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Long-term follow-up of patients with CLL treated with the selective Bruton's tyrosine kinase inhibitor ONO/GS-4059

Left running head: WALTER et al

Right running head: Long-term ONO/GS-4059 treatment in CLL

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To the Editor

The inhibitor of Bruton's tyrosine kinase (BTK) ibrutinib has transformed the treatment of chronic lymphocytic leukemia (CLL); many patients with previously untreatable disease may now enter durable remissions.[1,2] Nevertheless, the kinome of ibrutinib is broad, resulting in toxicities including bleeding, arthralgia, diarrhea, hypertension, and atrial fibrillation.[3-6] Up to 20% of patients discontinue ibrutinib due to toxicity.[7-9] More selective BTK inhibitors (BTKi's) include ONO/GS-4059, acalabrutinib, and BGB-3111. Preliminary data indicate that these drugs have comparable activity to ibrutinib, but with reduced toxicities.[10-12] However, long-term follow-up and response data have not yet been reported. We provide an updated, 3-year follow-up of treatment efficacy, safety, and laboratory correlates, including baseline mutational profiling of CLL patients in the Phase 1 ONO/GS-4059 extension study.

The ONO/GS-4059 POE001 Phase 1 clinical study (NCT01659255) was conducted to determine the safety and tolerability of ONO/GS-4059 in patients with relapsed/refractory (R/R) B-cell malignancies. Between September 2012 and January 2015, 90 patients were enrolled and treated with ONO/GS-4059. Patients continuing to respond or have stable disease could enroll in the long-term extension study (ONO/GS-US-1787, NCT02457559). In the CLL cohort (comprising 28 patients) treatment consisted of 9 cohorts receiving 20mg once daily (QD) to 600mg QD and twice-daily (BID) regimen of 300mg. Each site had Institutional Ethical Committee approval. Informed consent was obtained from all patients transferring to the extension study. DNA was extracted from peripheral blood from 27/28 CLL patients before trial therapy. Targeted sequencing was performed using the Illumina next-generation sequencing (NGS) platform from Sistemas Genomicos, Valencia (Spain), using a pre-designed CLL panel (Supplementary Table 1). Reads were aligned against the human reference genome version GRCh37/hg19. Filtering was performed using Picard-tools (https://broadinstitute.github.io/picard/) and SAMtools (http://samtools.sourceforge.net/). Confirmatory Sanger sequencing was performed on identified sequence variants and annotated using the Ensembl database

(www.ensembl.org). Only sequence variants leading to a change in amino acid composition and not reported in the dbSNP were scored as mutations. Sanger sequencing was used to determine *IGHV* status. Statistical analysis was performed on the modified intention-to-treat population (patients with ≥1 dose of study drug). Kaplan-Meier methodology was used to calculate progression-free survival (PFS). The date of definitive progression was the time point at which progression was first identified by radiographic, imaging, or clinical data, or death. Patients were censored if no PFS event was observed.

All 28 patients with R/R CLL enrolled were evaluable for efficacy and safety. The median number of prior treatments was 4 (range 2-9); 5 patients were primarily fludarabine refractory and 1 patient had received a prior PI3K inhibitor. Eleven (39%) were refractory to their last line of therapy. None had received prior BTKi treatment. Anticoagulant therapy was permitted; 6/28 patients were on anticoagulant therapy during the study. At the time of updated analysis (8 June 2016), 11 patients (39.3%) had discontinued treatment. (In comparison at 3 years, 47% had discontinued treatment with Ibrutinib[13].) Reasons for discontinuation were death (n=3), disease progression (n=4), adverse events (AEs) (n=3), and sponsor decision due to extended drug interruption (n=1); in one patient with AE, disease progression occurred concurrently (Supplementary Table 1). Subjects remaining on study were receiving doses of ONO/GS-4059 ranging from 40mg QD to 600mg QD or 300mg BID. No maximum tolerated dose in patients with CLL was identified. The median duration on study at censoring was 32.5 months. Estimated median PFS was 38.5 months (Figure 1a) and median overall survival was 44.9 months.

Responses (complete or partial) were initially observed in 24/25 (96%) evaluable patients.[10] Ibrutinib can result in prolonged lymphocytosis (duration >1 year), reported in 20% of patients in the Phase 1b/2 trial[14] and associated with 13q deletion. In our study, 23 patients (82%) exhibited lymphocytosis; mean fold increase above baseline was 4.5-fold.[10] In all instances, lymphocytosis following ONO/GS-4059 resolved by Cycle 6. As with other BTKi's,[11,15] changes in serum levels of CCL3 and CCL4 showed a significant decrease at

day 8, consistent with BCR signaling pathway blockade; TNF-alpha, IL10, IL6, and IL8 also showed a significant decrease 8 days post treatment initiation (Supplementary Figure 1a). Similar to data reported with acalabrutinib[11] but in contrast to ibrutinib,[16] immunoglobulin levels did not change significantly with long-term therapy with ONO/GS-4059 (Supplementary Figure 1b).

