Supplementary data

Supplementary methods

Dose-limiting toxicities

Dose-limiting toxicities (DLTs) were assessed from day -14 (following administration of the loading dose of cusatuzumab) to the end of Cycle 1. They were defined as any of the following drug-related events:

- Any grade ≥3 drug-related non-hematologic toxicity with a duration >14 days, except grade 3 events for which the patients had responded optimally to treatment with standard medication, with a duration >14 days
- Grade ≥3 infusion-related reactions
    - Reversible grade 3 infusion-related reactions (defined as an allergic reaction/hypersensitivity, fever, pain, bronchospasm, wheezing, or hypoxia) occurring during or within 24 hours after completion of the infusion and not recurring following a reduction in the infusion rate, provision of supportive care, and/or administration of corticosteroids were not considered to be DLTs
- Inability to administer the next dose due to a drug-related adverse event or a delay of the administration of the next dose due to toxicities for >14 days despite adequate medication
- Drug-related grade 4 febrile neutropenia
- Drug-related grade 4 anemia that could not be treated adequately by blood transfusions.

Isolated grade ≥3 laboratory abnormalities (including isolated lymphopenia) that resolved to baseline or to grade 2 within 21 days, without clinical sequelae or the need for therapeutic intervention, and grade 3 fatigue lasting for ≤14 days were not considered to be DLTs.

Eligibility criteria

Inclusion criteria:

1. Signed informed consent form indicating an understanding of the purposes, risks, and procedures required for the study, and a willingness and ability to participate in the study
2. Acute myeloid leukemia (AML) or high-risk myelodysplastic syndrome (MDS), according to the 2016 World Health Organization classification definition of ≥20% bone marrow blasts,[1] unsuitable for intensive treatment (including stem cell transplantation) with curative intent, but eligible to receive azacitidine treatment.

   Note: MDS patients could be rescreened if previously excluded on blast count
3. Aged ≥18 years
4. Expected life expectancy ≥3 months, at the discretion of the investigator
5. Eastern Cooperative Oncology Group performance status of 0, 1, or 2.
6. Women of childbearing potential had to have a negative serum pregnancy test at screening and within 48 hours before infusion of cusatuzumab on day -14, and be willing to use an effective contraceptive method (intrauterine devices, hormonal contraceptives, contraceptive pill, implants, transdermal patches, hormonal vaginal devices, or infusions with prolonged release) during the study and for at least 3 months after the last study drug administration
7. Men had to be willing to use an effective contraceptive method (e.g., condom, vasectomy) during the study and for at least 3 months after the last study drug administration.

Exclusion criteria:

1. Prior or concurrent malignancy, except for the following:
   - Adequately treated basal cell or squamous cell skin cancer
   - Carcinoma *in situ* of the cervix
   - Carcinoma *in situ* of the breast, or

- Incidental histological finding of prostate cancer (TNM stage T1a or T1b), or
- Any other cancer from which the patient had been disease-free for >2 years

2. Any previous chemotherapy or radiotherapy for AML or MDS, except hydroxyurea/hydroxycarbamide for leukocyte control (which had to be discontinued by the first day of azacitidine administration), local radiation therapy, and therapy for basal or squamous cell carcinoma of the skin
3. Treatment with any investigational product within 4 weeks before the first administration of cusatuzumab
4. Abnormal organ function, defined as follows (any single parameter to fulfill condition):
   - Aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) >3 × the upper limit of normal (ULN); or in case of liver infiltration by AML, AST and/or ALT >5 × ULN
   - Alkaline phosphatase (AP) >2.5 × ULN; or in case of liver infiltration by AML, AP >5 × ULN
   - Serum (total) bilirubin >1.5 × ULN; or in case of liver infiltration by AML, serum (total) bilirubin >5 × ULN
   - Serum creatinine >2.5 × ULN or glomerular filtration rate (Modification of Diet in Renal Disease) <40 mL/min for patients with creatinine levels above the normal limit
5. Use of immunosuppressive agents in the past 4 weeks before the first administration of cusatuzumab on day -14. For regular use of systemic corticosteroids, patients could only be included after stepwise discontinuation and had to be free of steroids for a minimum of 5 days before the first administration of cusatuzumab
6. Any known active or chronic infection, including human immunodeficiency virus, and hepatitis B or C virus infection.
7. Any other concurrent disease or medical condition that was likely to interfere with study procedures or results, or that in the opinion of the investigator would constitute a hazard for participating in this study

