SUPPLEMENTARY APPENDIX

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Supplementary Materials and Methods

a. Supplementary Exclusion and inclusion criteria

All participants were required to have Eastern Cooperative Oncology Group performance status of ≤2, a peripheral blast count of <20,000/mm^3 at the time of first treatment, and adequate organ function. Patients with a prior history of allogeneic hematopoietic cell transplantation, active untreated autoimmune disorders, or active central nervous system leukemia were excluded.

b. Supplementary Safety

All patients received premedication with antihistamines, acetaminophen and steroids (10-20 mg of dexamethasone) prior to dosing with flotetuzumab. The research protocol provided recommendations for the management of infusion related reaction (IRR) and cytokine release syndrome (CRS) including dose interruptions/reductions, tocilizumab use, and steroid use (**Table S7** in the Supplementary Appendix).

Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. CRS was graded according to Lee criteria.[1] DLTs were defined as nonhematologic toxic effects of Grade 3 or higher, or prolonged myelosuppression with the persistence of Grade 4 neutropenia or thrombocytopenia in the absence of leukemia (blast count, <5%) at least 42 days after the end of treatment. Serious adverse events (SAEs) were those that resulted in death, were life-threatening, led to hospitalization or prolongation of hospitalization, caused clinically significant incapacity, led to congenital anomaly/birth defect or, were deemed to be an important medical event.

c. Supplementary Efficacy

Clinical efficacy was assessed by the investigators with the use of modified 2003 International Working Group response criteria for AML.[2] In addition, complete remission with partial hematologic recovery (CRh), defined as <5% blasts in the bone marrow (BM), no evidence of disease, and partial recovery of peripheral blood (PB) counts (platelets >50×10^9/L and ANC

>0.5×10^9/L), was added as a response criterion.[3] Response rate, OS, event-free survival (EFS), and duration of response (DOR) are presented based on subgroup analysis.

d. Supplementary PK and PD assessments

Serum concentrations of flotetuzumab were measured using a validated electrochemiluminescence-based sandwich assay with a lower limit of quantitation of 5 pg/mL. Details of PK and PD assessments are shown in **Table S1**.

e. Supplementary Cytotoxicity Assays on Hematopoietic Precursors

Fresh CD34 enriched bone marrow from normal donors (purchased from All Cells) were mixed with CD3 enriched donor matched T cells, frozen, thawed and rested in media 100 IU of IL-2 for 24 hours prior (purchased from All Cells) at a ratio of 1:1, 10:1 and 0:1 (E:T), and resuspended in serum-free stem cell media with cytokines. The samples were then plated in 384-well microtiter plates, maintaining a total of 15,000 cells per well, and screened against a 10-point dose-response of flotetuzumab (1.6973aM to 1.6973nM) in triplicate using Notable Labs' automated platform. Specimens were treated for 72 hours and assayed using high-throughput, multi-parametric flow cytometry. Hematopoietic cells were stained with the following antibodies to identify hematopoietic stem and progenitor population (HSC), common myeloid progenitor (CMP), megakaryocyte-erythroid progenitor (MEP), and granulocyte-macrophage progenitor (GMP): CD19 PACBLUE, CD3 PACBLUE, CD16 BV510, CD34 BV605, CD45RA FITC, CD123 PE, CD90 PE-CY7, CD33 APC, CD38 APC-CY7, and DAPI, and were then incubated for 20 minutes at 4°C and analyzed on an iQue Plus flow cytometer. Viability of each population was normalized to vehicle control (DMSO) was used to evaluate cytotoxicity. Expression (percentage positive) and levels (gMFI or a geometric mean fluorescence intensity) of CD123 were assessed against a fluorescence minus one (FMO) control.

f. Supplementary CD123 Receptor Density Assay

Cell surface expression of CD123 on AML blasts was determined by flow cytometry. Briefly,

cryopreserved PB and BM mononuclear cells were thawed, washed, and resuspended in staining buffer (PBS supplemented with 0.5% bovine serum albumin and 2 mM EDTA). Cells were incubated for 30 min at room temperature with pre-titrated saturating dilutions of the following fluorochrome-labeled monoclonal antibodies (BD Biosciences, San Jose, CA unless where designated; clone designated in parenthesis): CD14 (M5E2), CD33 (WM53), CD34 (581), CD45 (HI30), CD117 (104D2), CD123 (32703; R&D Systems, Minneapolis, MN) and HLA-DR (G46-6). Dead cells were excluded by staining with 2 μg/ml 7-amino-actinomycin D (BD Biosciences) for 5 minutes prior to analysis. Samples were analyzed on a Gallios (Beckman Coulter, Brea, CA) or ZE5 (Bio-Rad, Hercules, CA) flow cytometer and data were analyzed using FlowJo software (LLC, Ashland, OR). AML blasts were identified using a $CD45^{dim}/SSC^{low}$ gating strategy with concomitant expression of CD33, CD34, CD117, and/or HLA-DR when appropriate based on pathological reports. The antibody-binding capacity (ABC) per AML blast was determined for anti-CD123 using saturating concentrations of antibody and Quantum Simply Cellular beads (Bangs Laboratories, Inc, Fishers, IN) as previously described.[4] The ABC value represents the mean value of the maximum capacity of each cell to bind the anti-CD123 antibody.

g. Supplementary Gene Expression Analysis

The NanoString PanCancer IO360™ assay was used to interrogate the expression of 770 genes, including the abundance of 14 immune cell types and 32 immuno-oncology signatures in BM samples of a subgroup of patients (n=38) with relapsed/refractory AML treated with flotetuzumab at the recommended phase 2 dose (RP2D; 38 BM samples collected at baseline and 34 BM samples collected on treatment (post-cycles 1 [n=25] and 2 [n=9]). Signature scores were calculated as pre-defined linear combinations (weighted averages) of biologically relevant gene sets as previously described.[5]

Supplementary Table 1: Lead in dose (LID) Dosing Schema (ng/kg/day).

Dosing cycle 1 (ng/kg/day)							
Lead in dose (LID)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1-step	100	100	100	100	0	0	0
2-step	30	30	30	100	100	100	100
Multi-step	30	60	100	200	300	400	500

Supplementary Table 2: Distribution of Flotetuzumab Cycles Received.

AML patients at all dose levels (n=88); % (n)				
One cycle	Two cycles	Three cycles	Four cycles	Six cycles
65.9% (58)	23.9% (21)	5.7 (5)	3.4% (3)	1.1 (1)
AML patients at RP2D (n=50); % (n)				
One cycle	Two cycles	Three cycles	Four cycles	Six cycles
56% (28)	32% (16)	8% (4)	4% (2)	0

Supplementary Table 3: Median (Range) of Individual Parameter Estimates by Dose.

