Supplementary Materials to:

Minimal Residual Hairy Cell Leukemia Eradication With Moxetumomab Pasudotox: Phase I Results and Long-term Follow-up

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Flow cytometry

Specimens were processed within 12 hours of collection. Red blood cells were pre-lysed by incubating with hypertonic ammonium chloride solution (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA) for 10 minutes at room temperature (maintained at 21–23^oC) at a ratio of 1:9 (volume of sample: volume of lysing solution). Specimens were then washed twice with phosphate buffered saline to remove cytophilic antibodies before determining cell number. Cells were then stained for 30 minutes at room temperature with a panel of antibodies (Table S1) as previously described.[1] Viability of lysed and washed cells was assessed by staining with the fluorescent dye 7-amino-actinomycin D (7-AAD) for 10 minutes at room temperature (7-AAD only stains non-viable cells). All cells were fixed in 1.0% paraformaldehyde and stored at 4°C for up to 12 hours before acquisition. Specimens were acquired using an 8-color multiparametric approach on a 3-laser FACSCanto II (BD Biosciences, San Jose, CA) with DiVa 6.1.1 software and analyzed by FCS Express 3 software (DeNovo Software, Los Angeles, CA). One to 1.5 million cells were acquired for each cocktail. For analysis of MRD flow cytometric data were evaluated for hairy cells in the following sequence: all cells; CD19 positive cells; and mononuclear cells (based upon light scatter characteristics). To detect residual hairy cell leukemia, cells coexpressing CD19, CD11c, CD25, CD103 and CD123 were identified and quantitated. The presence of residual hairy cell leukemia was confirmed in all cases by identifying cells coexpressing CD19, CD20, CD22, bright CD11c, and monoclonal light chains. The validated limit of detection of minimal residual hairy cell leukemia is 0.002% of cells, similar to the 10-4 limit reported for chronic lymphocytic leukemia.[2,3]

Table S1. Panel of Antibodies for Staining in Flow Cytometry Procedure

FITC	PE	PerCP	PC7	APC	AH7	v450	v500
CD16	CD19	CD3	CD13	CD34	CD14	CD56	CD45
CD57	TCR gd	CD4	CD3	CD5	CD8	CD56	CD45
CD103	CD25	CD123	CD19	CD23	CD20	CD11c	CD45
Kappa-monoclonal	Lambda-monoclonal	CD20	CD19	CD10	CD14	CD11c	CD45
Lambda-polyclonal	CD22	CD5	CD19	Kappa-polyclonal	CD20	11c	CD45

Abbreviations: APC, allophycocyanin conjugate; FITC, fluorescein isothiocyanate; PC7, proprotein convertase 7; PE, phycoerythrin; PerCP, peridinin-chlorophyll-protein complex conjugate; TCR gd, T-cell receptor gamma delta.

Table S2. Patient characteristics prior to treatment

	Dose Level — µg/kg Every Other Day × Three Doses			
Parameter	**5 to 40 (N = 16)**	**50 (extension) (N = 21)**	**50 (combined) (N = 33)**	**Total (N = 49)**
Age, median (range) — yr	58 (40–77)	54 (40–73)	55 (40–74)	57 (40–77)
Male-Female - no.	13-3	18-3	28-5	41-8
Prior purine analog courses no (%)				
1	1 (6)	3 (14)	3 (9)	4 (8)
2	7 (44)	12 (57)	17 (52)	24 (49)
≥3	8 (50)	6 (29)	13 (39)	21 (43)
Prior rituximab courses, no. (%)	8 (50)	14 (67)	22 (67)	30 (61)
Prior splenectomy, no. (%)	4 (25)	1 (5)	4 (12)	8 (16)
Evaluable for spleen size, no.	12	20	28	40
Maximum spleen size, median (range), mm	150 (112–194)	145 (95–225)	145 (95–225)	145 (95–225)
White blood cells — cells/nL*	1.90 (0.76–25.1)	1.85 (0.69–10.0)	1.89 (0.69–63.7)	1.89 (0.69–63.7)
Neutrophils — cells/nL*	0.75 (0.27–5.82)	0.86 (0.17–4.39)	0.86 (0.08–6.86)	0.78 (0.08–6.86)
Lymphocytes — cells/nL*	1.17 (0.16–14.1)	0.65 (0.24–12.0)	0.81 (0.24–58.6)	0.86 (0.16–58.6)
Hemoglobin — g/dL*	11.2 (6–14.4)	10.1 (7.1–12.7)	10.1 (6.7–14.2)	10.3 (6.0–14.4)
Platelets — cells/nL*	65 (6–167)	59 (23–90)	60 (22–125)	60 (6–167)
Circulating HCL cells/mm^3, median, n (Range)	61 (n=16) (1.6–14,566)	12 (n=21) (0.8–11,195)	19 (n=32) (0.8–60,444)	35 (n=48) (1–60,444)

When not otherwise indicated, the number of patients evaluable for each measurement is the number enrolled.
*Data are from the National Cancer Institute database.
Abbreviations: HCL, hairy cell leukemia.