8. Known hypersensitivity to cusatuzumab or azacitidine and its analogs in general, or to any other component of the study drug formulation
9. Congestive heart failure New York Heart Association (NYHA) Class III and IV, cardiac arrhythmias (except atrioventricular block type I and II, atrial fibrillation/flutter, or bundle brunch block) or other signs and symptoms of relevant cardiovascular disease
10. Pregnant women, nursing mothers, lactating women, and women of childbearing potential who were unwilling to use effective contraceptive methods (intrauterine devices, hormonal contraceptives, contraceptive pill, implants, transdermal patches, hormonal vaginal devices, or infusions with prolonged release) during the study from the time of consent and for at least 3 months after the last study drug administration
11. Men who were unwilling to use effective contraception for the duration of the study and for at least 3 months after the last study drug administration
12. Patients who were unwilling or unable to follow the protocol requirements.

Assessments

Disease assessments were based on peripheral blood counts and bone marrow (aspirate or biopsy) evaluations, as per clinical practice guidelines,[2-4] and were classified according to established criteria for AML (**Table S1**). Bone marrow samples for disease assessment were taken on Cycle 1 day 1 (pre-azacitidine), and on day 1 (pre-azacitidine) of every odd cycle from Cycle 3 onwards until complete remission (CR) or CR with incomplete recovery (CRi), and at the end-of-treatment visit. Additional samples could be taken as instructed by the treating physician. Bone marrow aspirate was assessed by experienced hematopathologists at local pathology laboratories and there was no central assessment performed.

Minimal residual disease assessments on bone marrow aspirates were performed at either Inselspital Bern or Covance Laboratory using multiparameter flow cytometry (based on quantification of leukemia-associated immunophenotype [LAIP]-positive cells) according to European LeukemiaNet guidelines.[5] Samples were collected as per the response evaluations.

Minimal residual disease-negative status was defined as $<10^{-3}$ LAIP-positive cells.

Blood samples for pharmacokinetic assessments of cusatuzumab were taken on day -14 (pre-, and 0, 2, 24, and 96 hours post-cusatuzumab), Cycle 1 day 1 (pre-azacitidine), Cycle 1 day 3 (pre-azacitidine, and 0, 2, and 24 hours post-cusatuzumab), Cycle 1 day 7 (pre-azacitidine), Cycle 1 day 17 (pre- and 0 hours post-cusatuzumab), Cycle 2 day 3 (pre-azacitidine and 0 hours post-cusatuzumab), Cycle 2 day 17 (pre- and 0 hours post-cusatuzumab), Cycle ≥3 day 3 (pre-azacitidine and 0 hours post-cusatuzumab), Cycle ≥3 day 17 (pre- and 0 hours post-cusatuzumab), and at the end-of-treatment and follow-up visits. Serum concentrations of cusatuzumab were analyzed using a validated enzyme-linked immunosorbent assay (ELISA) method.

The immunogenicity of cusatuzumab was evaluated in venous blood samples, taken up to 4 hours prior to cusatuzumab infusion on day -14, on days 3 (pre-azacitidine) and 17 (pre-cusatuzumab) in Cycles 1-4 and in Cycle 8 (if applicable), and at the end-of-treatment and follow-up visits. Antidrug antibodies were detected in serum using an enzyme-linked immunoassay (ELISA) method. The primary antibody was derived from llama immunizations and germlined to human antibody framework.