Max dose (mg/kg)	N	CL (L/hr)	V_1 (L)	V_2 (L)	Q (L/hr)	$t_{1/2,dist}$ (hr)	$t_{1/2,term}$ (hr)
3	1	12.1 (12.1; 12.1)	63.7 (63.7; 63.7)	82.4 (82.4; 82.4)	14.4 (14.4; 14.4)	1.31 (1.31; 1.31)	11 (11; 11)
10	4	9.29 (5.14; 9.55)	40.7 (31.3; 44.1)	53.8 (37.7; 208)	10.4 (9.18; 12)	1.03 (0.926; 1.95)	9.15 (7.21; 47.6)
30	5	5.57 (4; 9.64)	67.8 (27.5; 145)	47.3 (28; 99.4)	14 (9.54; 15.3)	1.2 (0.857; 3.31)	15.3 (7.85; 37.7)
100	4	4.13 (3.18; 10.2)	34.5 (15.5; 60.7)	27.1 (20.8; 36.3)	11.2 (10.1; 13.1)	0.87 (0.49; 0.96)	10.1 (5.98; 15.4)
300	7	8.49 (3.57; 13.8)	67 (47.5; 124)	57.9 (19.9; 90.2)	11.4 (10.5; 15.7)	1.58 (0.929; 2.26)	12.9 (4.75; 35.1)
500	69	9.25 (2.65; 43.5)	47.6 (12.8; 152)	56.5 (7.75; 1780)	12.6 (9.31; 20.9)	1.04 (0.308; 3.73)	9.77 (3.69; 236)
700	8	11.1 (2.18; 14.7)	58.7 (18.7; 105)	53.5 (14.6; 118)	12.1 (9.87; 13.3)	1.09 (0.595; 1.95)	12.2 (3.22; 34.8)

CL: Clearance; Q: Inter-Compartment Clearance; V_1: Central Volume; V_2: Peripheral Volume; t1/2,dist: distribution half-life; t1/2,term: terminal half-life. Flotetuzumab clearance (CL, 8.75 L/h), central volume (V1, 51.4 L), and peripheral volume of distribution (V2, 57.8 L) were higher than those of therapeutic monoclonal antibodies, consistent with the small molecular weight of 59 KD of flotetuzumab and reflecting its distribution in the large interstitial space and renal clearance. Patients with higher exposure had better best overall response, with the probability of complete response (< 5% BM blasts) significantly increasing with flotetuzumab exposure (P=0.007; **Fig. S4**).

Supplementary Table 4: IRR/CRS Events Decrease with Ongoing Treatment.

Cycle	**Cycle 1**				**Cycle 2**				**Cycle 3**				**> Cycle 3**
Week	1	2	3	4	1	2	3	4	1	2	3	4	
% of total	27.3	21.6	10.6	9	3.3	5.3	5.3	5.7	2.9	1.2	1.2	0.8	8.2

Supplementary Table 5: Guidelines for Management of IRR/CRS.

Guidelines for Management of IRR/CRS	
• ***Early intervention at the first signs of IRR/CRS.*** • ***In particular, the use of tocilizumab to forestall worsening of IRR/CRS.***	
Grade 1 infusion reactions including CRS and infusion-related events	1 Slow the infusion rate by 10-20%; 2 Monitor the patient for worsening of condition; 3 Administer IV fluids, diphenhydramine hydrochloride (or institutional equivalent) 50 mg IV, acetaminophen 1000 mg PO or ibuprofen 400 mg PO or paracetamol 1000 mg PO for fever, and oxygen and bronchodilators for mild bronchospasm, as appropriate; 4 Administer tocilizumab (4-8 mg/kg IV) for grade 1 IRR/CRS that does not resolve within 2 hours • *Corticosteroids should not be used for grade 1 IRR/CRS.* • *Continue infusion at reduced rate and slowly increase infusion rate to the original rate in 2 steps after stabilization or resolution of symptoms every 4-6 hours, as tolerated. A more gradual increase in the rate of infusion may be undertaken after consultation with the Medical Monitor.*
Grade 2 infusion reactions including CRS and infusion-related events	1 Slow the infusion rate by 20-50%; 2 Monitor the patient for worsening of condition; 3 Administer IV fluids, diphenhydramine hydrochloride (or institutional equivalent) 50 mg IV, acetaminophen 1000 mg PO or ibuprofen 400 mg PO or paracetamol 1000 mg PO for fever, and oxygen and bronchodilators for mild bronchospasm, as appropriate; 4 Administer vasopressors (at doses < 20 ng/min of norepinephrine, or equivalent) as needed for circulatory support; 5 Tocilizumab (8 mg/kg IV) should be used for grade 2 IRR/CRS that does not resolve within 2 hours or that requires the use of supplemental oxygen > 4L by nasal cannula or low dose vasopressors; 6 Corticosteroids may be used for grade 2 IRR/CRS that does not respond to other measures, including tocilizumab. • *Continue infusion at reduced rate and slowly increase infusion rate to the original rate gradually by half-rate increments (i.e., if rate is reduced from 5 mL/hour to 2.5 mL/hour increase to ~3.75 mL/hour, then 4.5 mL/hour, then 5 mL/hour) after stabilization or resolution of symptoms every 4-6 hours, as tolerated. A more gradual increase in the rate of infusion may be undertaken after consultation with the Medical Monitor.*
Grade 3 infusion reactions including CRS and infusion-related events	1 Stop infusion, aspirate MGD006; 2 All measures as above. Additional dose of tocilizumab may be given; 3 Grade 3 CRS refractory to tocilizumab should be treated with any of the following: a. Corticosteroids. Dexamethasone (or equivalent) of greater than 30 mg may be required b. Etanercept (or equivalent anti-TNF-α) 50 mg IV, and/or

	• *Resume the infusion at previously tolerated dose once the infusion reaction has resolved or decreased to grade 1. Increase dose rate to the original rate, as prescribed by protocol, e.g., 500 ng/kg/day, by increasing the dose in near double increments as tolerated after stabilization or resolution of symptoms every 4-6 hours. A more gradual increase in the rate of infusion may be undertaken after consultation with the Medical Monitor.* • *Discontinue the infusion if not resolved to Grade 1 within 72 hours*
Grade 4 infusion reactions including CRS and infusion-related events	1 Stop infusion, aspirate MGD006, do not re-administer; 2 Administer supportive care as needed; 3 Following agents have been described in the management of severe IRR/CRS and may be required: a. Higher doses of corticosteroids above dexamethasone 30 mg (or equivalent) b. Tocilizumab (anti-IL-6 receptor) 8 mg/kg IV, and/or c. Etanercept (anti-TNF-α) 50 mg IV

Supplementary Table 6: Comparison between Patients that Did and Did Not Receive Tocilizumab for CRS Treatment.

	Tocilizumab	**No tocilizumab**
Number of subjects	8	13
Age (median, range)	74 (53-82)	63 (49-82)
Females (%, n)	37.5% (3/8)	46.2% (6/13)
Baseline BM blasts (%; mean ± SEM)	46.8 ± 7.7	36.4 ± 10.2
Number of CRS events	13	27
CRS Grade (median, range)	1 (1-2)	1 (1-2)
CRS duration (days; mean ± SEM)	1.3 ± 0.2	1.8 ± 0.2

Legend: BM = bone marrow; SEM = standard error of the mean; CRS = cytokine release syndrome.

Supplementary Table 7: Recovered Blood Counts On Treatment with Flotetuzumab.

