

Entospletinib and obinutuzumab in patients with relapsed/refractory chronic lymphocytic leukemia and B-cell malignancies

Therapeutic resistance and intolerance of Bruton tyrosine kinase (BTK) inhibitors is an emerging unmet medical need in chronic lymphocytic leukemia (CLL).^{1,2} Entospletinib is a small molecule inhibitor of spleen tyrosine kinase (SYK), which is integral to the activation of BTK and the B-cell receptor (BCR) signaling cascade.³ SYK is overexpressed in CLL and its inhibition induces apoptosis of CLL cells in pre-clinical models.^{4,5} We have shown that BAFF-mediated SYK activation triggered BCR signaling and rendered protection of CLL cells from spontaneous apoptosis *in vitro*.⁵ Single agent entospletinib was efficacious in treatment of patients with relapsed/refractory (R/R) CLL who had progressed following chemoimmunotherapy.⁶ Here we report the results of a single arm, open label, investigator-initiated phase I/II clinical trial which evaluated safety and efficacy of entospletinib in combination with obinutuzumab, a glycoengineered monoclonal anti-CD20 antibody, in patients with R/R CLL and non-Hodgkin lymphoma (NHL) (clinicaltrials.gov. Identifier: NCT03010358).

Patients enrolled in the phase I dose-escalation portion of the trial included adult patients with CLL⁷ or NHL (phase I part of the study only) after ≥ 1 prior therapy. Participants had an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 , aspartate transaminase (AST) and alanine transaminase (ALT) $< 2.5\times$, bilirubin $< 2\times$ upper limit of normal and creatinine clearance (CrCl)

≥ 50 mL/min.

Complex karyotype (CK) was determined as presence of ≥ 3 cytogenetic abnormalities on a CpG-stimulated karyotype. Gene mutations were identified using a 76-gene next-generation sequencing panel (GeneTrails[®]). Bone marrow examinations were required to confirm complete response (CR), with minimal residual disease (MRD) assessment using 8-color flow cytometry (MRD-undetectable at $< 10^{-4}$).

Patients were enrolled at two dose levels to receive entospletinib 200 mg (dose level 1 [DL1]) or 400 mg (dose level 2 [DL2]) twice-daily orally according to a standard 3+3 design (*Online Supplementary Table S1*). The primary endpoint for the phase I portion of the study was to determine the maximum tolerated dose (MTD) and/or the recommended phase II dose (RP2D) of the combination. All patients received single agent entospletinib as part of a 7-day run-in. Thereafter, patients received entospletinib on days 1-28 of each 28-day cycle continuously, and obinutuzumab intravenously in standard doses for 6 cycles.

Adverse events were graded according to Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.03 and international workshop CLL (iwCLL) criteria.⁷ Dose limiting toxicity (DLT) was defined as grade ≥ 3 non-hematological toxicity (except grade 3 nausea, vomiting, diarrhea or asymptomatic grade 3-4 laboratory abnormalities reversible within 72 hours; grade 3 infusion reactions, grade 3 tumor lysis syndrome); grade 4 neutropenia lasting > 7 days or febrile neutropenia; grade 4 thrombocytopenia/anemia or grade 3 thrombocytopenia with bleeding.

