)	4(31)	2 (17)	9 (23)	30 (27)
Headache	8 (26)	6 (46)	3 (25)	6 (15)	28 (25)
Anemia	9 (29)	4 (31)	2 (17)	8 (21)	27 (24)
Dyspnea	9 (29)	4 (31)	0	6 (15)	23 (20)
Dizziness	9 (29)	1 (8)	2 (17)	9 (23)	22 (19)
Abdominal pain	9 (29)	3 (23)	1 (8)	6 (15)	21 (19)
Upper respiratory tract infection	6 (19)	4 (31)	1 (8)	5 (13)	21 (19)
Peripheral edema	9 (29)	4 (31)	2 (17)	3 (8)	20 (18)
Muscle spasms	6 (19)	2 (15)	1 (8)	4 (10)	18 (16)
Sinusitis	3 (10)	1 (8)	3 (25)	7 (18)	16 (14)
Contusion	5 (16)	3 (23)	0	3 (8)	16 (14)
Vomiting	5 (16)	2 (15)	1 (8)	5 (13)	15 (13)
Pneumonia	4 (13)	1 (8)	0	6 (15)	15 (13)

	CC-292 dosing[a]				
	750 mg once daily (n = 31)	**1,000 mg once daily (n = 13)**	**375 mg twice daily (n = 12)**	**500 mg twice daily (n = 39)**	**Total[b] (N = 113)**
Constipation	4 (13)	2 (15)	2 (17)	4 (10)	14 (12)
Urinary tract infection	6 (19)	1 (8)	1 (8)	3 (8)	14 (12)
Decreased appetite	6 (19)	2 (15)	1 (8)	4 (10)	14 (12)
Arthralgia	5 (16)	0	4 (33)	4 (10)	13 (12)
Dyspepsia	4 (13)	0	0	5 (13)	12 (11)
Hypocalcemia	3 (10)	2 (15)	1 (8)	5 (13)	12 (11)
Hyponatremia	2 (6)	2 (15)	0	7 (18)	11 (10)

[a] All values presented as n (%). [b] Total includes all dose levels, including 125 mg, 250 mg, 400 mg, and 625 mg once daily. TEAEs, treatment-emergent adverse events.

Supplementary Table S6. Pharmacokinetic profile of CC-292 (N = 113).

Cohort	Dose (mg)	Geometric mean $AUC_{0–24}$ (ng*hr/mL)	Geometric mean C_{max} (ng/mL)
Once daily			
DL 1	125	1,675	695
DL 2	250	2,155	653
DL 3	400	5,242	1,593
DL 4	625	3,636	1,054
DL 5	750	7,972	1,851
DL 6a	1,000	16,230	3,047
Twice daily			
DL 6b	375	9,729	1,534
DL 7	500	14,697	1,958

$AUC_{0–24}$, area under the curve 0–24 hours; C_{max}, maximum concentration; DL, dose level.

Supplementary Table S7. Dose levels of responding patients.

			CC-292 dosing[a]			
			750 mg once daily (n = 31)	**1,000 mg once daily (n = 13)**	**375 mg twice daily (n = 12)**	**500 mg twice daily (n = 39)**
del(11q) (n = 14)	PR	(n = 6)	3 (21)	1 (7)	0	2 (14)
	PRL	(n = 4)	3 (21)	0	0	1 (7)
del(17p) (n = 16)	PR	(n = 7)	0	0	1 (6)	6 (38)
	PRL	(n = 4)	3 (19)	0	0	1 (6)
Unmutated *IgVH*	PR	(n = 10)	3 (19)	2 (13)	2 (13)	3 (19)
(n = 16)	PRL	(n = 0)	0	0	0	0

[a] All values presented as n (%). *IgVH,* immunoglobulin heavy chain variable; PR, partial response; PRL, PR with lymphocytosis.

Supplementary Table S8. Duration of treatment for patients continuing treatment as of December 21, 2013.