	Percentage Bone Marrow Blasts	**Lab Visit**	**Platelets ($\times 10^9$/L)**	**Neutrophils ($\times 10^9$/L)**
Patient 2 (CRh)				
Screening	8		76	1.42
Start of Tx		Cycle 1 Day 1	77	2.35
End Cycle 1 BM Bx	0	Cycle 1 Day 26	29	3.56
		Cycle 2 Day 22	**64**	**1.06**
Cycle 2 Day 25	**0**			
		Cycle 3 Day 1	**54**	**0.76**
End of Tx		**End of Treatment**	**64**	**0.97**
Patient 3 (CR)				
Screening	39		28	0.1
Start of Tx		Cycle 1 Day 1	17	0.1
End Cycle 1 BM Bx	10	Cycle 1 Day 26	23	0.2
End Cycle 2 BM Bx	2	Cycle 2 Day 25	60	2.8
		Cycle 3 Day 1	**110**	**2.4**
Cycle 4 Day 25	5	Cycle 4 Day 25	56	0.7
End of Tx		Cycle 4 Day 26	44	0.5
Patient 7 (CR)				
Screening	70		43	3.52
Start of Tx		Cycle 1 Day 1	74	1.44
End Cycle 1 BM Bx	56	Cycle 1 Day 25	32	1.93
End Cycle 2 BM Bx	4	Cycle 2 Day 26	14	0.42
End Cycle 3 BM Bx	**2**	**Cycle 3 Day 25**	**103**	**1.2**
End of Cycle 4 BM Bx	25	Cycle 4 Day 25	52	
End of Tx		Cycle 4 Day 26	44	0.79
Patient 8 (CR)				
Screening	70		46	0.1
Start of Tx		Cycle 1 Day 1	38	0
End Cycle 1 BM Bx	1	Cycle 1 Day 25	26	0
End Cycle 2 BM Bx	0	Cycle 2 Day 25	91	2.9
		Cycle 2 Day 26	**104**	**2.8**
End of Tx		Cycle 3 Day 26	153	1.7
Patient 9 (CR)				
Screening	16		231	0.97
Start of Tx	16	Cycle 1 Day 1	189	0.82
End Cycle 1 BM Bx	**4**	**Cycle 1 Day 23**	**202**	**1.89**
End of Tx		**Cycle 2 Day 26**	**229**	**1.29**
End Cycle 2 BM Bx	0			

Patient 10 (CRh)				
		Screening	15	5.1
Start of Tx	50	Cycle 1 Day 1	21	6.1
End Cycle 1 BM Bx	**0**	**Cycle 1 Day 26**	**50**	**1.4**
End of Tx			**52**	**2.7**

Supplementary Table 8: Recovered Blood Counts Off Treatment with Flotetuzumab.

	Percentage Marrow Blasts	**Lab Visit**	**Platelet (10^9/L)**	**Neutrophils (10^9/L)**
Patient 1 (CR)				
Screening			83	0.54
Start of Tx	6	Cycle 1 Day 1	87	1.14
End of Tx		Cycle 1 Day 18	46	2.41
	0	**Unscheduled**	**115**	**1.38**
Patient 4 (CR)				
		Screening	127	0
Start of Tx	5	Cycle 1 Day 1	93	0.1
Cycle 1 Day 25	1	Cycle 1 Day 25	13	0
End Cycle 1 BM Bx	1	Cycle 1 Day 25	13	0
End of Tx		Cycle 1 Day 28	9	0.1
Unscheduled	**3**	**Unscheduled**	**133**	**1.9**
Patient 5 (CR)				
Screening			228	0.2
Start of Tx	7	Cycle 1 Day 1	159	0.3
End Cycle 1 BM Bx	1	Cycle 1 Day 25	18	0
End of Treatment	1	Cycle 1 Day 25	18	0
End of Tx		Cycle 2 Day 5	78	0.1
		Unscheduled	**135**	**1.5**
Patient 6 (CRh)				
Screening	100*		179	16.1
Start of Tx		Cycle 1 Day 1	226	12
End Cycle 1 BM Bx	2	Cycle 1 Day 25	9	0.8
End of Tx		Cycle 1 Day 28	23	1.8
		Unscheduled	40	13.7
		Unscheduled	**63**	**9.9**

*Aspirate was hemodiluted but biopsy IHC confirmed leukemia.

Supplementary Table 9: Reasons for Discontinuation in Patients Treated at the RP2D (n=50).

Adverse Event	Physician Decision (i.e., end of study, transition to HSCT or subsequent therapy,)	Patient Decision (i.e. transition to comfort care)	Treatment failure/ Progressive Disease	Death
7	13	7	20	3

Supplementary Table 10: Response Rates in Patients Treated with Flotetuzumab in Earlier Salvage Attempt.

Prior lines of therapy	Numbers of responding patients per line of therapy	Flotetuzumab (CR/CRh/CRi rate)
2	5	55.6% (5/9)
3	1	46.2% (6/13)
4	3	40.9% (9/22)
≥5	0	32.1% (9/28)

Legend: CR = complete remission; CRh = complete remission with partial hematopoietic recovery; CRi = complete remission with incomplete hematopoietic recovery.

Supplementary Table 11: Gene ontologies (GO) and KEGG Pathways Captured by the Top 10 Genes Associated with Complete Response to Flotetuzumab.

GO term	Description	Count in gene set	FDR
GO:0005102	Signaling receptor binding	25 of 1513	1.63×10^{-12}
GO:0042605	Peptide antigen binding	7 of 22	1.00×10^{-10}
GO:0003823	Antigen binding	8 of 56	3.98×10^{-10}
GO:0019838	Growth factor binding	9 of 126	4.05×10^{-9}
GO:0005518	Collagen binding	7 of 61	2.31×10^{-8}
GO:0005161	Platelet-derived growth factor receptor binding	5 of 15	6.26×10^{-8}
GO:0005515	Protein binding	38 of 326605	1.25×10^{-7}
GO:0005021	Vascular endothelial growth factor-activated receptor activity	4 of 7	4.24×10^{-7}
GO:0046977	TAP binding	4 of 7	4.24×10^{-7}
GO:0044877	Protein-containing complex binding	15 of 968	4.26×10^{-7}
GO:0048407	Platelet-derived growth factor binding	4 of 11	1.26×10^{-6}
Pathway	**Description**	**Count in gene set**	**FDR**
hsa05165	Human papillomavirus infection	20 of 317	1.56×10^{-20}
hsa04330	Notch signaling pathway	10 of 48	1.49×10^{-14}
hsa05166	HTLV-I infection	14 of 250	1.43×10^{-13}
hsa05206	MicroRNAs in cancer	12 of 149	2.12×10^{-13}
hsa04658	Th1 and Th2 cell differentiation	10 of 888	1.49×10^{-12}
hsa04612	Antigen processing and presentation	9 of 66	5.40×10^{-12}
hsa04145	Phagosome	10 of 145	1.12×10^{-10}
hsa05200	Pathways in cancer	151 of 515	6.68×10^{-10}
hsa04015	Rap1 signaling pathway	10 of 203	1.82×10^{-9}
hsa04514	Cell adhesion molecules (CAMs)	9 of 139	1.82×10^{-9}
hsa05330	Allograft rejection	6 of 35	9.90×10^{-9}

FDR = false discovery rate.

Supplementary Table 12: AUROC Curves Measuring the Predictive Ability of European Leukemia-Net (ELN) Risk Category and Our 10-Gene Signature Score for Anti-leukemic Activity from Flotetuzumab.