Targeted NGS mutational data at time of trial entry, along with *IGHV* mutation and interphase FISH data, are shown in Supplementary Table 1. Twenty-one of 25 patients exhibited unmutated *IGHV* gene segments. Seven of 21 patients with unmutated *IGHV* gene segments have discontinued treatment. One patient with mutated *IGHV* utilizing V_H3-21 progressed. Although no formal correlative analysis was possible due to small sample size, no differences in response or PFS according to chromosome 17p deletion or *TP53* mutation were observed. Seven of 10 patients with *TP53* mutation remain on therapy; of the 3 that discontinued study treatment, 1 progressed,1 was withdrawn due to an AE and 1 died from septicemia. The patient with the *TP53* mutation who progressed with a *TP53* mutation in the DNA binding domain (DeltaL252T253) had an initial response for 31 cycles on a dose of 40mg QD ONO/GS-4059. Since this patient lacked *BTK* and *PLCG2* mutations (data not shown), the dose of ONO/GS-4059 was increased to 600mg QD. The patient responded for a further 12 months, associated with a second lymphocytosis comparable to that seen initially (1.74-fold increase; initial 1.81-fold increase) and lymph nodal response (Figure 1b). One of 3 patients with *ATM* mutation has progressed on study (930 days). Eight patients had *SF3B1* mutations, 5 of whom have discontinued treatment (1 due to progression). *NOTCH1* mutations were found in 7 patients, 3 of whom have come off study (1 due to progression). As previously reported, *NOTCH1* and *SF3B1* were mutually exclusive.[17] No mutations appeared to predict shorter PFS with ONO/GS-4059, but the sample sizes were too small for statistical analysis. Mutations not previously identified in CLL include a mutation in *MEK1* (E203K) in 1 patient with early progression, previously reported in

metastatic melanoma resistant to vemurafenib,[18] and a *POT1* mutation E67K. No mutations in *MYD88, PLCG2,* or *BRAF* were observed.

ONO/GS-4059 continued to be well tolerated. Extended follow-up did not reveal new safety or toxicity concerns, and updated treatment-emergent AEs (TEAEs; frequency ≥15%) are shown in Table 1**.** Most TEAEs were grade 1 or 2. The most common AEs were bruising (35.7% all grades), neutropenia (35.7% all grades), and anemia (32.1% all grades). Only one grade 3 bleeding event (3.6%; hematoma) occurred on study in a patient not receiving anticoagulation therapy. Twelve patients (42.9%) had ≥grade 3 infections. There were no ≥grade 3 events reported for other AEs of interest with the BTKi class, including hypertension and atrial fibrillation. One patient had an AE of weight gain; 14 patients (50%) had a grade 1-3 weight gain. Similar weight gain has been reported with acalabrutinib.[11]

Interestingly, no cases of Richter's transformation have been reported in patients receiving ONO/GS-4059. Richter's transformation in patients receiving ibrutinib tends to occur early.[8,19]

In conclusion, these data strongly support the ongoing evaluation of ONO/GS-4059 in CLL. Patients with high-risk CLL genetics responded with minimal toxicity. Identification of significant differences in toxicity profiles between BTKis awaits direct comparative studies. However, the tolerability of ONO/GS-4059 shown here with extended follow-up may confer advantages, particularly in the context of combination therapies and in ibrutinib intolerant patients.

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Table 1. Updated TEAEs (frequency >15%) for CLL patients.

Adverse event	Grade 1-2	Grade 3-4	Total (%)
Bruising	10 (35.7%)	0 (0%)	10 (35.7%)
Neutropenia	3 (10.7%)	7 (25.0%)	10 (35.7%)
Nasopharyngitis	9 (32.1%)	0 (0%)	9 (32.1%)
Anemia	6 (21.4%)	3 (10.7%)	9 (32.1%)
Fall	9 (32.1%)	0 (0%)	9 (32.1%)
Cough	8 (28.6%)	0 (0%)	8 (28.6%)
Arthralgia	8 (28.6%)	0 (%)	8 (28.6%)
Basal cell carcinoma	8 (28.6%)	0 (0%)	8 (28.6%)
Lower respiratory tract infection	3 (10.7%)	4 (14.3%)	7 (25.0%)
Diarrhea	5 (17.9%)	2 (7.1%)	7 (25.0%)
Pyrexia	6 (21.4%)	1 (3.6%)	7 (25.0%)
Hematoma	6 (21.4%)	1 (3.6%)	7 (25.0%)
Abdominal pain (upper and lower)	6 (21.4%)	0 (%)	6 (21.4%)
Herpes zoster infection	6 (21.4%)	0 (%)	6 (21.4%)
Constipation	6 (21.4%)	0 (%)	6 (21.4%)
Dry skin	6 (21.4%)	0 (%)	6 (21.4%)
Macules	6 (21.4%)	0 (%)	6 (21.4%)
Petechiae	5 (17.9%)	1 (3.6%)	6 (21.4%)
Purpura	5 (17.9%)	1 (3.6%)	6 (21.4%)
Oropharyngeal pain	6 (21.4%)	0 (%)	6 (21.4%)
Paraesthesia	6 (21.4%)	0 (%)	6 (21.4%)
Bronchitis	5 (17.9%)	0 (%)	5 (17.9%)
Vomiting	5 (17.9%)	0 (%)	5 (17.9%)
Erythema	5 (17.9%)	0 (%)	5 (17.9%)
Fatigue	5 (17.9%)	0 (%)	5 (17.9%)
Musculoskeletal pain	5 (17.9%)	0 (%)	5 (17.9%)
Thrombocytopenia	1 (3.6%)	4 (14.3%)	5 (17.9%)