Patient number	Duration	
	Days	Weeks
1	532	76.0
2	491	70.1
3	491	70.1
4	484	69.1
5	470	67.1
6	377	53.9
7	253	36.1
8	216	30.9
9	197	28.1
10	357	51.0
11	336	48.0
12	835	119.3
13	790	112.9
14	538	76.9
15	538	76.9
16	461	65.9
17	254	36.3
18	237	33.9
19	189	27.0
20	251	35.9
21	790	112.9

Patient number	Duration	
	Days	Weeks
22	498	71.1
23	527	75.3
24	461	65.9
25	217	31.0
26	506	72.3
27	385	55.0
28	266	38.0
29	211	30.1
30	189	27.0
31	548	78.3
32	617	88.1
33	240	34.3
34	210	30.0
35	588	84.0
36	512	73.1
37	464	66.3
38	590	84.3
39	538	76.9
40	267	38.1
41	237	33.9
42	518	74.0
43	225	32.1

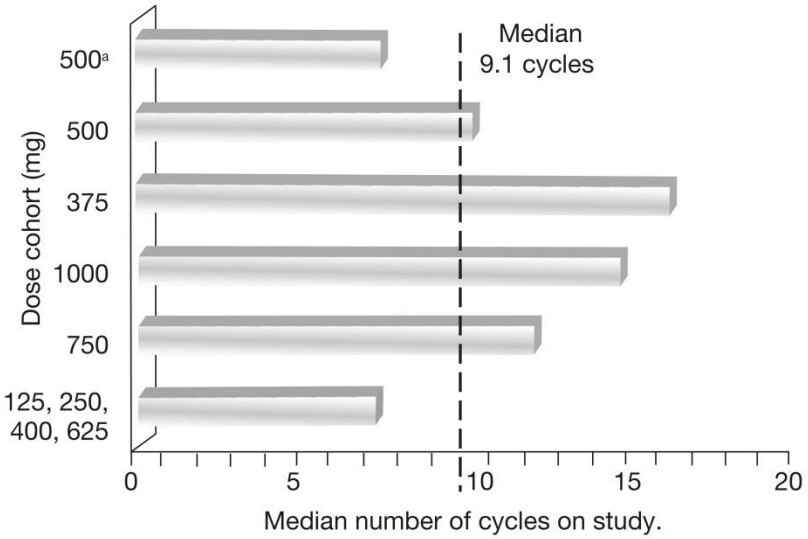
Supplementary Figure S1. Median number of cycles per dose cohort vs current patient disposition. Patients received continuous dosing in 28-day cycles until progressive disease or intolerable toxicity.

[a] Expansion cohort.

Supplementary Figure S2. BTK receptor occupancy and outcomes in lymph node tissue. On-treatment levels of free BTK in lymph node biopsy tissue were determined at 4 hours post-dose (n = 11). Free BTK was determined quantitatively using ELISA (lower limit of quantification < 12.5 pg/μL), and the presence of BTK protein ascertained by Western blot. For 1 patient, no BTK protein was detected by Western blot, and hence the absence of detectable free BTK by ELISA is not meaningful. For 1 patient (**), a total BTK ELISA was available at the time of biopsy analysis, enabling BTK occupancy to be quantitatively measured at 96%. This patient was also the only patient with a detectable level of free BTK. The remaining patients with a BTK band present by Western blot had no detectable free BTK, implying high receptor occupancy because in control (non-malignant breast) lymph node samples, high levels of free BTK were detected by ELISA whenever a band was present by Western blot.

b.i.d., twice daily; BTK, Bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia; ELISA, enzyme-linked immunosorbent assay; iNHL, indolent non-Hodgkin lymphoma; PD, progressive disease; PR, partial response; PR-L, PR with lymphocytosis; SD, stable disease; WM, Waldenström macroglobulinemia.