Variable	***Area***	***S.E.***	***P value***	***95% CI***	
				Lower bound	***Upper bound***
ELN risk	0.672	0.10	0.122	0.476	0.869
10-gene signature score	0.854	0.067	**0.001**	0.724	0.985
ELN risk + 10-gene signature score	0.904	0.055	**0.000**	0.797	1.000

SE = standard error; CI = confidence interval. PIF = primary induction failure; ER = early relapse; LR = late relapse (as defined in Materials and Methods). AUROC = 1.0 would denote perfect prediction and AUROC = 0.5 would denote no predictive ability. AUROC curves were estimated using the SPSS software package, as previously published.[5,6]

Legends to Supplementary Figures

Fig. S1: **Study design showing the progression of the patient cohorts.**

Fig. S2: **Pharmacokinetic/Pharmacodynamic Findings. A)** Exposure (Cmean cycle 1 weeks 1-4) versus maximum scheduled dose. **B)** Dose intensity versus maximum scheduled dose. **C)** Exposure (Cmean cycle 1 week 1 or 2) versus peak cytokine release syndrome (CRS) severity cycle 1 days 1-15 (red stars = patients treated at 700 ng/kg/day; blue stars = patients treated at 500 ng/kg/day). **D)** Dose intensity (cycle 1 week 1 or 2) versus peak CRS severity cycle 1 days 1-15 (red stars = patients treated at 700 ng/kg/day; blue stars = patients treated at 500 ng/kg/day).

Fig. S3: **Effect of flotetuzumab on normal hematopoietic progenitor cells.** *In vitro*, five bone marrow samples were collected from normal donors and exposed to increasing doses of flotetuzumab, from 1.7×10^{-9} nM to 1.7×10^{-0} nM; cell counts were normalized to untreated controls. No effect was seen on hematopoietic stem cells (HSC), megakaryocyte-erythroid progenitors (MEP), granulocyte-macrophage progenitors (GMP) or common myeloid progenitors (CMP) at concentrations equivalent to the Cmax at 500 ng/kg/day.

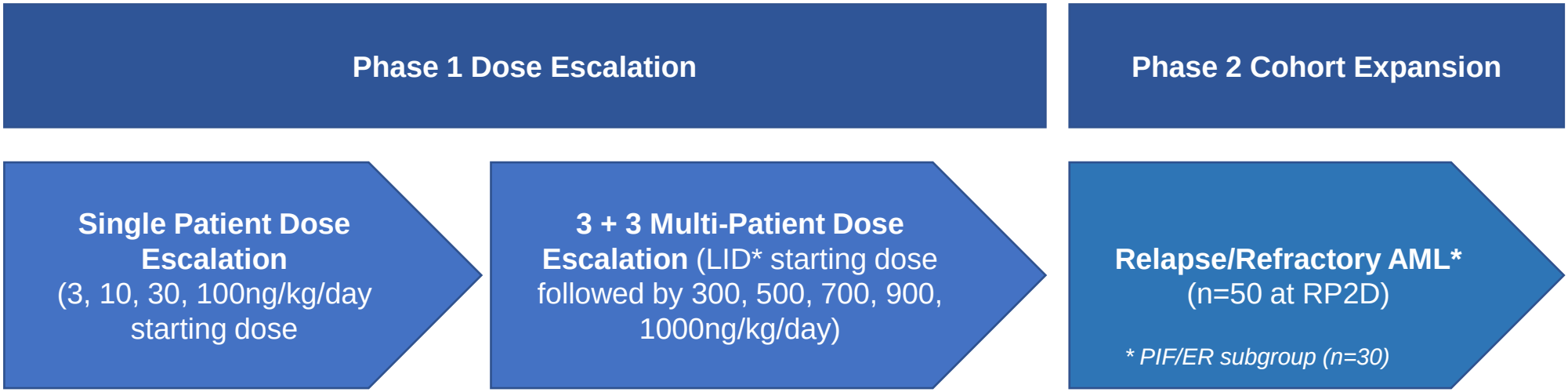
Fig. S4: Complete responses and AML status at study entry. Number of patients with primary induction failure (PIF)/early relapse (ER), late relapse (LR) and failure of hypomethylating agents (HMA) who achieve complete response to flotetuzumab (defined as either CR, CRh or CRi). Data were compared using the χ^2 test.

Fig. S5: Swimmer plot of all patients (n=50) treated at the RP2D. CR = complete remission; CRh = CR with partial hematologic recovery; CRi = CR with incomplete hematologic recovery; HSCT = hematopoietic stem cell transplantation.

Fig. S6: CD123 expression in patients receiving flotetuzumab immunotherapy. A) Bar graph of CD123 receptor density (no. of binding sites/cell) measured by flow cytometry in primary induction failure (PIF) and early relapse (ER; n = 22) and late relapse (LR) AML (n = 7) treated at the recommended phase 2 dose (RP2D) for whom data was available. The unpaired t-test was used for data comparisons. **B-C**) Dot plots summarizing median CD123 receptor density (no. of binding sites/cell) and percentage of $CD123^+$ AML blasts measured by flow cytometry in primary induction failure and early relapse (n = 22) treated at RP2D for whom response data was available.

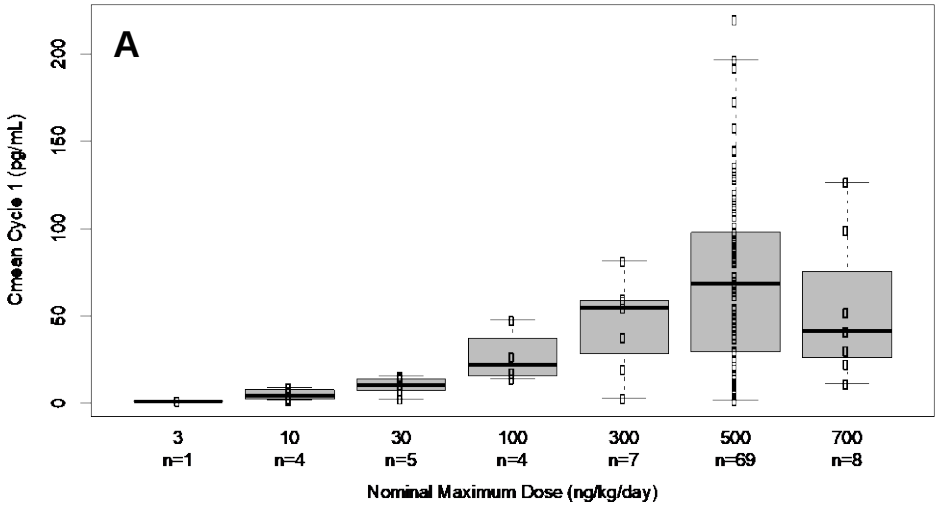
Fig. S7: ***CD123*** **mRNA expression is increased in patients with unfavorable ELN risk and with chemotherapy-refractory AML**. **A**) *CD123* mRNA expression was correlated with cytogenetic risk (2017 ELN categories) in a large public dataset of primary AML cases (GEO Series accession number GSE134589). Red bars denote median values. KW = Kruskal Wallis statistics. **B**) *CD123* mRNA expression in AML patients who achieved complete remission (n=206) after induction chemotherapy and in individuals with primary induction failure (PIF) and early relapse (ER). Black bars denote median values. Data were compared using the Mann-Whitney *U* test for unpaired determinations.

