A phase I study of the fully human, fragment crystallizable-engineered, anti-CD-33 monoclonal antibody BI 836858 in patients with previously-treated acute myeloid leukemia

In recent years, several research programs in acute myeloid leukemia (AML) have investigated the use of therapeutic monoclonal antibodies, which primarily elicit their effects through direct cell killing (apoptosis), via antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP).[1,2] Attention has been particularly focused on the myeloid differentiation antigen CD33,[2] which is expressed on the surface of leukemic blast cells of almost all AML patients.[1] While the activity of unconjugated anti-CD33 antibodies such as lintuzumab has been generally disappointing to date,[3-6] clinical experience with gemtuzumab ozogamicin, a humanized anti-CD33 antibody-drug conjugate, provides proof-of-principle for targeting CD33 in patients with AML.[7,8]

BI 836858 is a fully humanized IgG1 unconjugated anti-CD33 monoclonal antibody.[1,9] Unlike lintuzumab, BI 836858 was fragment crystallizable (Fc)-engineered for increased binding to FcγRIIIa (CD16),[9] a receptor found on the surface of natural killer (NK) cells and known to be involved in ADCC signaling.[1,10] Accordingly, BI 836858 demonstrated superior ADCC to lintuzumab in AML cells in the laboratory.[9] Here, we report the findings of a phase I dose escalation study of BI 836858 in patients with relapsed or refractory (R/R) AML, according to World Health Organization 2016 criteria (clinicaltrials gov. Identifier: NCT01690624).

Details of the study are available on request. Briefly, BI 836858 was administered as an intravenous (i.v.) infusion every 7 days in a 14-day treatment cycle (days 1 and 8). Premedication was obligatory prior to the first three administrations (acetaminophen/paracetamol; antihistamine; glucocorticoid). The starting dose of BI 836858 was 10 mg, and in the absence of dose-limiting toxicities (DLT), dose escalation up to 320 mg was planned. Patients could receive up to eight repeated administrations of BI 836858 and in the case of clinical benefit and acceptable tolerability were allowed to continue treatment beyond that until disease progression. In the first seven patients, infusions were administered over a 3-hour period; however, due to the occurrence of a high number of infusion-related reactions (IRR), administration was adapted to a stepwise rate-controlled infusion with an increased premedication glucocorticoid dose (100 mg prednisolone or equivalent). If no IRR were apparent after first administration, the dose was reduced to 50 mg for adminstrations two to four, 25 mg for administration 5 and zero thereafter. Also, the protocol was adapted according to tumor load; patients with >5,000 leukocytes/μL peripheral blood were excluded. The primary endpoints for the study were the maximum tolerated dose (MTD) and number of patients with DLT during the MTD evaluation period (the first 2 treatment cycles). Secondary efficacy endpoints included best overall response according to International Working Group Criteria and progression-free survival (PFS), defined as the time from first treatment with BI 836858 until disease progression, relapse or death.

Fifty-five patients were screened and 27 were treated with BI 836858 (10-40 mg; Table 1). The median duration of treatment was 21 days (range, 1-99 days), with a median of three infusions given (range, 1-14 infusions). The DLT evaluation period was not completed by 13 patients for reasons other than DLT (progressive disease, n=7; fatal intracranial hemorrhage, n=1; other adverse event [AE], n=2; patient refusal, n=1; not evaluable per protocol, n=1; persistent disease/lack of efficacy, n=1), therefore the MTD analysis set comprised 14 patients. Two patients in the 10 mg cohort had DLT. One patient had drug-related grade 3 elevated alanine aminotransferase (ALT) and aspartate transaminase (AST) 2 days after the first dose of BI 836858, which resolved within 8 days and 5 days, respectively. No change was made to study treatment for this patient. A second patient had a treatment-related grade 3 liver function test increased 3 days after the first dose of BI 836858, which resolved within 6 days. BI 836858 was discontinued in this patient. Following protocol amendment, no further DLT were reported in the 10, 20 or 40 mg dose cohorts. The MTD was not reached because the study was prematurely terminated by the sponsor, based on interim pharmacodynamic evaluations.