Figures and figure legends

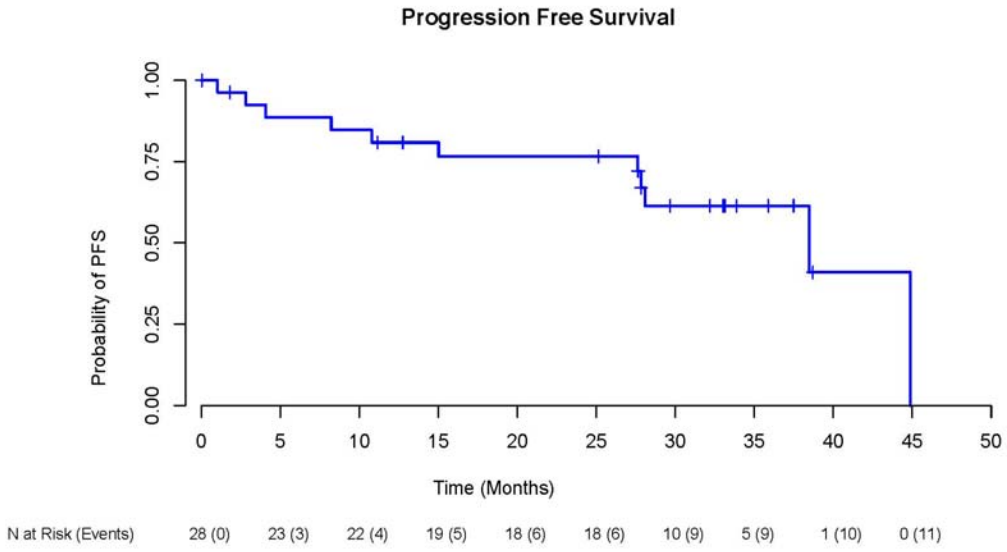

Figure 1a. Updated PFS curve for CLL patients. Mean duration on study was 26.6 months and estimated median PFS was 38.5 months.

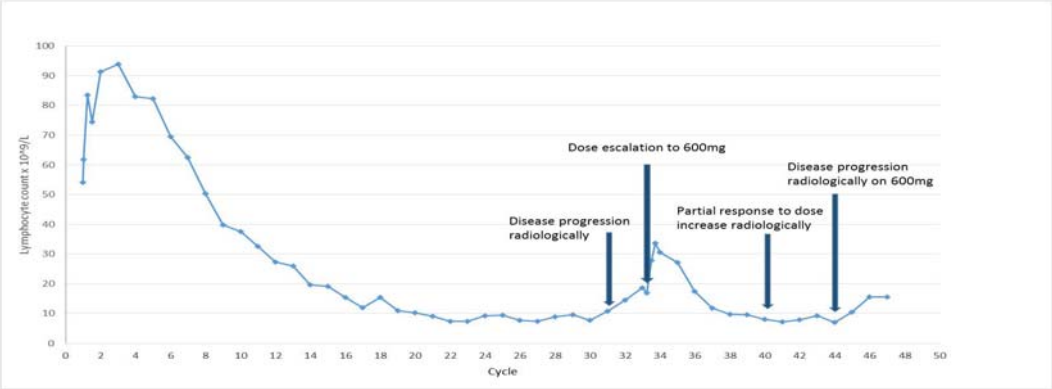

Figure 1b. Case example: Recurrent lymphocytosis shown in *TP53* mutant CLL patient following an initial lymph nodal response for 31 cycles on 40mg OD ONO/GS-4059; the patient subsequently responded for a further 12 months to 600mg OD, with a second lymphocytosis (1.74- fold increase; initial 1.81- fold increase) and lymph nodal response.

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