Table S3. Treatment-Related Grade 3 and 4 Adverse Events at 50 μg/kg*

Adverse Event, no. (%)	Grade	Extension (N = 21)	Combined (N = 33)
Alanine transaminase increased	3	3 (15)	3 (9)
Gamma glutamyl transferase increased	3	1 (5)	2 (6)
Lymphopenia	3 and 4	1 (5)	2 (6)
White blood cell count decreased	3 and 4	2 (10)	2 (6)
Febrile neutropenia	3	0	1 (3)
Hypoalbuminemia	3	0	1 (3)
Pyrexia	3	0	1 (3)
Haptoglobin decreased	3	1 (5)	1 (3)
Neutrophil count decreased	3	1 (5)	1 (3)

*The only treatment-related grade 3/4 event observed at doses less than 50 μg/kg was grade 3 haptoglobin decrease at 30 μg/kg, which was associated with grade 2 hemolytic uremic syndrome.

Table S4. Clinical Treatment-Related Adverse Events Observed in Two or More Patients

Adverse Event, no. (%)	Dose Level (μg/kg every other day × 3 doses)							
	3 (N = 3)	10 (N = 3)	20 (N = 3)	30 (N = 3)	40 (N = 4)	50 (extension) N = 21)	50 (combined) (N = 33)	Total (N = 49)
Edema peripheral	1 (33.3)	1 (33.3)	1 (33.3)	3 (100)	3 (75.0)	6 (28.6)	12 (36.4)	21 (42.9)
Pyrexia	1 (33.3)	1 (33.3)	2 (66.7)	3 (100)	0	9 (42.9)	14 (42.4)	21 (42.9)
Myalgia	0	0	0	2 (66.7)	1 (25.0)	12 (57.1)	17 (51.5)	20 (40.8)
Headache	1 (33.3)	1 (33.3)	1 (33.3)	2 (66.7)	1 (25.0)	9 (42.9)	12 (36.4)	18 (36.7)
Nausea	1 (33.3)	0	0	1 (33.3)	2 (50.0)	9 (42.9)	12 (36.4)	16 (32.7)
Fatigue	1 (33.3)	1 (33.3)	1 (33.3)	3 (100)	0	7 (33.3)	8 (24.2)	14 (28.6)
Edema	1 (33.3)	1 (33.3)	0	1 (33.3)	1 (25.0)	5 (23.8)	8 (24.2)	12 (24.5)
Hypotension	0	0	0	2 (66.7)	0	7 (33.3)	10 (30.3)	12 (24.5)
Chills	1 (33.3)	0	0	1 (33.3)	0	6 (28.6)	9 (27.3)	11 (22.4)
Capillary leak	1 (33.3)	0	0	1 (33.3)	0	1 (4.8)	6 (18.2)	8 (16.3)

syndrome								
Dizziness	0	0	0	0	1 (25.0)	5 (23.8)	6 (18.2)	7 (14.3)
Weight increased	1 (33.3)	0	0	0	0	1 (4.8)	6 (18.2)	7 (14.3)
Arthralgia	0	0	0	0	0	4 (19.0)	5 (15.2)	5 (10.2)
Back pain	0	0	0	0	0	4 (19.0)	4 (12.1)	4 (8.2)
Diarrhea	1 (33.3)	0	0	0	1 (25.0)	2 (9.5)	2 (6.1)	4 (8.2)
Vomiting	0	0	0	0	1 (25.0)	2 (9.5)	3 (9.1)	4 (8.2)
Decreased appetite	1 (33.3)	0	0	0	0	2 (9.5)	2 (6.1)	3 (6.1)
Hyperhidrosis	0	0	0	1 (33.3)	0	2 (9.5)	2 (6.1)	3 (6.1)
Sinus tachycardia	0	0	0	1 (33.3)	0	1 (4.8)	1 (3.0)	2 (4.1)
Abdominal pain	0	0	0	0	0	2 (9.5)	2 (6.1)	2 (4.1)
Constipation	0	0	0	1 (33.3)	0	1 (4.8)	1 (3.0)	2 (4.1)
Face edema	0	0	1 (33.3)	1 (33.3)	0	0	0	2 (4.1)
Localized edema	0	0	0	0	1 (25.0)	1 (4.8)	1 (3.0)	2 (4.1)