The most common AE were febrile neutropenia (44%), nausea (44%), IRR (41%) and anemia (37%; Table 2). Febrile neutropenia (41%) was the most frequent grade 3 AE. Grade 4 AE were reported in six patients (22%). Seventeen (63%) patients had AE considered related to BI 836858 (9 in the 10 mg cohort, 5 in the 20 mg cohort, and 3 in the 40 mg cohort). The most common were IRR (11 patients [41%]). The rate of IRR was higher prior to adaptation of the infusion protocol (57% *vs.* 35% after adaptation) with all IRR occurring in patients with a white blood cell (WBC) count of $>10x10^3/\mu L$. The majority of IRR occurred during the first six infusions. All cases of IRR were manageable with established supportive care measures. No patients experienced AE that led to dose reductions. Six (22%) patients discontinued treatment due to AE, including one with grade 3 febrile neutropenia (20 mg dosing cohort), one with grade 2 leukocytosis (10 mg), one grade 3 cardiac failure (40 mg), one grade 3 IRR (20 mg), one grade 3 liver function test increased (10 mg) and one patient with a grade 4 neutrophil count decreased (10 mg). Twenty-three patients (85%) had serious AE (SAE); five SAE were considered to be related to the study drug and were recorded in four patients (grade 3 IRR; grade 3 IRR and grade 3 liver function test increased; grade 3 non-cardiac chest pain; grade 3 elevated ALT/AST). Sixteen patients died during the study (8 during the on-treatment period; 4 of which were due to disease progression and 4 were due to unknown reasons). None of the deaths were related to study treatment. Special search categories for AE (i.e., user-defined search categories) were used for this study to adequately monitor the frequency and severity of anticipated AE (e.g., potential class effects). The most frequent user-defined AE were neutropenia (48%), nausea (44%), IRR (41%) and drug-related hepatic disorders (33%). Laboratory evaluation demonstrated that 24 (89%) patients had low WBC counts (grade 3: 33%; grade 4: 52%)

BI 836858 exhibited two-compartmental pharmacokinetic behavior, with maximum plasma concentrations generally achieved at the end of the infusion (Table 3). From 10 to 40 mg BI 836858, maximum plasma concentrations for patients with R/R AML patients increased in a dose-proportional manner (*Online Supplementary Figure S1*). BI 836858 exposure and apparent terminal half-life ($t_{1/2}$) increased with increasing doses (Table 3). Total plasma clearance was low and decreased in a dose-dependent manner. The volume of distribution was small, at approximately 6-7 L. No accumulation of BI 836858 was observed in plasma after weekly doses of up to 20 mg.

Slight accumulation was observed in patients receiving 40 mg. Anti-drug antibodies were detected in one of 25 patients with R/R AML after the first infusion in cycle 1; however, this patient had tested positive prior to treatment commencement.

Baseline absolute counts of CD33⁺ blasts in peripheral blood and bone marrow were highly variable, ranging from 15% to 95% and 28% to 95% of total blasts, respectively. Overall, total blood blasts remained unchanged following treatment with BI 836858. However, pharmacodynamic analyses showed decreased levels of CD33⁺ blasts in blood after adminis-

Table 1. Baseline demographics and characteristics of patients with relapsed/refractory acute myeloid leukemia treated with BI 836858.

