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Chronic lymphocytic leukemia

Phase 1 first-in-human trial of the anti-CD37 antibody BI 836826 in relapsed/refractory chronic lymphocytic leukemia

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To the Editor:

Chronic lymphocytic leukemia (CLL) remains incurable for most patients, requiring repeated therapy [1]. The tetraspanin CD37 is predominantly expressed on mature B cells, with low-level expression on T cells, granulocytes, and monocytes, and is widely expressed in mature B-cell malignancies, including CLL [2]. Upon cross-ligation, CD37 can function as a cell death receptor in CLL cells [3]. Thus, CD37 is an attractive target for CLL.

BI 836826 is a chimeric mouse–human monoclonal antibody that targets human CD37 with a dual mode of action that induces antibody-dependent cell-mediated cytotoxicity and has intrinsic proapoptotic activity [4]. BI 836826 has demonstrated stringent antitumor effects in preclinical cell-based assays and xenograft models, with superior activity versus rituximab [4]. These data warrant evaluation of BI 836826 in CLL and other mature B-cell malignancies. Here, we report a phase 1 first-in-human, open-label, dose-escalation study to evaluate the maximum tolerated dose (MTD), safety and efficacy of BI 836826 in relapsed/refractory CLL (NCT01296932).

Eligible patients had relapsed/refractory CLL according to the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) definition [5], and had received ≥2 prior treatment regimens (Supplementary Patients and Methods). The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and the protocol approved by the relevant institutional review boards. Patients provided written informed consent.

BI 836826 was administered at a starting dose of 1 mg (Supplementary Patients and Methods; Suppl. Fig. 1). Dose escalation followed a modified 3 + 3 design based on protocol-defined dose-limiting toxicities (DLTs; Supplementary Materials and Methods) during the first treatment cycle. Primary endpoints were MTD and number of patients with DLTs during cycle 1. MTD was defined as the highest

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dose of BI 836826 for which the incidence of DLT was no more than 1/6 patients during cycle 1. Patients received up to four treatment cycles, with additional cycles permitted in the case of clinical benefit. An expansion cohort was planned but did not occur because the trial was terminated due to the lack of recruitment after new drugs (idelelisib/ibrutinib) received marketing approval.

Adverse events (AEs) were graded according to the NCI-CTCAE (National Cancer Institute-Common Terminology Criteria for Adverse Events) (version 4.0) and collected up to 42 days after the last dose of BI 836826 (on-treatment period). Efficacy endpoints included (amongst others): best percentage change in peripheral lymphocyte count from baseline; number of patients with improved hemoglobin levels, platelet counts and neutrophil counts (Supplementary Patients and Methods); and best overall response by investigator assessment according to IWCLL criteria [5]. Blood samples for pharmacokinetic analysis were taken pre dose and at prespecified timepoints post dose (Supplementary Patients and Methods). Pharmacodynamic assessments were undertaken centrally and included assessment of circulating B lymphocyte antigen (CD19+) and CD19+/CD37+ cell counts (Supplementary Patients and Methods). Statistical analyses were descriptive and exploratory; no formal statistical tests were performed. Primary endpoints were based on the MTD evaluable set; the treated set was used for all other analyses.

Forty-five patients were screened across ten sites in three countries from November 2011 to October 2015. Thirty-seven patients were treated in eight cohorts: 1 mg ($n = 3$), 3 mg ($n = 3$), 9 mg ($n = 6$), 25 mg ($n = 6$), 50 mg ($n = 3$), 100 mg ($n = 3$), 200 mg ($n = 6$), 400 mg ($n = 3$), and 800 mg ($n = 4$). Median age (range) was 68 (44–80) years, 70% were male, and 65% had a del(17p) and/or *TP53* mutation (Suppl. Table 1). Patients received a median of 7 cycles (range 1–68); 13 patients (35.1%) received 8 cycles and five (13.5%) received >8 cycles. At the time of primary analysis, 36 patients had discontinued treatment due to: progressive disease ($n = 10$), DLT ($n = 2$), patient refusal ($n = 5$), other AE ($n = 7$), or other reasons ($n = 12$). Two patients (400 mg; 9 mg escalating to 25 mg after 16 cycles) derived prolonged clinical benefit and remained on treatment for nearly 3 years (70 and 68 cycles, respectively).