Bone pain	0	0	0	1 (33.3)	0	1 (4.8)	1 (3.0)	2 (4.1)
Neck pain	0	0	0	0	0	2 (9.5)	2 (6.1)	2 (4.1)
Cough	0	0	0	0	0	1 (4.8)	2 (6.1)	2 (4.1)
Dyspnea	0	0	0	1 (33.3)	0	1 (4.8)	1 (3.0)	2 (4.1)
Productive cough	0	0	0	0	0	0	2 (6.1)	2 (4.1)
Hypertension	0	1 (33.3)	0	1 (33.3)	0	0	0	2 (4.1)

Table S5. Laboratory Treatment-Related Adverse Events Observed in Two or More Patients

	Dose Level (μg/kg every other day × 3 doses)							
Adverse event, no. (%)	**3 (N = 3)**	**10 (N = 3)**	**20 (N = 3)**	**30 (N = 3)**	**40 (N = 4)**	**50 (extension) (N = 21)**	**50 (Combined) (N = 33)**	**Total (N = 49)**
Hypoalbuminemia	2 (66.7)	1 (33.3)	1 (33.3)	3 (100)	3 (75.0)	16 (76.2)	24 (72.7)	34 (69.4)
Alanine transaminase increased	1 (33.3)	1 (33.3)	1 (33.3)	3 (100)	2 (50.0)	16 (76.2)	23 (69.7)	31 (63.3)
Aspartate transaminase increased	2 (66.7)	1 (33.3)	1 (33.3)	2 (66.7)	1 (25.0)	17 (81.0)	23 (69.7)	30 (61.2)
Blood creatinine increased	0	0	0	1 (33.3)	0	6 (28.6)	8 (24.2)	9 (18.4)
Proteinuria	0	0	0	0	0	8 (38.1)	9 (27.3)	9 (18.4)
Hemorrhage urinary tract	0	0	0	1 (33.3)	1 (25.0)	4 (19.0)	5 (15.2)	7 (14.3)
Gamma glutamyl	0	0	0	0	0	4 (19.0)	6 (18.2)	6 (12.2)

transferase increased								
White blood cell decreased	1 (33.3)	0	0	0	0	2 (9.5)	3 (9.1)	4 (8.2)
Lymphopenia	0	0	0	0	0	1 (4.8)	3 (9.1)	3 (6.1)
Blood albumin decreased	0	0	0	0	0	0	2 (6.1)	2 (4.1)
Blood magnesium decreased	0	0	0	0	1 (25.0)	0	1 (3.0)	2 (4.1)
Haptoglobin decreased	0	0	0	1 (33.3)	0	1 (4.8)	1 (3.0)	2 (4.1)
Hemoglobinuria	0	0	0	0	0	1 (4.8)	2 (6.1)	2 (4.1)
Hemolytic uremic syndrome	0	0	0	1 (33.3)	0	0	1 (3.0)	2 (4.1)
Platelet count decreased	0	0	0	0	1 (25.0)	1 (4.8)	1 (3.0)	2 (4.1)
Protein urine present	0	0	0	0	1 (25.0)	0	1 (3.0)	2 (4.1)

Table S6. Change from Baseline in Hematology (NCI Extension Cohort)

Parameter	Baseline	Visit	Visit	Change from Baseline			
	Mean		Mean	Mean	SD	Median	Range
Hemoglobin (g/dL)	10.5	Nadir on-study	9.2	-1.3	1.2	-1.5	(-2.9, 2.5)
		Zenith on-study	13.5	3.0	1.9	2.2	(0.1, 5.6)
		End of treatment	12.9	2.4	2.2	2.2	(-0.9, 5.6)
Platelets (10^3/uL)	59.4	Nadir on-study	49.6	-9.8	7.4	-8.0	(-26.0, 0.0)
		Zenith on-study	284.7	225.3	96.4	210.0	(14.0, 420.0)
		End of treatment	212.1	152.7	93.7	134.0	(5.0, 387.0)
Neutrophils (10^3/uL)	1.0	Nadir on-study	0.5	-0.5	0.8	-0.3	(-3.6, 0.2)
		Zenith on-study	5.9	4.9	2.8	4.2	(0.9, 11.6)
		End of treatment	3.2	2.2	1.5	2.0	(-0.6, 5.6)

Abbreviations: NCI, National Cancer Institute.