Characteristics	BI 836858 dose 10 mg N=12	20 mg N=8	40 mg N=7	All patients N=27
Male	6 (50)	6 (75)	3 (43)	15 (56)
Race				
White	12 (100)	6 (75)	6 (86)	24 (89)
Black/African American	0	2 (25)	0	2 (7)
Multiple*	0	0	1 (14)	1 (4)
Age, years				
Median (range)	63.5 (36-77)	62.0 (45-67)	70.0 (63-81)	67.0 (36-81)
<65	6 (50)	4 (50)	1 (14)	11 (41)
≥65	6 (50)	4 (50)	6 (86)	16 (59)
ECOG PS				
0	4 (33)	1 (13)	1 (14)	6 (22)
1	8 (67)	5 (63)	3 (43)	16 (59)
2	0	2 (25)	3 (43)	5 (19)
Type of AML				
De novo	7 (58)	3 (38)	5 (71)	15 (56)
Secondary	5 (42)	5 (63)	2 (29)	12 (44)
Blasts in bone marrow				
Median, % (range)	18.0 (4.0-96.0)	56.0 (0-90.0)	50.0 (1.0-92.0)	41.5 (0-96.0)
Bone marrow blasts category				
<30%	7 (58)	2 (25)	2 (29)	11 (41)
≥30%	4 (33)	6 (75)	5 (71)	15 (56)
Missing	1 (8)	0	0	1 (4)
Previous systemic anti-leukemia therapies				
Median, n (range)	2.0 (1-7)	3.5 (1-9)	2.0 (1-5)	3.0 (1-9)
≥1 line of iHD	10 (83)	8 (100)	7 (100)	25 (93)
≥1 line of pLD	3 (25)	3 (38)	3 (43)	9 (33)
≥1 line of ASCT	4 (33)	2 (25)	1 (14)	7 (26)
≥1 line of other	2 (17)	1 (13)	0	3 (11)

*American Indian/Alaskan native and White. Data are n (%), unless otherwise stated. iHD: intensive high dose; pLD: palliative low dose; ECOG PS: Eastern Cooperative Oncology Group performance status; R/R AML: relapsed or refractory acute myeloid leukemia; ASCT: allogeneic stem cell transplantation.

Table 2. All-cause adverse events by MedDRA preferred terms and highest CTCAE grade in patients with relapsed or refractory acute myeloid leukemia treated with BI 836858 during the on-treatment period.

AE, n (%)	All grades	Grade 1/2	Grade 3	Grade 4	Grade 5
Total with AE	27 (100)	2 (7)	11 (41)	6 (22)	8 (30)
Febrile neutropenia	12 (44)	1 (4)	11 (41)	0	0
Nausea	12 (44)	12 (44)	0	0	0
Infusion-related reactions	11 (41)	8 (30)	3 (11)	0	0
Anemia	10 (37)	1 (4)	8 (30)	1 (4)	0
Dyspnea	9 (33)	8 (30)	1 (4)	0	0
Platelet count decreased	8 (30)	0	0	8 (30)	0
Constipation	7 (26)	7 (26)	0	0	0
White blood cell count decreased	7 (26)	1 (4)	1 (4)	5 (19)	0
Hypokalemia	7 (26)	6 (22)	1 (4)	0	0
Fatigue	6 (22)	5 (19)	1 (4)	0	0
Sepsis	4 (15)	0	1 (4)	2 (7)	1 (4)
Atrial fibrillation	3 (11)	0	3 (11)	0	0

Adverse events (AE) shown are those occurring in >20% of patients for all grades and grade 1/2, >10% for grade 3 and all grade 4. CTCAE: common terminology criteria for adverse events; R/R AML: relapsed or refractory acute myeloid leukemia. Medical dictionary for drug regulatory activities (MedDRA) version used for reporting: 21.0.

Table 3. Summary of pharmacokinetic parameters following a single infusion of BI 836858 in patients with relapsed or refractory acute myeloid leukemia (n=25, infusion duration 3-6 hours in different dose groups).

	Pharmacokinetic parameters*							
	$AUC_{0-\infty}$ ng.h/mL	$AUCO_{-\infty, norm}$ (ng.h/mL)/mg	C_{max} ng/mL	$C_{max, norm}$ (ng/mL)/mg	t_{max} hours	$t_{½}$ hours	CL mL/min	V_{ss} L
10 mg† (n=12)	23,400 (137)	2,340 (137)	873 (207)	87.3 (207)	4.43 (3.00-7.00)	11.3 (64.3)	7.11 (137)	7.01 (53.0)
20 mg‡ (n=6)	97,900 (114)	4,890 (114)	3,270 (35.4)	164 (35.4)	6.10 (5.00-8.80)	21.4 (91.7)	3.40 (114)	6.30 (29.4)
40 mg‡ (n=7)	296,000 (256)	7,400 (256)	6,100 (82.1)	152 (82.1)	5.87 (5.00-8.45)	34.5 (107)	2.25 (256)	6.86 (69.5)