Two of 35 evaluable patients experienced a DLT in cycle 1; grade 3 hypophosphatemia (200 mg cohort) and grade 4 neutropenia lasting >7 days (800 mg cohort; Suppl. Table 2). Based on on-treatment DLTs and AEs leading to discontinuation, 800 mg was not considered tolerable. No DLTs were observed in patients receiving 400 mg. Doses between 400 and 800 mg were not explored as the trial was discontinued. A posthoc Bayesian logistic regression model indicated that 600 mg might be acceptable for further development.

Table 1 Overall summary of AEs, and treatment-related AEs occurring in >10% of patients

	Any grade *n* (%)	Grade 3 or 4 *n* (%)
Any AE	37 (100)	31 (83.8)
Treatment-related AE	35 (94.6)	27 (73.0)
Any SAE	18 (48.6)	13 (35.1)
Treatment-related SAE	12 (32.4)	8 (21.6)
AE leading to treatment discontinuation	10 (27.0)	7 (18.9)
Most common treatment-related AEs		
Infusion-related reaction	26 (70.3)	3 (8.1)
Chills	22 (59.5)	0 (0.0)
Pyrexia	18 (48.6)	1 (2.7)
Neutropenia	17 (45.9)	16 (43.2)
Thrombocytopenia	12 (32.4)	9 (24.3)
Anemia	10 (27.0)	6 (16.2)
Dyspnea	8 (21.6)	2 (5.4)
Nausea	8 (21.6)	0 (0.0)
Leukopenia	7 (18.9)	6 (16.2)
Hypertension	5 (13.5)	2 (5.4)
Vomiting	5 (13.5)	0 (0.0)
Aspartate aminotransferase increased	5 (13.5)	0 (0.0)
C-reactive protein increased	5 (13.5)	0 (0.0)
Alanine aminotransferase increased	4 (10.8)	1 (2.7)
Hypotension	4 (10.8)	1 (2.7)
Asthenia	4 (10.8)	0 (0.0)
Hyperhidrosis	4 (10.8)	0 (0.0)

AE adverse event, *SAE* serious adverse event

Thirty-five patients (94.6%) had ≥1 treatment-related AE (Table 1); the most frequent were infusion-related reactions (IRRs), chills, and pyrexia. The most frequent treatment-related grade 3/4 AEs were neutropenia, thrombocytopenia, anemia, and leukopenia. Twelve patients (32.4%) had treatment-related serious AEs, most frequently neutropenia (8.1%), IRRs, pyrexia, and leukopenia (all 5.4%). There were no cases of drug-related tumor lysis syndrome. Ten patients (27.0%) had AEs that resulted in discontinuation of BI 836826. AEs that most frequently resulted in discontinuation were thrombocytopenia and IRRs (both 5.4%). There were no fatal on-treatment AEs. IRRs were observed at all dose levels, most frequently in cycle 1 (Suppl. Fig. 2). IRRs generally occurred during infusion and quickly responded to standard management including treatment interruption and symptomatic therapy. IRRs decreased in frequency and severity following introduction of a modified, stepwise, infusion scheme (Supplementary Patients and Methods).