Fig. S8: The tumor immunological microenvironment (TME) in patients receiving flotetuzumab immunotherapy. Unsupervised hierarchical clustering (Euclidean distance, complete linkage) of immune cell type-specific scores and biological activity scores (as defined in **Fig. 4A**) in baseline bone marrow (BM) samples from 38 patients with relapsed/refractory (R/R) AML treated with flotetuzumab immunotherapy (color-coded *per* response). ClustVis, an online tool for clustering of multivariate data, was used for data analysis and visualization.[7]



*For LID, see Supplementary Table 1: Lead In Dose (LID) Dosing Schema

Fig. S1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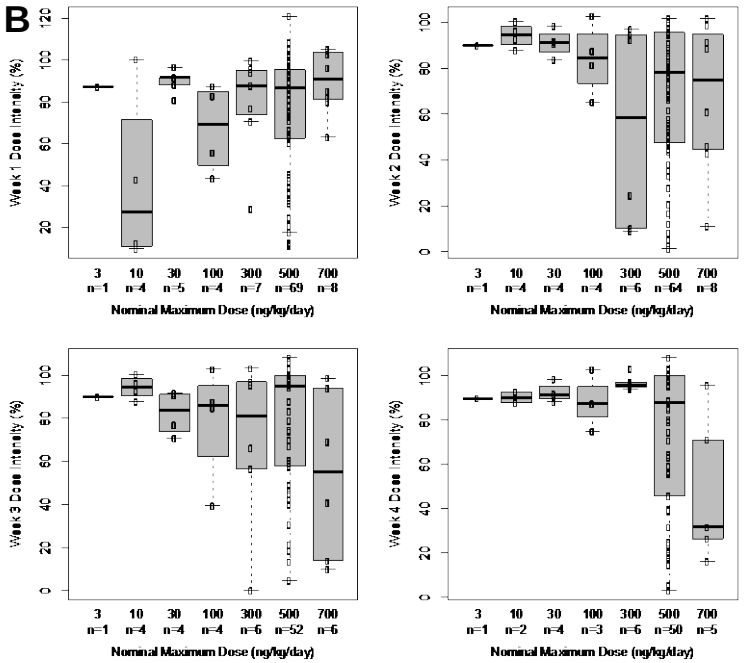
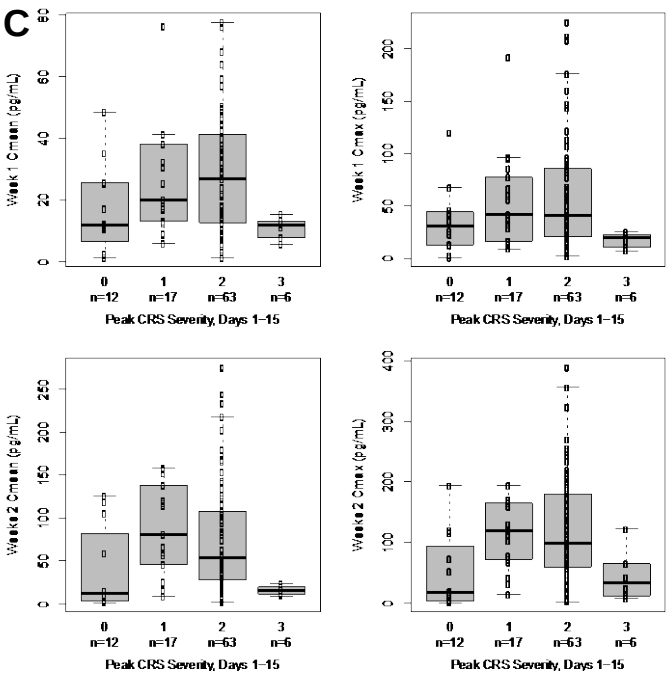
Cmean Cycle 1 (pg/mL)
0
50
100
150
200
3
n=1
10
n=4
30
n=5
100
n=4
300
n=7
500
n=69
700
n=8
Nominal Maximum Dose (ng/kg/day)


B
Week 1 Dose Intensity (%)
Week 2 Dose Intensity (%)
Week 3 Dose Intensity (%)
Week 4 Dose Intensity (%)
3 n=1
10 n=4
30 n=5
100 n=4
300 n=7
500 n=69
700 n=8
Nominal Maximum Dose (ng/kg/day)
3 n=1
10 n=4
30 n=4
100 n=4
300 n=6
500 n=64
700 n=8
Nominal Maximum Dose (ng/kg/day)
3 n=1
10 n=4
30 n=4
100 n=4
300 n=6
500 n=52
700 n=6
Nominal Maximum Dose (ng/kg/day)
3 n=1
10 n=2
30 n=4
100 n=3
300 n=6
500 n=50
700 n=5
Nominal Maximum Dose (ng/kg/day)


C
Week 1 Cmean (pg/mL)
Week 1 Cmax (pg/mL)
Week 2 Cmean (pg/mL)
Week 2 Cmax (pg/mL)
0 n=12
1 n=17
2 n=63
3 n=6
Peak CRS Severity, Days 1–15

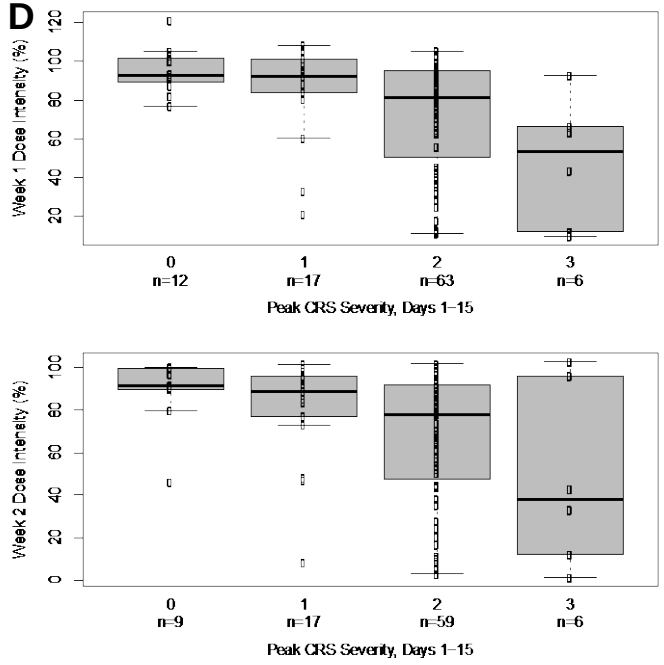

D
Week 1 Dose Intensity (%)
Week 2 Dose Intensity (%)
0 n=12
1 n=17
2 n=63
3 n=6
Peak CRS Severity, Days 1–15
0 n=9
1 n=17
2 n=59
3 n=6
Peak CRS Severity, Days 1–15