*Shown are geometric mean (%gCV) non-compartmental pharmacokinetic parameters of BI 836858 except for t_{max} which is shown as median (range); †infusion range from 3-6 hours; ‡5-hour infusion. $AUC_{0-\infty}$: area under the plasma concentration-time curve over the time interval from zero extrapolated to infinity; CL: clearance; C_{max}: maximum concentration; gCV: geometric coefficient of variance; NA: not assessable; NC: not calculated; norm: normalized to administered dose; R/R AML: relapsed or refractory acute myeloid leukemia; t_{max}: time point at which maximum concentration is reached; t.: apparent half-life; V_{ss}: volume of distribution at steady state

tration of 40 mg BI 836858, indicating target engagement (*Online Supplementary Figure S2*). In the 40 mg dose cohort, $CD33^+$ blasts were undetectable in the bone marrow of five of seven patients on cycle 1 day 8, suggesting target saturation. Conversely, in the 10 mg cohort, the majority of patients had detectable $CD33^+$ blasts at this time point. Most patients had low NK counts (CD3-negative, CD16-positive, CD56-positive) in the blood and bone marrow at screening (*Online Supplementary Figure S3*). Peripheral blood NK cells were below the lower limit of normal (<30 cells/μL), within the lower end of normal range (30-150 cells/μL), normal (>150 cells/μL) and missing in 30%, 56%, 11% and 4% of patients, respectively. Of note, there was a transient decline in NK cell counts at cycle 1 day 4 compared to the screening values in patients across all treatment cohorts. NK cells in the bone marrow ranged from very few to 7%. In both blood and bone marrow, there were no significant changes in the numbers of activated NK cells (expressing CD69 or CD158b) during treatment (*data not shown*). Monocyte counts were also below, or at the very end of the lower limit of the normal range in the majority (59%) of patients.

No responses to treatment were detected; 23 patients (85%) were removed from the study due to lack of response with persistent AML, two (7%) patients were not evaluable, and two (7%) did not have a post-baseline assessment. Median PFS was 29 days (95% confidence interval: 27-50). There were no notable changes in the percentage of myeloid blasts in the bone marrow during the treatment period.

To conclude, this study provides valuable information about the safety, efficacy, pharmacokinetics and pharmacodynamics of BI 836858 in patients with R/R AML which may have important implications for potential future development of immunotherapies in this setting. BI 836858 had predictable and manageable tolerability with no unexpected AE. However, although there was evidence of target engagement in the blood and to some extent in the bone marrow, no responses to BI 836858 were observed. The primary objective of MTD was not met due to premature termination. We hypothesize that the low levels of baseline effector cells observed in this study were relevant to the lack of efficacy of BI 836858. Other studies indicate that baseline NK cell phenotype and function is defective in patients with AML.[11] Interestingly, phenotypic and functional abnormalities of NK cells appear to be partially restored in AML patients achieving a complete remission (CR).[11] As BI 836858 relies on ADCC for functionality, the low levels of effector cells detected in the patient population, and the lack of pharmacodynamic effects (see *Online Supplementary Appendix*), underpinned the decision to terminate the study based on a lack of perceived benefit over risk.