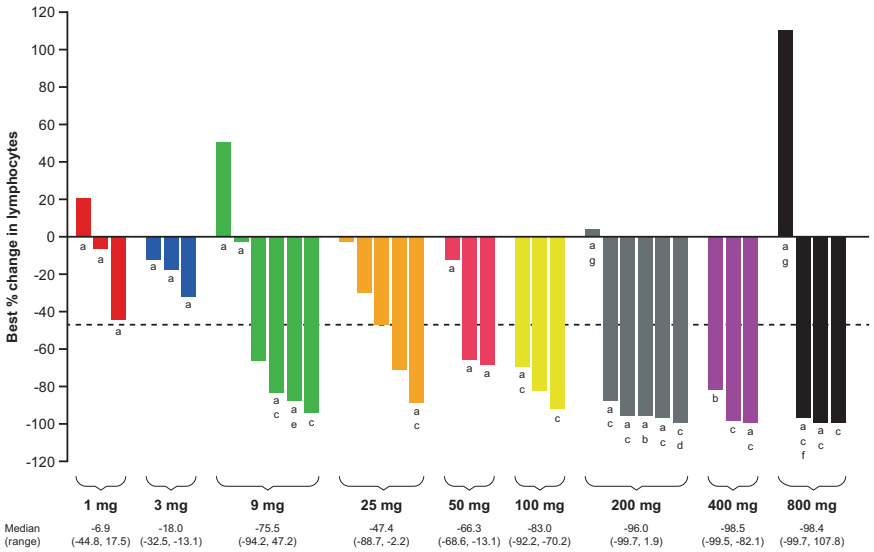

Fig. 1 Best percentage change from baseline in peripheral blood lymphocyte count by patient. **a** Patients presented with del(17p) and/or *TP53* mutation. **b** Patients had a baseline lymphocyte count of $<4 \times 10^9$/L. **c** Patients had a best percentage change from baseline to a lymphocyte count of $<4 \times 10^9$/L. **d** Patient was randomized to 200 mg but was escalated to 400 mg. **e** Patient was randomized to 9 mg but was escalated to 25 mg. **f** Patient was randomized to 800 mg but was de-escalated to 600 mg in cycle 3 and received 800 mg again by error in cycle 4. **g** Patients received only the predose, i.e., the first part of the split infusion of BI 836826 in cycle 1, which was 10% of the total dose, but maximally 10 mg

Baseline neutrophil, hemoglobin, and platelet counts were low in 21.6%, 35.1%, and 51.4% of patients, respectively (Table 1). During treatment, at least one neutrophil, hemoglobin, or platelet measurement declined to $<0.5 \times 10^9$/L (grade 4), <80 g/L (grade 3), or $<25 \times 10^9$/L (grade 4) in 62.1%, 43.2%, or 29.7% of patients, respectively. The degree of preexisting cytopenia in these patients is shown in Suppl. Fig. 3. Neutrophil and platelet counts declined immediately after the end of the infusion, with recovery prior to the next dose in most patients (Suppl. Fig. 4). Fifteen (40.5%) patients had grade 4 neutropenia lasting ≥7 days; none had concomitant CTCAE grade ≥3 infections. Five patients (13.5%) had grade 4 thrombocytopenia lasting ≥7 days; none had concomitant bleeding events of CTCAE grade ≥3.

Clinically relevant reductions in lymphocyte count were observed in all dose cohorts ≥9 mg (Fig. 1), including patients with a del(17p) or *TP53* mutation. Improvements in hemoglobin, neutrophil, and platelet counts are summarized in the Supplementary results. Thirteen patients had an objective response (35.1%; all PRs). At doses ≥9 mg and ≥200 mg, objective response rates (ORRs) were 41.9% (13/31 patients) and 61.5% (8/13 patients), respectively. ORR in patients with a del(17p) mutation and/or a *TP53* mutation was 45.8% (11/24 patients). ORR in rituximab-refractory patients was 47.6% (10/21 patients). All evaluable patients achieved disease control (complete response, PR, or stable disease).

Plasma exposure was highly variable and increased more than dose proportionally (Suppl. Table 3). No accumulation of plasma concentrations was observed after repeated administration. Following the end of infusions, plasma concentrations decreased rapidly and were below the limit of quantification by 336 h for doses <400 mg. Plasma clearance decreased with increasing doses, suggesting target-mediated drug clearance. Also, the volume of distribution decreased with increasing doses, with moderate variability across all dose groups.

At screening, CD37 expression on B cells was observed in all 35 evaluable patients. The median percentage of CD19+/CD37+ B cells was 86.4 (range 12.7–95.3; standard deviation: 21.3). A change in peripheral CD19+ B cells was seen at ≥9 mg, with a reduction following the first infusion. A clinically relevant and sustained reduction became evident from doses approximately ≥100 mg (Suppl. Fig. 5).

These data represent the first human experience with BI 836826, and indicate that it is tolerable up to at least 400 mg; additional doses of 400–800 mg remain unexplored due to study termination. The AE profile was similar to other Fc-modified antibodies [6–8], consisting predominantly of IRRs, neutropenia, and thrombocytopenia, which were manageable with supportive care. The frequency of IRRs and those resulting in treatment discontinuation were similar to those observed with other antibodies such as obinutuzumab and XmAb5574 in patients with CLL and were observed despite premedication [7, 8]. IRRs with BI 836826 are not considered a barrier to further development, and may be reduced by combination strategies that debulk CLL

burden prior to initial infusion. Neutropenia and thrombocytopenia were not associated with severe infections or bleeding. Their rapid kinetics and recovery suggest that cytopenia may be related to direct effects of BI 836826 on mature peripheral blood cells, as supported by preclinical data (unpublished). Prolonged administration of BI 836826 was possible without cumulative toxicity, with two patients receiving almost 3 years of treatment.