Bone marrow aspirate and/or whole blood samples were used for the pharmacodynamic evaluations. Soluble CD27 (sCD27) levels in serum were measured on days 1 and 17 of each treatment cycle and at the end-of-treatment and follow-up visits using electrochemiluminescence methodology with the R-PLEX Human CD27 Antibody Set [Meso Scale Discovery]; the lower and upper assay detection limits were 12.2 and 50,000 pg/mL, respectively. To determine the cell-surface expression of pharmacodynamic markers, flow cytometric analysis was performed on bone marrow samples following red blood cell lysis, as described previously.[6]

Supplementary tables and figures

Table S1. Response criteria (investigator assessed).

Response	Definition[a]
Complete remission (CR)[b]	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count >1.0 × 10^9/L (1,000/µL); platelet count >100 × 10^9/L (100.000/µL); independence of red cell transfusions
CR with incomplete recovery (CRi)	All CR criteria except for residual neutropenia (<1.0 × 10^9/L [1,000/µL]) or thrombocytopenia (<100 × 10^9/L [100.000/µL])
Morphologic leukemia-free state (MLFS)	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required
Partial remission (PR)	Relevant in the setting of phase I and II clinical trials only; all hematologic criteria of CR; decrease in bone marrow blast percentage to 5-25%; and decrease in pretreatment bone marrow blast percentage by at least 50%
Treatment failure	
Resistant disease	Failure to achieve CR or CRi (general practice; phase II/III trials), or failure to achieve CR, CRi, or PR (phase I trials); only includes patients surviving >7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination
Death in aplasia	Deaths occurring >7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring >7 days following completion
Relapse	Bone marrow blasts >5%; or reappearance of blasts in the blood; or development of extramedullary disease

[a]Definitions were based on those given by Cheson et al[2], Döhner et al[3], and NCCN[4].

[b]All criteria must be fulfilled; marrow evaluation should be based on a count of 200 nucleated cells in an aspirate with spicules; if ambiguous, consider repeat exam after 5-7 days; flow cytometric evaluation may help to distinguish between persistent leukemia and regenerating normal marrow; a marrow biopsy should be performed in cases of dry tap, or if no spicules are obtained; no minimum duration of response required.

Table S2. Responses to cusatuzumab plus azacitidine by genetic risk classification per ELN 2017 criteria.

	Phase I					Phase II	
ELN 2017 risk classification	**1 mg/kg**	**3 mg/kg**	**10 mg/kg**	**20 mg/kg**	**All doses**	**10 mg/kg**	**Total**
Response, n (%)	**(n=3)**	**(n=3)**	**(n=3)**	**(n=3)**	**(n=12)**	**(n=26)**	**(N=38)**
Favorable	n=0	n=1	n=0	n=1	n=2	n=2	n=4
CR	0	1 (100)	0	1 (100)	2 (100)	1 (50)	3 (75)
CRi	0	0	0	0	0	1 (50)	1 (25)
Intermediate	n=2	n=2	n=2	n=0	n=6	n=3	n=9
CR	2 (100)	1 (50)	2 (100)	0	5 (83.3)	2 (66.7)	7 (77.8)
Adverse	n=1	n=0	n=1	n=2	n=4	n=21	n=25
CR	0	0	0	2 (100)	2 (50)	2 (9.5)	4 (16)
CRi	1 (100)	0	1 (100)	0	2 (50)	2 (9.5)	4 (16)
NE	0	0	0	0	0	2 (9.5)	2 (8)

CR: complete remission; CRi: complete remission with incomplete recovery; ELN: European LeukemiaNet; NE: not evaluable.

Table S3. Summary of the most common serious TEAEs (occurring in ≥5% of all patients) following treatment with cusatuzumab plus azacitidine.