In contrast to the current study, lintuzumab conferred objective responses including CR in a phase I dose escalation trial in R/R AML.[12] Given that BI 836858 was a similar design to lintuzumab (both were fully humanized IgG1 monoclonal antibodies, though BI 836858 was Fc engineered to increase ADCC) and was considered superior to lintuzumab in preclinical experiments,[9] it is not clear why it did not show clinical activity in this phase I study. Lintuzumab is dependent on engagement of several immune effector cells including macrophages/monocytes (that facilitate ADCP)[13] as well as NK cells (that facilitate ADCC).[13] The impact of BI 836858 on immune effector cell function in patients requires more evaluation. It is possible that differences in internalization kinetics of CD33 following engagement with lintuzumab or BI 836858 may influence immunogenicity. While internalization is slower with BI 836858 and this correlates with superior ADCC in cell-based assays,[9] it is uncertain how this may influence other effector functions. Also, lack of efficacy could potentially relate to pre-administration of glucocorticoids. Despite steps to reduce glucocorticoid dose as soon as possible, administration may interfere with NK cell function, as indicated in previous preclinical studies.[14,15] However, unpublished observations suggest that BI 836858 may activate NK cells when combined with decitabine, despite premedication with glucocorticoids. Further clinical studies have been designed to assess combination regimens that may potentiate the activity of BI 836858 by increasing effector immune function. For example, based on preclinical evidence that decitabine increased BI 836858 activity via upregulation of the NK group 2D ligand,[9] a phase II study is exploring the efficacy and safety of this combination (clinicaltrials gov. Identifier: NCT02632721). Other BI 836858-based combinations are also being assessed in a multi-sub-study phase Ib/II trial (clinicaltrials gov. Identifier: NCT03013998). These studies will include post-remission therapy when immune effectors might be more numerous and active.

Sumithira Vasu,[1] Jessica K. Altman,[2] Geoffrey L. Uy,[3] Martin S. Tallman,[4] Ivana Gojo,[5] Gerard Lozanski,[6] Ute Burkard,[7] Annika Osswald,[7] Pamela James,[8] Björn Rüter[8] and William Blum[9]

[1]Division of Hematology, Department of Internal Medicine, The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA; [2]Division of Hematology/Oncology, Northwestern University, Chicago, IL, USA; [3]Washington University School of Medicine, Siteman Cancer Center, St Louis, MO, USA; [4]Leukemia Service, Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, New York, NY, USA; [5]The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA; [6]Department of Pathology, The Ohio State University, Columbus, OH, USA;

[7]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riß, Germany; [8]Boehringer Ingelheim Pharmaceuticals, Inc, Ridgefield, CT, USA and [9]Department of Hematology and Medical Oncology, Emory University School of Medicine, Winship Cancer Institute, Atlanta, GA, USA

Correspondence:

WILLIAM BLUM - william.g.blum@emory.edu

doi:10.3324/haematol.2020.274118

Received: October 22, 2020.

Accepted: November 25, 2021.

Pre-published: December 2, 2021.

Disclosures: SV reports receiving funding from Boehringer Ingelheim for Advisory Board participation. JKA reports being part of an advisory council or committee for Astellas, Novartis, Cancer Expert Now, Agios, Glycomimetics, Theradex, AbbVie and Daiichi Sankyo, and being a speaker for PeerView, prIME Oncology, France Foundation. GLU reports receiving honoraria from Astellas and consulting fees from Genentech and Jazz. MST reports being part of an advisory council or committee for AbbVie, Daiichi-Sankyo, Orsenix, KAHR, Rigel, Delta Fly Pharma, Tetraphase, Oncolyze, Jazz Pharma, Roche, Biosight and Novartis, receiving grants or funds from AbbVie, Orsenix, ADC Therapeutics, Biosight, Glycomimetics, Rafael and Amgen, and other potential financial relationship with UpToDate. IG reports receiving grants or funds from Merck, Amgen, Amphivena, Celgene and Genentech. GL reports receiving funding of flow cytometry analysis of samples for this study from Boehringer Ingelheim. UB and AO report employment with Boehringer Ingelheim Pharma GmbH & Co. KG. PJ reports no conflict of interests. BR reports employment and other potential financial relationship with Boehringer Ingelheim Pharmaceuticals Inc. WB reports receiving consulting fees from Amerisource Bergen and grants or funds from Leukemia and Lymphoma Society, Xencor, Forma, Celyad and Novartis.

Contributions: JKA, GLU, MST, BR and WB designed the study; SV, WB, MT, JKA, MST, GLU and GL conducted the study, analyzed data, wrote and edited the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work, which includes ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgements: the authors would like to thank the patients and their families, the research staff and Anne Esler for statistical analysis. Medical writing assistance during the preparation of this manuscript was provided by Lynn Pritchard, Ashfield MedComms, an Ashfield Health Company, which was funded by Boehringer Ingelheim.