BI 836826 demonstrated notable clinical activity, achieving a clearance of CLL cells from peripheral blood. At doses that showed the strongest pharmacodynamic effect on B-lymphocyte counts, the ORR was 61.5%. Responses were also observed in patients with del(17p) or *TP53* mutations. Although phase 1/2 studies are limited by small patient numbers and dose variations, response rates with BI 836826 compare favorably to otlertuzumab or rituximab as single agents in CLL [9, 10], and are in the range observed with ofatumumab and obinutuzumab [6, 11]. Moreover, there is preclinical rationale to use BI 836826 in combination with cytotoxics and nonchemotherapy agents such as idelalisib [12, 13].

Overall, this study indicates that BI 836826 treatment in patients with CLL is a valid approach with acceptable tolerability and notable efficacy, especially in difficult-to-treat patients with poor-risk features including del(17p) or *TP53* mutations. CD37 is a promising therapeutic target that warrants further clinical investigation in CLL.

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Author contributions UUK managed study, revised and approved the final manuscript. SS performed research and collected and interpreted data, revised and approved the final manuscript. TAS, FO, J-FR, WS, FL, BE, LY, and TZ performed research and collected data, revised and approved the final manuscript. PB designed research, interpreted data, wrote, revised and approved the final manuscript. UVW analyzed data, revised and approved the final manuscript.

Compliance with ethical standards

Conflict of interest SS reports consultancy, speaker honoraria and research grants from AbbVie, Amgen, AstraZeneca, Boehringer Ingelheim, Celgene, Gilead, GSK, Hoffmann La-Roche, Janssen, and Novartis. BE reports consultancy for Gilead, Roche, Janssen, Abbvie, Novartis, Celgene, and research funding from Gilead, Roche, Janssen, Abbvie, and honoraria from Gilead, Roche, Janssen, Abbvie, Novartis, Celgene. UVW, UUK, and PB report employment for Boehringer Ingelheim Pharma GmbH & Co KG. FL reports consultancy, research funding, honoraria and payment for expert testimony from Novartis, Incyte, and BMS. TZ holds a patent (WO2012007576A1). The remaining authors have nothing to disclose.

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Minimal resdiual disease

getITD for *FLT3*-ITD-based MRD monitoring in AML

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To the Editor:

The clinical relevance of measurable residual disease (MRD) monitoring has been well recognized in acute myeloid leukemia (AML) [1] and respective assays have been established for several recurrent leukemic markers [2, 3]. However, although internal tandem duplications in the *FLT3* gene (*FLT3*-ITDs) are the most common poor prognosis AML drivers [4], they have remained a challenging target: Their heterogeneity makes conventional PCR-based methods either insensitive or laborious [5]. Yet with recently approved FLT3-kinase inhibitors available [6], a specific monitoring of *FLT3*-mutation loads, and thus response to targeted therapy, is of particular interest. Next-generation sequencing (NGS) workflows for *FLT3*-ITD monitoring have been described, but were previously either proprietary or undisclosed [7, 8], unable to detect and correctly annotate all of the tested ITDs [9, 10], or used in conjunction with manual analysis with inherently subjective results [10].

We have therefore developed a new method based on targeted high-coverage NGS and our novel, open-source analysis program *getITD*. For assay assessment, we sequenced 3 human AML cell lines (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany), 2 healthy volunteers, and 57 samples from 28 AML patients who were all included in the AMLSG BiO Registry study (NCT01252485) and gave their informed consent according to the Declaration of Helsinki. We show that our workflow detects ITDs of a broad range of lengths, insertion sites and variant allele frequencies (VAFs) with high accuracy and precision, is fully objective without any requirement for manual analysis, and thus applicable to routine clinical monitoring. Sample and method details are provided as supplementary information. *getITD* is freely available at https://github.com/tjblaette/getitd.

To demonstrate our assay's specificity, we analyzed three *FLT3*-ITD negative control samples (peripheral blood of healthy volunteers, $n = 2$; AML cell line HL-60). No ITDs were reported, indicating an assay specificity of 100% (coverage: 1.1–4.2 million, mean 2.6 million paired-end reads). To assess sensitivity, we analyzed two serial dilutions of *FLT3*-ITD positive in *FLT3*-ITD-negative DNA from AML cell lines MOLM-14 (21 bp ITD, 67% VAF [11]), PL-21 (126 bp ITD, 33% VAF [11]), and HL-60 (*FLT3*-ITD negative [11]). For each of the ITD-positive cell lines, we sequenced undiluted DNA and 3–4 serial 1:10 dilutions in HL-60 (1.1–2.9 million, mean 2.0 million paired-end reads). The expected ITDs were detected in all samples and VAF estimates were accurate and decreased

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