Fig. S2

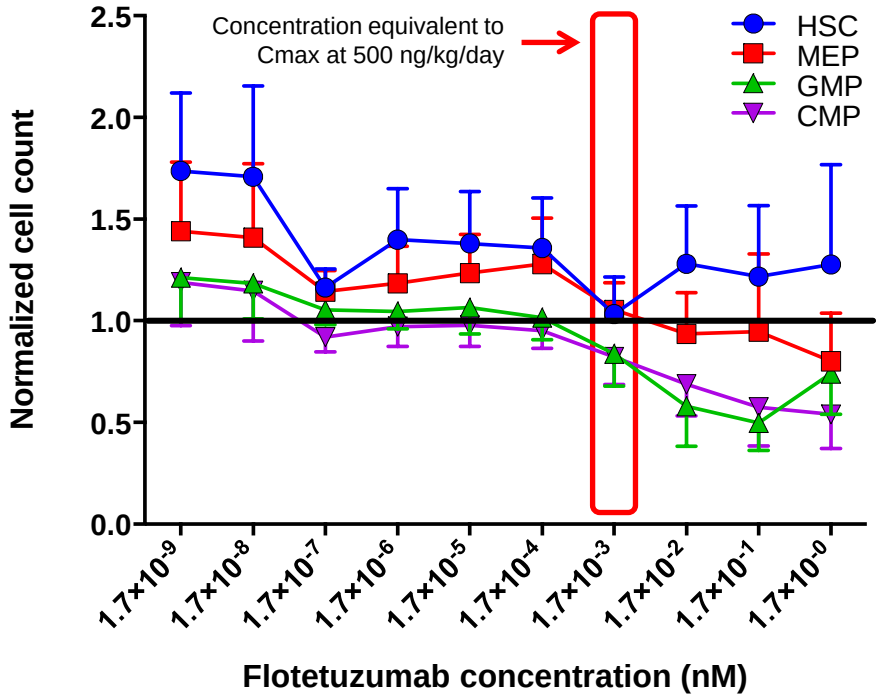

Concentration equivalent to
Cmax at 500 ng/kg/day
HSC
MEP
GMP
CMP
Normalized cell count
2.5
2.0
1.5
1.0
0.5
0.0
1.7×10-9
1.7×10-8
1.7×10-7
1.7×10-6
1.7×10-5
1.7×10-4
1.7×10-3
1.7×10-2
1.7×10-1
1.7×10-0
Flotetuzumab concentration (nM)

Fig. S3

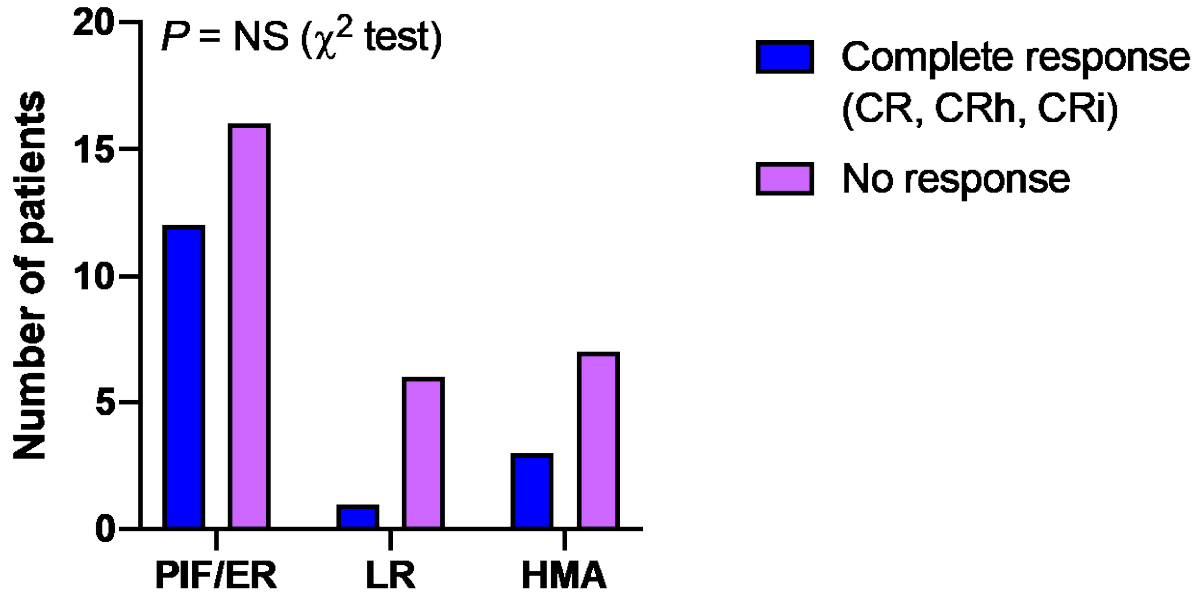

P = NS (χ2 test)
Number of patients
20
15
10
5
0
PIF/ER
LR
HMA
Complete response
(CR, CRh, CRi)
No response