Funding: this work was supported by Boehringer Ingelheim.

Data sharing statement: the clinical study report (including appendices, but without line listings) and other clinical documents related to this study may be accessed on request. Prior to providing access, the documents and data will be examined, and, if necessary, redacted and de-identified to protect the personal data of study participants and personnel, and to respect the boundaries of the informed consent of the study participants. See https://trials.boehringer-ingelheim.com/data_sharing/sharing.html#accordion-1-2 for further details. Bona fide, qualified scientific and medical researchers may request access de-identified, analyzable patient-level study data, together with documentation describing the structure and content of the datasets. Researchers should use https://vivli.org/ to request access to raw data from this study.

References

1. Walter RB. Investigational CD33-targeted therapeutics for acute myeloid leukemia. Expert Opin Investig Drugs. 2018;27(4):339-348.
2. Williams BA, Law A, Hunyadkurti J, Desilets S, Leyton JV, Keating A. Antibody therapies for acute myeloid leukemia: unconjugated, toxin-conjugated, radio-conjugated and multivalent formats. J Clin Med. 2019;8(8):1261.
3. Feldman EJ, Brandwein J, Stone R, et al. Phase III randomized multicenter study of a humanized anti-CD33 monoclonal antibody, lintuzumab, in combination with chemotherapy, versus chemotherapy alone in patients with refractory or first-relapsed acute myeloid leukemia. J Clin Oncol. 2005;23(18):4110-4116.
4. Borthakur G, Rosenblum MG, Talpaz M, et al. Phase 1 study of an anti-CD33 immunotoxin, humanized monoclonal antibody M195 conjugated to recombinant gelonin (HUM-195/rGEL), in patients with advanced myeloid malignancies. Haematologica. 2013;98(2):217-221.
5. Sekeres MA, Lancet JE, Wood BL, et al. Randomized phase IIb study of low-dose cytarabine and lintuzumab versus low-dose cytarabine and placebo in older adults with untreated acute myeloid leukemia. Haematologica. 2013;98(1):119-128.
6. Feldman E, Kalaycio M, Weiner G, et al. Treatment of relapsed or refractory acute myeloid leukemia with humanized anti-CD33 monoclonal antibody HuM195. Leukemia. 2003;17(2):314-318.
7. Castaigne S, Pautas C, Terre C, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. Lancet. 2012;379(9825):1508-1516.
8. Hills RK, Castaigne S, Appelbaum FR, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. Lancet Oncol. 2014;15(9):986-996.
9. Vasu S, He S, Cheney C, et al. Decitabine enhances anti-CD33 monoclonal antibody BI 836858-mediated natural killer ADCC against AML blasts. Blood. 2016;127(23):2879-2889.
10. Romain G, Senyukov V, Rey-Villamizar N, et al. Antibody Fc engineering improves frequency and promotes kinetic boosting of serial killing mediated by NK cells. Blood. 2014;124(22):3241-3249.
11. Stringaris K, Sekine T, Khoder A, et al. Leukemia-induced phenotypic and functional defects in natural killer cells predict failure to achieve remission in acute myeloid leukemia. Haematologica. 2014;99(5):836-847.
12. Raza A, Jurcic JG, Roboz GJ, et al. Complete remissions observed in acute myeloid leukemia following prolonged exposure to lintuzumab: a phase 1 trial. Leuk Lymphoma. 2009;50(8):1336-1344.
13. Sutherland MK, Yu C, Lewis TS, et al. Anti-leukemic activity of lintuzumab (SGN-33) in preclinical models of acute myeloid leukemia. MAbs. 2009;1(5):481-490.
14. Kumai T, Oikawa K, Aoki N, Kimura S, Harabuchi Y, Kobayashi H. Assessment of the change in cetuximab-induced antibody-dependent cellular cytotoxicity activity of natural killer cells by steroid. Head Neck. 2016;38(3):410-416.
15. Eddy JL, Krukowski K, Janusek L, Mathews HL. Glucocorticoids regulate natural killer cell function epigenetically. Cell Immunol. 2014;290(1):120-130.