Patients with ≥1 serious TEAE,[a] n (%)	Dose group				Total (N=38)
	1 mg/kg (n=3)	3 mg/kg (n=3)	10 mg/kg (n=29)	20 mg/kg (n=3)	
Febrile neutropenia	2 (66.7)	0	9 (31)	2 (66.7)	13 (34.2)
Sepsis[b]	0	0	10 (34.5)	1 (33.3)	11 (28.9)
Pneumonia[c]	1 (33.3)	0	7 (24.1)	2 (66.7)	10 (26.3)
General physical health deterioration	0	0	1 (3.4)	2 (66.7)	3 (7.9)
Acute coronary syndrome	0	0	1 (3.4)	1 (33.3)	2 (5.3)
Cardiac failure	0	0	1 (3.4)	1 (33.3)	2 (5.3)
Cellulitis	0	1 (33.3)	1 (3.4)	0	2 (5.3)
Device-related infection	0	0	2 (6.9)	0	2 (5.3)
Pyrexia	0	1 (33.3)	1 (3.4)	0	2 (5.3)

[a]TEAEs are defined as AEs with onset or worsening on or after the date of the first dose of study treatment up to and including 30 days after date of last dose of study medication. TEAEs are listed in decreasing frequency of any-grade TEAE in the total study population (N=38).

[b]Sepsis includes the following preferred terms: Enterobacter sepsis, Escherichia sepsis, Pseudomonal bacteremia, sepsis, septic shock, and Staphylococcal bacteremia.

[c]Pneumonia includes the following preferred terms: pneumonia.

AE: adverse event; TEAE: treatment-emergent adverse event.

Table S4. Summary of pharmacokinetic parameters following cusatuzumab administration on day -14 (loading dose) and on Cycle 1 day 3.