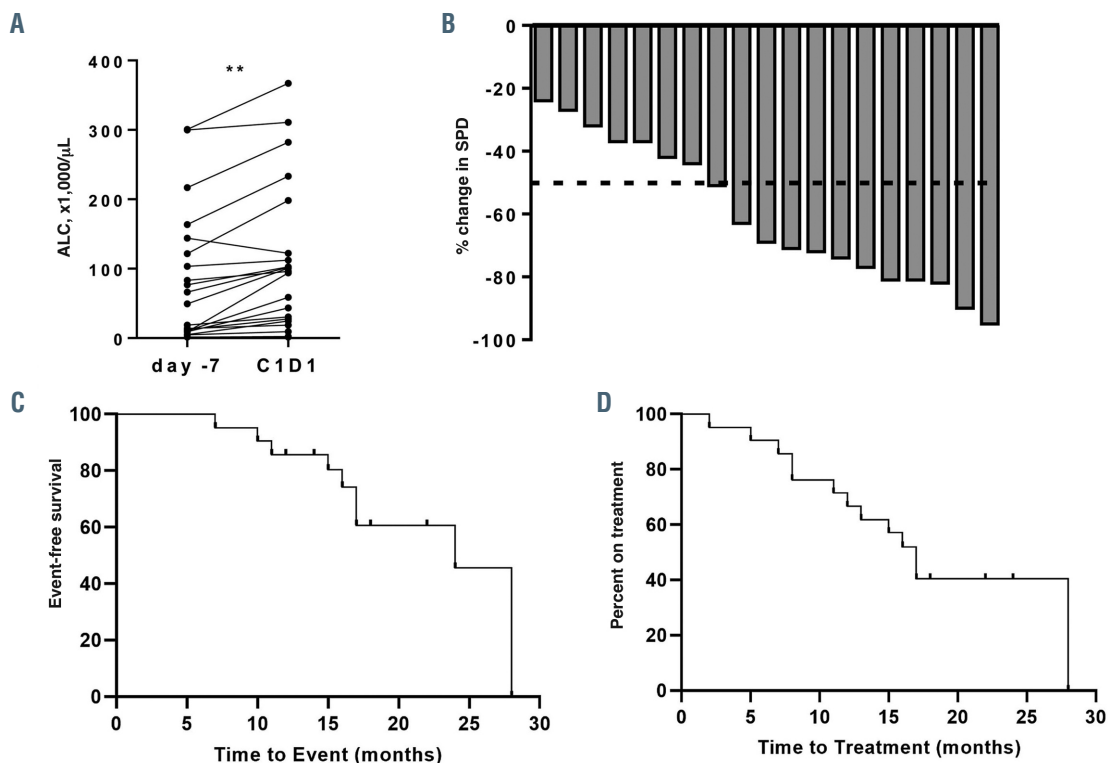


Figure 1. Efficacy of entospletinib and obinutuzumab in patients with chronic lymphocytic leukemia. (A) Redistribution lymphocytosis. Peripheral blood absolute lymphocyte count (ALC) prior to (day -7) and after 7 days of treatment with entospletinib single agent, prior to introduction of obinutuzumab (cycle 1 day 1 [C1D1]). ** $P < 0.01$. (B) Nodal response (maximum percent change in the sum of the product [SPD] of the longest perpendicular dimensions) in patients with chronic lymphocytic leukemia evaluable lymphadenopathy. (C) Event-free survival. (D) Duration of treatment.

Table 1. Adverse events.

Adverse events, N (%)	All Patients (N=23)	
	Any Grade	Grade ≥ 3
Adverse Events - All	22 (95.7)	15 (65.2)
Hematologic Adverse Events		
Neutrophil count decreased	11 (47.8)	10 (43.5)
Platelet count decreased	5 (21.7)	4 (23.5)
Anemia	3 (13.0)	1 (4.3)
Febrile neutropenia	3 (13.0)	3 (13.0)
Lymphocyte count increased	1 (4.3)	1 (4.3)
Non-hematologic Adverse Events		
Fatigue	11 (47.8)	2 (8.7)
Infusion related reaction	10 (43.5)	4 (17.4)
Nausea	8 (34.8)	1 (4.3)
Diarrhea	7 (30.4)	0
Fever	7 (30.4)	0
Alanine aminotransferase increased	5 (21.7)	3 (13.0)
Aspartate aminotransferase increased	5 (21.7)	3 (13.0)
Other Infection	5 (21.7)	1 (4.3)
Creatinine increased	4 (17.4)	0
Pneumonia	3 (13.0)	1 (4.3)
Chills	3 (13.0)	1 (4.3)
Dizziness	3 (13.0)	0
Vomiting	2 (8.7)	1 (4.3)
Tremor	2 (8.7)	1 (4.3)
Tumor lysis syndrome	2 (8.7)	1 (4.3)

Once the RP2D was determined, a phase II study enrolled patients with R/R CLL only. CR was the primary endpoint.⁷ Secondary endpoints included objective response rate (ORR), event-free survival (EFS), (defined as the interval between the first study treatment and objective signs of disease recurrence, subsequent anti-leukemic therapy, or death), and safety. Patients with CLL enrolled at the RP2D level in the phase I were included in the phase II part of the study.

The planned sample size of 17 patients in the phase II part of the study provided 83% power to detect a difference in CR of 0.30 (0.20 vs. 0.50) using a one-sided binomial test ($P < 0.05$). The null hypothesis (H_0)=2 was based on CLL11 study data (CR 21%).⁸ Patients who received at least one dose of the study therapy were evaluable for safety. Response rates and exact 95% Confidence Interval (CI) were estimated by Clopper-Pearson method and EFS by the Kaplan-Meier method. Data cut-off date for analysis was January 1, 2020. The study was approved by the Oregon Health and Science University Institutional Review Board and conducted in accordance with the principles of the Declaration of Helsinki.