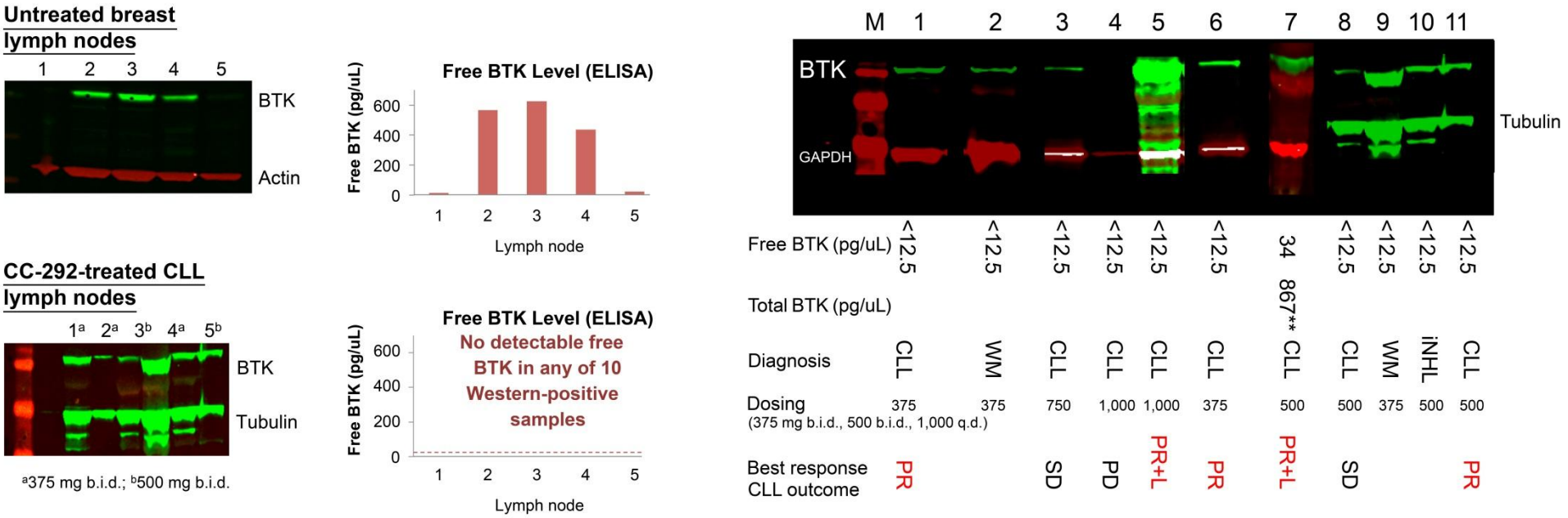
Figure S1



[a] Expansion cohort

Enrolled (n)	Still on treatment, n (%)	
27	13	(48)
12	4	(33)
12	6	(50)
13	2	(15)
31	6	(18)
18	3	(17)

Figure S2


Untreated breast lymph nodes
1 2 3 4 5
BTK
Actin
Free BTK Level (ELISA)
Free BTK (pg/uL)
600
400
200
0
1 2 3 4 5
Lymph node
CC-292-treated CLL lymph nodes
1a 2a 3b 4a 5b
BTK
Tubulin
a375 mg b.i.d.; b500 mg b.i.d.
Free BTK Level (ELISA)
No detectable free BTK in any of 10 Western-positive samples
Free BTK (pg/uL)
600
400
200
0
1 2 3 4 5
Lymph node
M 1 2 3 4 5 6 7 8 9 10 11
BTK
GAPDH
Tubulin
Free BTK (pg/uL) <12.5 <12.5 <12.5 <12.5 <12.5 <12.5 34 <12.5 <12.5 <12.5 <12.5
Total BTK (pg/uL) 867**
Diagnosis CLL WM CLL CLL CLL CLL CLL CLL WM iNHL CLL
Dosing (375 mg b.i.d., 500 b.i.d., 1,000 q.d.) 375 375 750 1,000 1,000 375 500 500 375 500 500
Best response CLL outcome PR SD PD PR+L PR PR+L SD PR