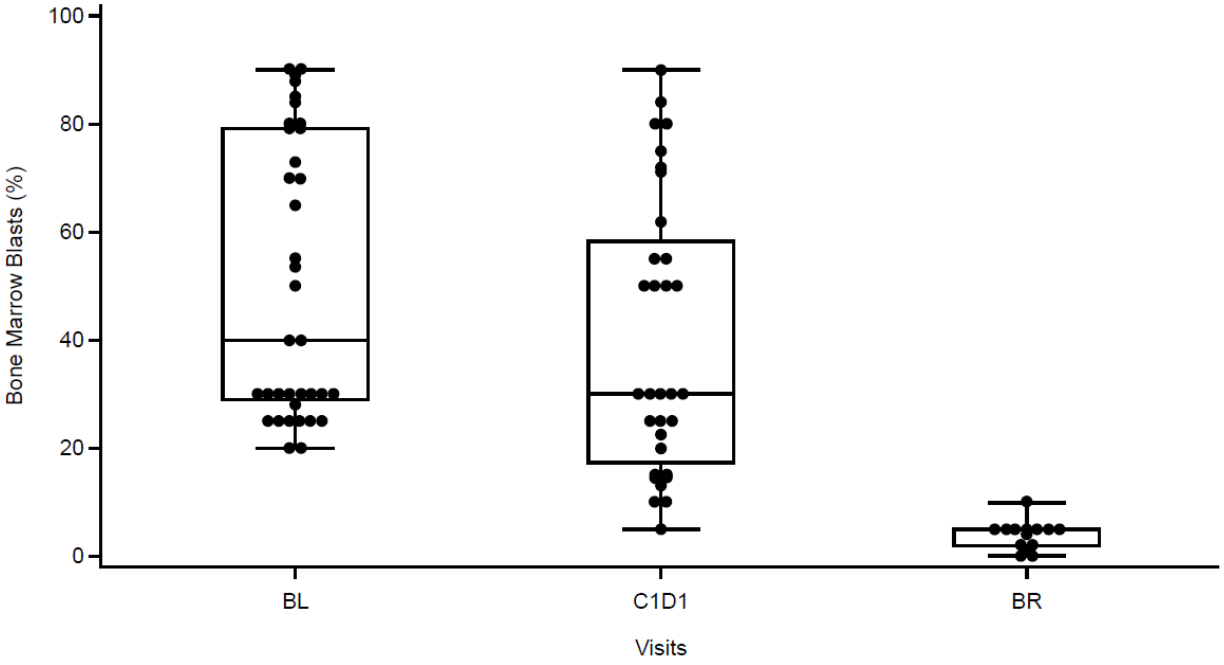
	Phase I				Phase II
	1 mg/kg	**3 mg/kg**	**10 mg/kg**	**20 mg/kg**	**10 mg/kg**
Post loading dose (day -14)	(n=3)	(n=3)	(n=3)	(n=3[a])	(n=25[a])
C_{max}, µg/mL	28.3 (13.5)	61.4 (13)	188 (38.4)	405 (32.4)	195 (81.3)
t_{max}, day	0.013	0.233	0.215	0.229	0.219
	(0.135-0.170)	(0.205-0.245)	(0.143-0.226)	(0.153-0.293)	(0.132-2.86)
AUC_{14d}, µg.h/mL	2,682 (237)	9,911 (1,689)	28,170 (3,135)	52,149 (13,479)	32,932 (25,722)
$t_{1/2}$, days	6.1 (1.6)	8.2 (1.3)	9.7 (2)	10.4 (1.4)	11.1 (4.1)
$C_{max, dn}$, µg/mL/mg	28.3 (13.5)	20.6 (4.37)	18.8 (3.78)	20.2 (1.53)	19.5 (8.05)
$AUC_{14d,dn}$, µg.h/mL/mg	2,682 (237)	3,316 (574)	2,825 (301)	2,603 (682)	3,308 (2,564)
Cycle 1 day 3	(n=3)	(n=3)	(n=3)	(n=3)	(n=23[b])
C_{trough}, µg/mL	3.09 (1.22)	12.2 (1.35)	40.3 (8.46)	64.1 (6.83)	40.6 (17.6)
C_{max}, µg/mL	22.2 (2.99)	79.2 (10.1)	-	452 (49.5)	233 (59.2)
t_{max}, day	0.0658	0.0763	-	0.260	0.139
	(0.0625-0.0692)	(0.0658-0.193)		(0.212-0.979)	(0.0692-0.910)
AUC_{14d}, µg.h/mL	2,351 (554)	10,851 (4,636)	-	78,762 (10,488)	38,298 (11,991)
$t_{1/2}$, days	-	7.4 (4.2)	-	-	8.3 (2.4)
$C_{max, dn}$, µg/mL/mg	22.2 (2.99)	26.4 (3.45)	-	22.6 (2.48)	23.2 (5.93)
$AUC_{14d,dn}$, µg.h/mL/mg	2,351 (554)	3,625 (1,555)	-	3,938 (524)	3,811 (1,192)

[a]n=23 for AUC_{14d} and $AUC_{14d,dn}$, and n=18 for $t_{1/2}$.

[b]n=21 for AUC_{14d} and $AUC_{14d,dn}$, and n=11 for $t_{1/2}$.

AUC_{14d}: area under the serum concentration–time curve from time 0 to 14 days; C_{max}: maximum serum concentration; C_{trough}: minimum serum concentration; Dn: dose normalized to 1 mg/kg; $t_{1/2}$: terminal elimination half-life; $t_{max:}$, time to maximum serum concentration.