A total of 24 patients (n=22 CLL and n=2 follicular lymphoma [FL]) were enrolled between 08/2017 and 10/2018 (Online Supplementary Table S2). One patient had CLL in Richter's transformation leading to ineligibility on study entry and was removed from subsequent analysis. Twelve patients were enrolled in the phase I part of the study. The phase II part of the study included 17 patients with CLL (of which six received entospletinib at DL2 on the phase I part of the study).

Among the 23 evaluable patients, the median follow-up was 17 months (range, 7-28 months). The median relative dose intensity of entospletinib was 96%. The median treatment duration was 16 months (range, 2-26). Four patients (17.4%) experienced permanent dose reductions due to toxicities. An additional nine patients had temporary dose holds/reductions. Thirteen patients (56.5%) discontinued study therapy due to: progressive disease (n=8),

Table 2. Clinical characteristics of patients with chronic lymphocytic leukemia.

Characteristics	All patients	
	N=21	(%)
Age		
< 65 years	7	(33.3)
≥ 65 years	14	(66.7)
Sex		
Male	15	(71.4)
Female	6	(28.6)
ECOG performance status		
0	8	(38.1)
1	12	(57.1)
2	1	(4.8)
$\beta 2$ -microglobulin (n=16)		
≤ 3.5 mg/L	5	(31.3)
> 3.5 mg/L	11	(68.7)
IgG* (n=20)		
<700 mg/dL	16	(80.0)
>700 mg/dL	4	(20.0)
FISH		
Deletion 13q	14	(66.7)
Trisomy 12	3	(14.2)
Deletion 11q	2	(9.5)
Deletion 17p	5	(23.8)
Complex Karyotype (CK, ≥ 3 abnormalities)		
CK + <i>TP53</i> aberration	6	(28.6)
≥ 5 abnormalities	5	(23.8)
	1	(4.8)
Mutational Status		
<i>TP53</i>	9	(42.9)
<i>Notch1</i>	2	(9.5)
<i>SF3B1</i>	4	(19.0)
High Risk CLL**	13	(61.9)
Prior Treatments		
1	6	(26.8)
2	8	(38.1)
≥ 3	7	(33.3)
Fludarabine	12	(57.1)
Bendamustine	14	(66.7)
Ibrutinib	7	(33.3)
Idelalisib	1	(4.8)

*Normal range: 700–1,600 mg/dL. **High-Risk CLL defined as complex karyotype (CK), *TP53* aberration, *NOTCH1* and *SF3B1* mutations. CLL: chronic lymphocytic leukemia; ECOG: Eastern Cooperative Oncology Group; FISH: fluorescence *in situ* hybridization.

adverse event related to entospletinib (n=1; recurrent AST/ALT abnormalities); a new diagnosis of breast cancer unrelated to study treatment (n=1), withdrawal of consent (n=2), and achievement of CR with undetectable MRD in the bone marrow after 12 cycles of therapy (n=1).

Twelve patients enrolled in the phase I part of the study received a median of 18 cycles (range, 7-28) of study therapy. Of six patients (four CLL, two FL) who received entospletinib 200 mg (DL1), one patient experienced a DLT (grade 3 asymptomatic AST/ALT abnormalities) attributed to entospletinib. No DLT were observed among the six patients who received entospletinib 400 mg (DL2). Thus, entospletinib 400 mg twice-daily was determined to be the RP2D in combination with obinutuzumab.

Treatment-related AE were reported in 95.7% of patients (Table 1). Grade 3 or higher AE occurred in 65.2%. The most common hematologic AE observed across all patients were neutropenia (47.8%), thrombocy-

topenia and anemia, as in earlier studies of SYK inhibitors.^{6,9,10} Neutropenia (43.5%; including four patients [17.4%] who had transient grade 4 neutropenia attributed to obinutuzumab), thrombocytopenia and anemia were the most common grade ≥ 3 hematologic toxicities. The median onset of neutropenia was 7 days after the first obinutuzumab infusion, median duration was 28 days. In six of 11 patients, first onset occurred during cycle one of therapy. Growth factor support was not required.