Fig. S4

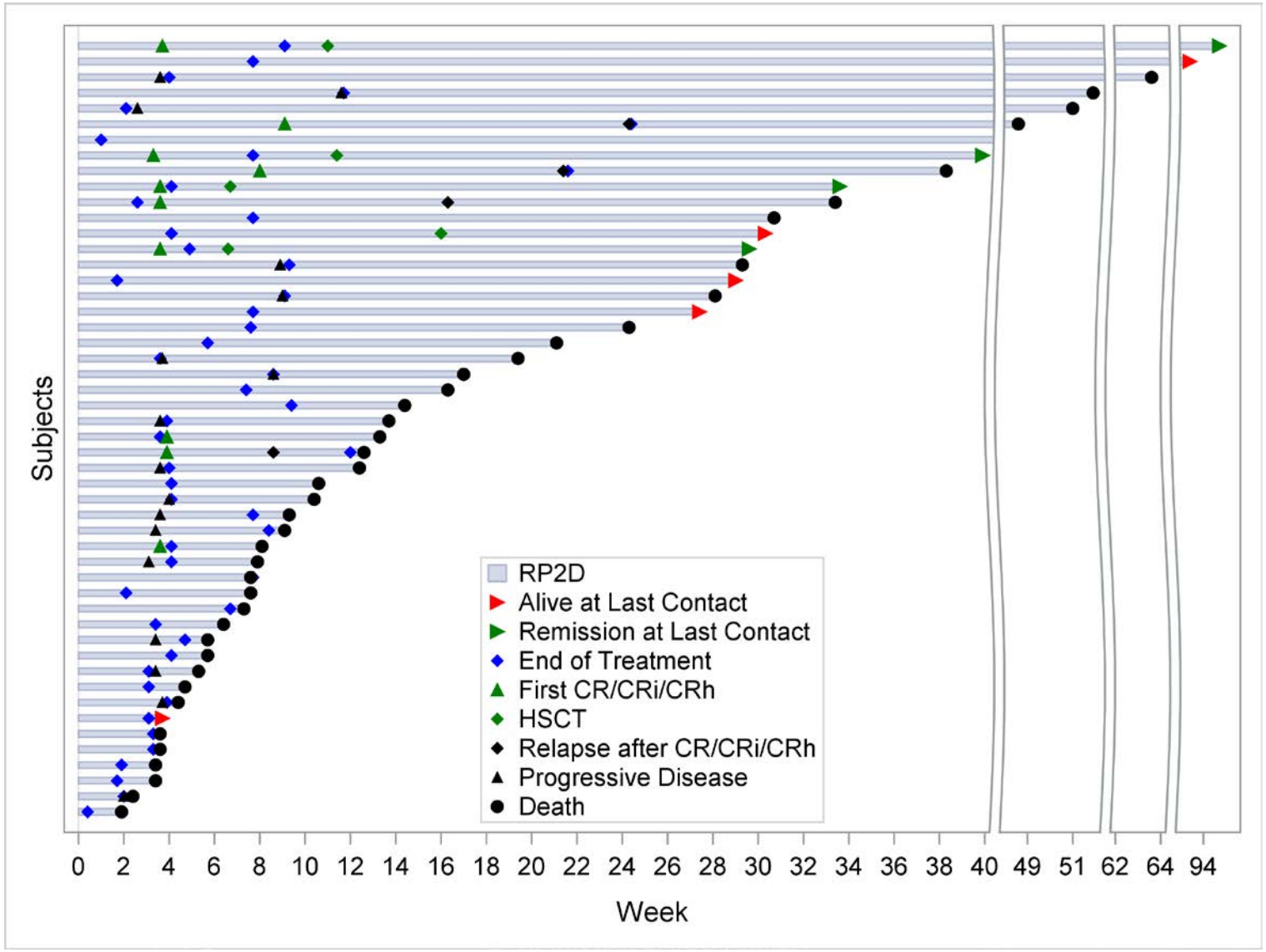
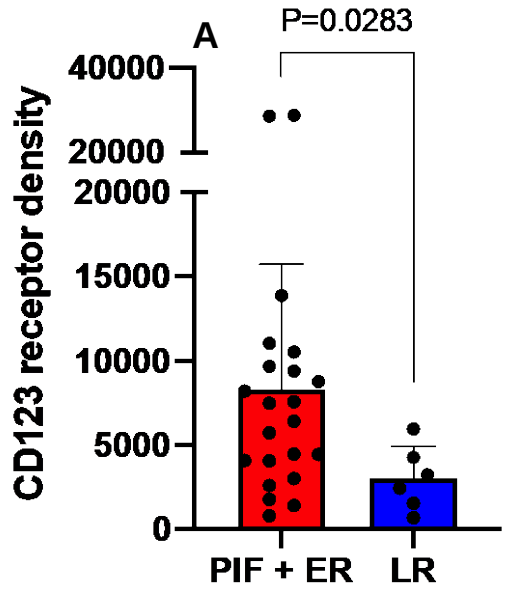
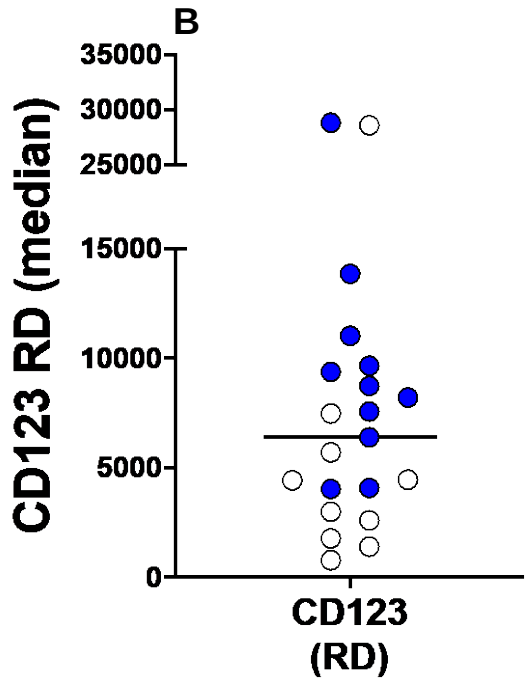
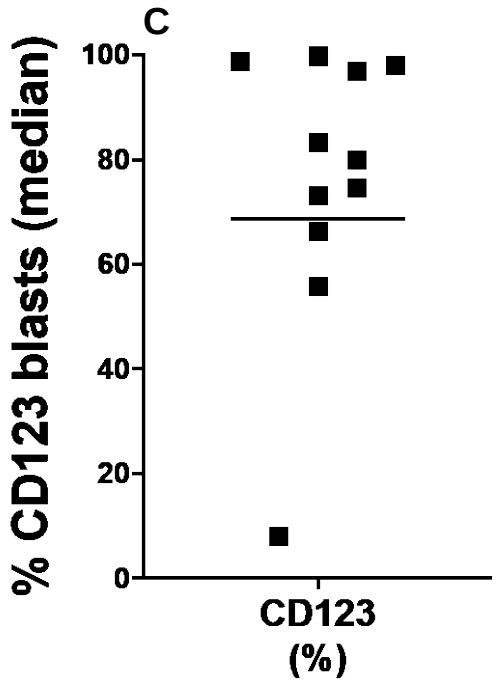

Subjects
RP2D
Alive at Last Contact
Remission at Last Contact
End of Treatment
First CR/CRi/CRh
HSCT
Relapse after CR/CRi/CRh
Progressive Disease
Death
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 49 51 62 64 94
Week

Fig. S5







	No response to flotetuzumab	Flotetuzumab anti-leukemic activity (>30% decrease in bone marrow blasts)
CD123 (RD)	●	●
CD123 (%)	■	■

Fig. S6

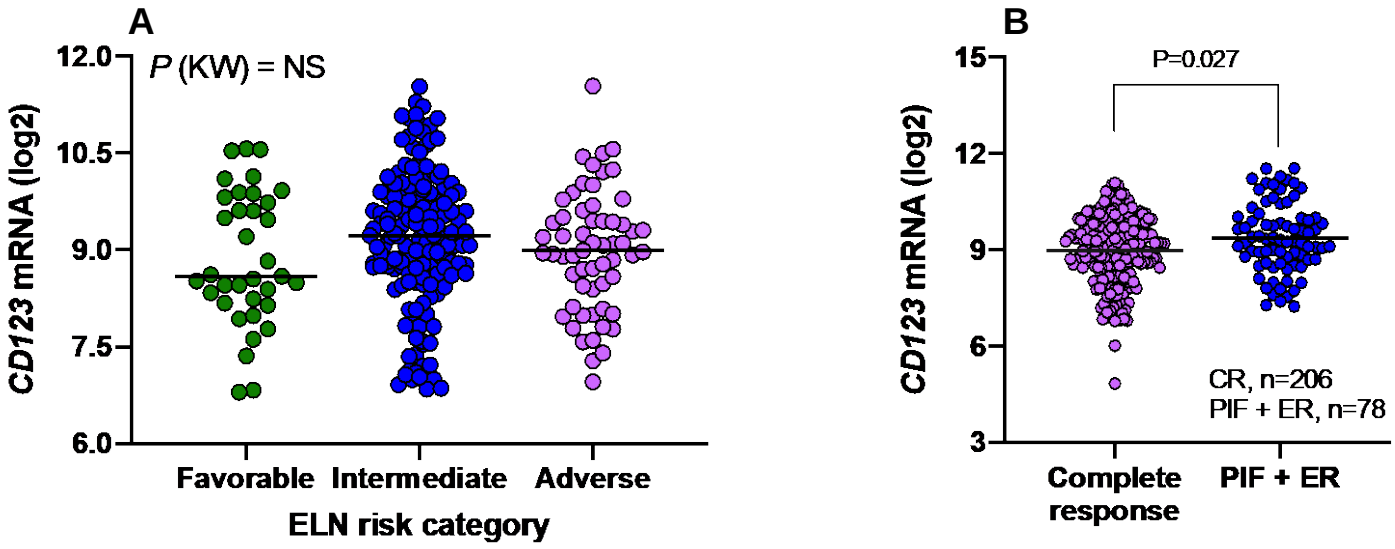

A
P (KW) = NS
CD123 mRNA (log2)
12.0
10.5
9.0
7.5
6.0
Favorable
Intermediate
Adverse
ELN risk category
B
P=0.027
CD123 mRNA (log2)
15
12
9
6
3
CR, n=206
PIF + ER, n=78
Complete response
PIF + ER