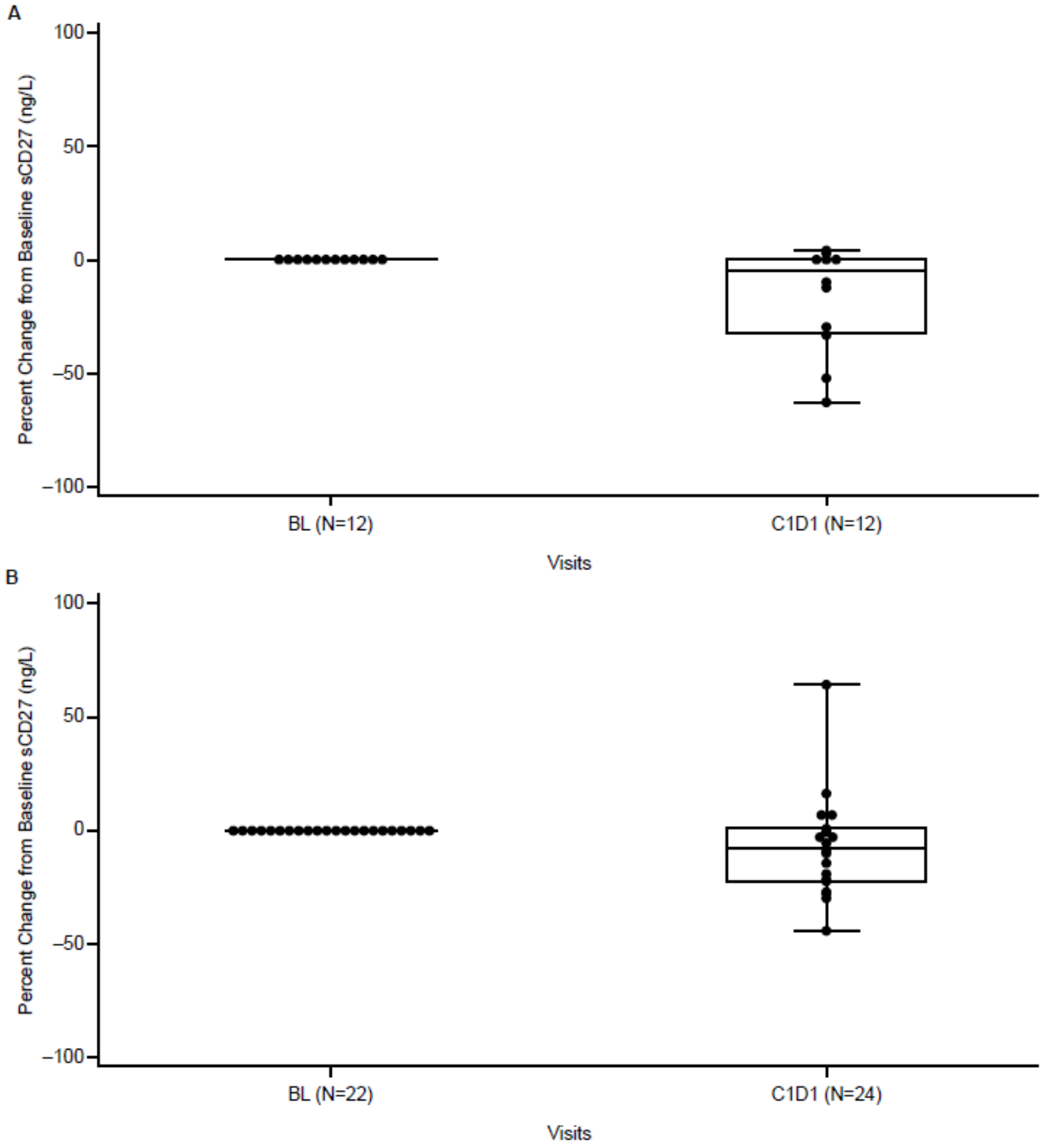
Figure S1. Box-and-whisker plot of bone marrow blasts at baseline (day -14, pre-cusatuzumab), on Cycle 1 day 1 (pre-combination treatment), and at best response in all patients treated with cusatuzumab plus azacitidine (both study phases combined), as determined by cytomorphology.



The BR timepoint only includes patients achieving an objective response (complete remission or complete remission with incomplete recovery). Bone marrow data are based on aspirate blast percentage; if aspirate was not available due to dry tap, biopsy blast percentage was used.