The most frequently occurring non-hematological AE of all grades were fatigue, infusion-related reaction and nausea. The most frequent grade 3-4 non-hematological AE were: infusion-related reaction (17.4%; all attributed to obinutuzumab) and increased AST/ALT abnormalities (13.0%), the latter adverse event reported in 10-20% of patients receiving SYK inhibitors.^{6,10-12} Despite the fact that patients had received a median of two prior therapies, including fludarabine and bendamustine, few patients (13%) developed febrile neutropenia or pneumonia. There were no grade 5 AE.

Of 23 patients, only one patient discontinued therapy due to AE (recurrent AST/ALT abnormalities which resolved upon cessation of entospletinib). This discontinuation rate (4.3%) compares favorably with those seen in early-phase trials and real-world analyses of BTK inhibitors over a similar follow-up period.^{1,13-15}

Efficacy of entospletinib+obinutuzumab was analyzed in the 21 patients with CLL, of which 17 received entospletinib at RP2D (400 mg twice daily). Among an additional four patients who were initially treated with 200 mg entospletinib, two escalated to RP2D after 12 and 13 treatment cycles.

Patients with CLL had a median age of 66 years (range, 48-76; Table 2). Ten patients (47.6%) had either complex karyotype (CK; n=6) or a *TP53* aberration (n=9). Including CK, *TP53* aberration, *NOTCH1* and *SF3B1* mutations, 13 patients (61.9%) had unfavorable cytogenetic and molecular characteristics, defined as "high-risk CLL". The median number of prior therapies was two (range, 1-6). Seven patients had received prior ibrutinib and one patient received the phosphoinositide-3 kinase (PI3K) inhibitor idelalisib. Of those, four patients discontinued ibrutinib due to intolerance, one per their discretion, and three patients discontinued ibrutinib and idelalisib for progressive disease. Baseline median absolute neutrophil count was 2,700/ μ L, hemoglobin 10.9 g/dL, platelet count 150,500/ μ L, immunoglobulin (Ig) G level 494 mg/dL (range, 176-1475 mg/dL).

Treatment with entospletinib for 7 days during run-in resulted in redistribution lymphocytosis (Figure 1A). Among the 21 efficacy-evaluable participants with CLL, the ORR was 66.7% (95% CI: 43.0-85.4). Three patients (14.3%, 95% CI: 3.1-36.3) achieved a CR, and 11 patients (52.4%) had a partial response (PR). The remaining seven patients had stable disease as their best response. Two of three patients with confirmed CR had undetectable MRD in the bone marrow. The median time to CR was 10.7 months (range, 7.0-17.9 months) and time to PR was 5.8 months (range, 2.8-6.5 months; Online Supplementary Table S3). Nineteen patients with evaluable lymphadenopathy demonstrated a reduction in lymph node sizes (Figure 1B). The low CR rate is consistent with that seen with BCR-signaling inhibitors.^{13,15}

Median EFS was 24 months, treatment duration – 17 months (95% CI: 16-28; Figure 1C and D). Nine patients with CLL (eight out of nine discontinued due to progressive disease) started subsequent therapy (six with BTK

inhibitors, two with venetoclax and one with PI3K inhibitor). All but one patient remains alive.

Thirteen patients with high-risk disease genetics had an ORR of 53.8% (five PR and two CR). Five patients remain on treatment, with a median EFS of 24 months. Among the eight patients who had previously received kinase inhibitors, ORR was 62.5% (all PR) and median EFS was 17 months.

Among the 17 patients with CLL who received entospletinib at RP2D in phase II of the study ORR was 82.4% (64.7% PR, 17.6% CR). The median EFS has not been reached, and the estimated 2-year EFS is 64%. This compares favorably with entospletinib monotherapy in patients with R/R CLL (ORR 61%, median PFS 13.8 months).⁶

Thus, the combination of entospletinib and obinutuzumab has an acceptable safety profile and shows a strong efficacy signal warranting continued exploration in CLL.

Adam S. Kittai,^{1*} Scott Best,¹ Bria Thurlow,¹ Vi Lam,¹ Taylor Hashiguchi,¹ Shaun Goodyear,¹ Daniel O. Persky,² Craig Okada,¹ Byung Park,¹ Stephen E. Spurgeon¹ and Alexey V. Danilov^{1,3}

¹Knight Cancer Institute, Oregon Health & Science University, Portland, OR; ²University of Arizona Cancer Center, Tucson, AZ and ³City of Hope Comprehensive Cancer Center, Duarte, CA, USA

*Current address: Ohio State University, Columbus, OH, USA

Correspondence:

ALEXEY V. DANILOV - adanilov@coh.org

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