Fig. S7

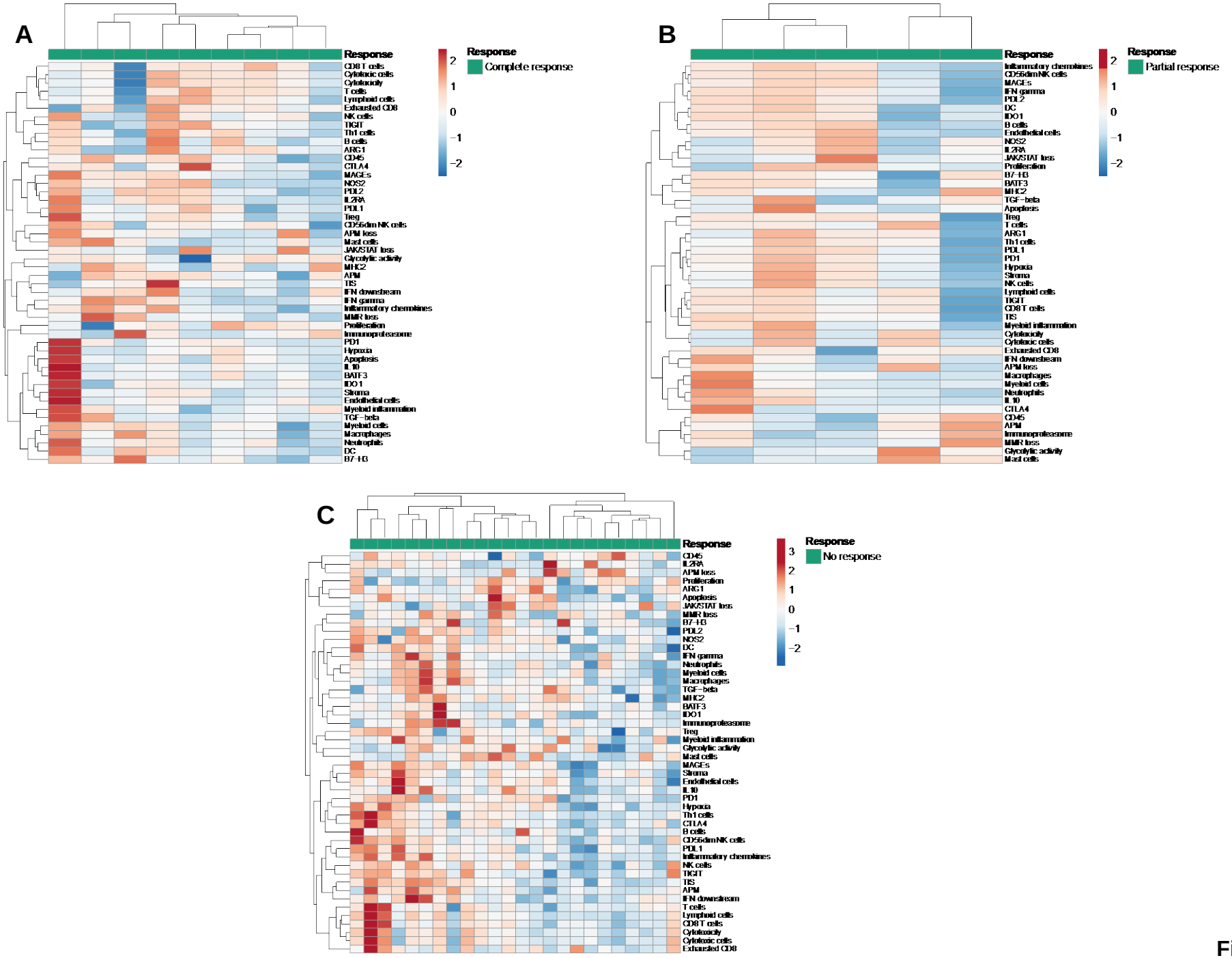

A
Response
CD8 T cells
Cytolytic cells
Cytotoxicity
T cells
Lymphoid cells
Exhausted CD8
NK cells
TIGIT
Th1 cells
B cells
ARG1
CD45
CTLA4
MAGEs
NOS2
PDL2
IL2RA
PDL1
Treg
CD56dim NK cells
APM loss
Mast cells
JAK/STAT loss
Glycolytic activity
MHC2
APM
TIS
IFN downstream
IFN gamma
Inflammatory chemokines
MMR loss
Proliferation
Immunoproteasome
PD1
Hypoxia
Apoptosis
IL10
BATF3
IDO1
Stroma
Endothelial cells
Myeloid inflammation
TGF−beta
Myeloid cells
Macrophages
Neutrophils
DC
B7−H3
2
1
0
−1
−2
Response
Complete response
B
Response
Inflammatory chemokines
CD56dim NK cells
MAGEs
IFN gamma
PDL2
DC
IDO1
B cells
Endothelial cells
NOS2
IL2RA
JAK/STAT loss
Proliferation
B7−H3
BATF3
MHC2
TGF−beta
Apoptosis
Treg
T cells
ARG1
Th1 cells
PDL1
PD1
Hypoxia
Stroma
NK cells
Lymphoid cells
TIGIT
CD8 T cells
TIS
Myeloid inflammation
Cytotoxicity
Cytolytic cells
Exhausted CD8
IFN downstream
APM loss
Macrophages
Myeloid cells
Neutrophils
IL10
CTLA4
CD45
APM
Immunoproteasome
MMR loss
Glycolytic activity
Mast cells
2
1
0
−1
−2
Response
Partial response
C
Response
CD45
IL2RA
APM loss
Proliferation
ARG1
Apoptosis
JAK/STAT loss
MMR loss
B7−H3
PDL2
NOS2
DC
IFN gamma
Neutrophils
Myeloid cells
Macrophages
TGF−beta
MHC2
BATF3
IDO1
Immunoproteasome
Treg
Myeloid inflammation
Glycolytic activity
Mast cells
MAGEs
Stroma
Endothelial cells
IL10
PD1
Hypoxia
Th1 cells
CTLA4
B cells
CD56dim NK cells
PDL1
Inflammatory chemokines
NK cells
TIGIT
TIS
APM
IFN downstream
T cells
Lymphoid cells
CD8 T cells
Cytotoxicity
Cytolytic cells
Exhausted CD8
3
2
1
0
−1
−2
Response
No response

Fig. S8

References

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