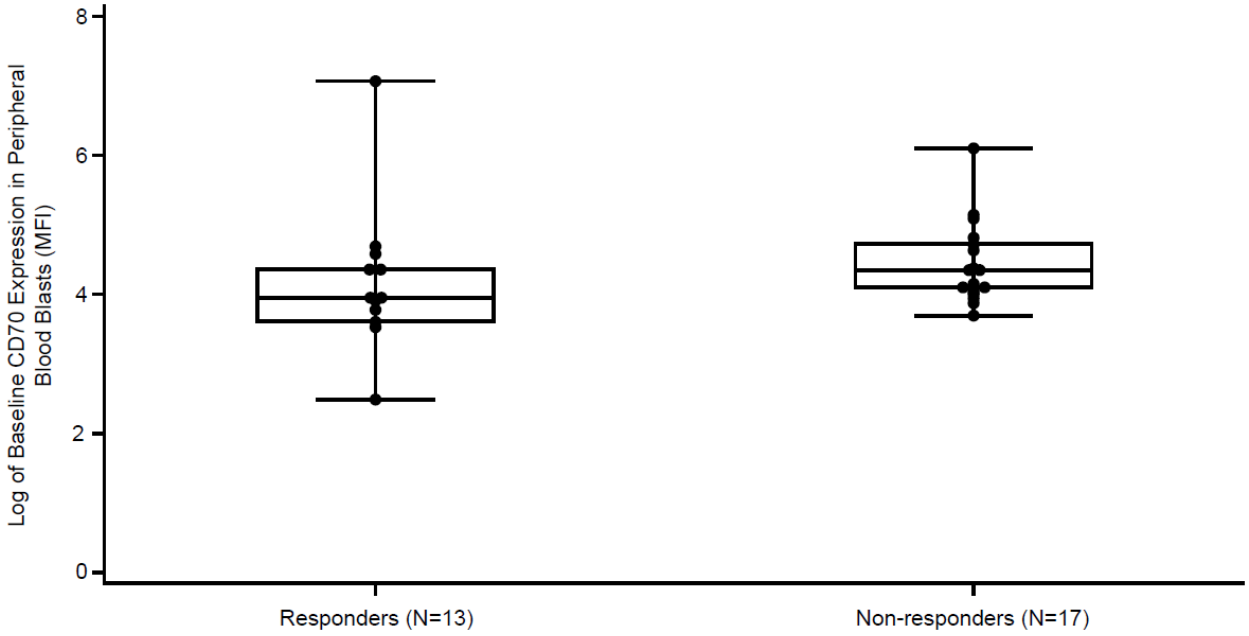
BL: baseline; BR: best response; C: Cycle; D: day.

Figure S2. Change in serum sCD27 levels in phase I (A) and phase II (B) patients treated with cusatuzumab monotherapy (day -14), all dose levels and responses combined per phase. Phase I and phase II sCD27 assays were run in different laboratories.



BL: baseline; C: Cycle; D: day; N: number; sCD27: soluble CD27.

Figure S3. Box-and-whisker plot of baseline CD70 expression in peripheral blood blasts in responders and non-responders to cusatuzumab plus azacitidine (all dose levels combined).



Responders include patients achieving complete remission or complete remission with incomplete recovery.

Abbreviations: MFI, mean fluorescence intensity.

References

1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.
2. Cheson B, Bennett J, Kopecky K, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003;21(24):4642-4649.
3. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015;373(12):1136-1152.
4. National Comprehensive Cancer Network® (NCCN). NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Acute Myeloid Leukemia. Version 1.2015.
5. Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 2018;131(12):1275-1291.
6. Riether C, Pabst T, Höpner S, et al. Targeting CD70 with cusatuzumab eliminates acute myeloid leukemia stem cells in patients treated with hypomethylating agents. Nat Med. 2020;26(9):1459-1467.