Table S7. Response of HCL to Moxetumomab Pasudotox at Different Dose Levels

Dose Level, μg/kg*	No.	ORR No. (%)	PR No. (%)	CR No. (%)	MRD-Negative CR no./No. (%)
5	3	3 (100)	3 (100)	0 (0)	0/3 (0)
10	3	3 (100)	1 (33)	2 (67)	0/2 (0)
20	3	2 (67)	0 (0)	2 (67)	0/2 (0)
30	3	2 (67)	1 (33)	1 (33)	0/3 (0)
40	4	3 (75)	1 (25)	2 (50)	1/3† (33)
50 (extension)	21	19 (91)	4 (19)	15 (71)	8/21 (38)
50 (combined)	33	29 (88)	8 (24)	21 (64)	11/32† (34)
Total	49	42 (86)	14 (29)	28 (57)	12/49 (25)

*All doses were administered every other day for three doses.
†One patient at 50 μg/kg with minimal residual disease-negative CR documented off study during follow-up was not included in this analysis. One CR at 40 μg/kg was not evaluated by bone marrow aspirate flow cytometry.
Abbreviations: CR, complete remission; HCL, hairy cell leukemia; MRD, minimal residual disease; ORR, objective response rate; PR, partial response.

Table S8. Flow Cytometry Minimal Residual Disease Status of Patients in CR at 50 μg/kg Every Other Day x Three Doses (National Cancer Institute extension cohort).

Patients, No.	Blood	Bone Marrow
11	Negative	Negative
8	Negative	Positive
1	Positive	Positive
1	Unknown	Unknown

Abbreviations: CR, complete remission.

Table S9. Pharmacokinetics of Moxetumomab Pasudotox at 50 µg/kg During Cycles 1 and 2

Parameter, median (range)	**Cycle 1 Day 1**	**Cycle 1 Day 5**	**Cycle 2 Day 1**	**Cycle 2 Day 5**
No. evaluable	N = 28	N = 29	N = 29	N = 29
Peak level (ng/mL)	326 (58–958)	758 (163–1440)	633 (149–1470)	796 (70–1787)
Half-life* (min)	56 (8–397)	107 (8–672)	106 (10–206)	115 (10–188)
Area under the curve* (µg/min/mL)	15 (5–131) ()	107 (6–356)	106 (8–300)	139 (7–374)
Volume of distribution (L)	21 (4–205)	6 (2–147)	6 (3–106)	6 (2–28)
Clearance — mL/min	261 (30–812)	38 (10–726)	36 (16–509)	29 (13–547)

*For patients wherein biexponential disappearance was relevant, the half-life chosen reflect the second (half-life β). Area under the concentration-time curve with biexponential parameters were included in the summary where relevant.

Table S10. Percentage Change in Pharmacokinetic Parameters From Day 1 to Day 5 at Moxetumomab Pasudotox 50-µg/kg Dose Level

Parameter, Median % Change (range)	**Change from Cycle 1 Days 1 to 5**	**Change from Cycle 2 Days 1 to 5**
No. evaluable	N = 28	N = 29
Peak level — ng/mL	118%–885% (213%) *P* < .001	47%–426% (121%) *P* = .002
Half-life	206% (8%–1995%) *P* < .001	122% (26%–451%) *P* < .001
Area under the curve	512% (92%–3068%) *P* < .001)	147% (68%–465%) *P* < .001
Volume of distribution	33% (2%–1262%) *P* = .009	90% (6%–165%) *P* = .06
Clearance	20% (3%–111%) *P* < .001	72% (22%–150%) *P* < .001

Table S11. Immunogenicity of Moxetumomab Pasudotox in HCL*

	Dose Level — µg/kg Every Other Day × Three Doses				
Parameter	**5 to 40**	**P value†**	**50 (Combined)**	**50 (extension)**	**Total**
No. of patients evaluable	14		32	21	46
>50% of 200 ng/mL, No. (%)	12 (86%)	0.70	24 (75%)	18 (86%)	36 (78%)
>75% of 1000 ng/mL - no. (%)	5 (36%)	0.35	17 (53%)	13 (62%)	22 (48%)
Evaluable per cycle, No.‡	13		29	21	42
>50% of 200 ng/mL, No. (%)	11 (85%)	0.47	21 (72%)	18 (86%)	32 (76%)
>75% of 1000 ng/mL, No. (%)	5 (38%)	0.51	16 (55%)	13 (62%)	21 (50%)
Cycles prior to neutralization for those patients who neutralized (N=36)					
>50% of 200 ng/mL, No. (range)	3.0 (1–8)		3.0 (1–7)	3.0 (1–7)	3.0 (1–8)
>75% of 1000 ng/mL, No. (range)	2.5 (1–4)		3.0 (2–7)	3.0 (2–7)	3.0 (1–7)

*Trends for higher rate of CR without MRD (8 of 15 vs. 3 of 17; *P* = .06) and longer CR duration (median undefined vs. 19.1 months, *P* = .05) were observed in patients without vs. with antibodies neutralizing more than 75% of the activity of 1000 ng/m of moxetumomab pasudotox.
†P values indicate comparison between patients receiving doses of 50 µg/kg vs. those receiving 5 to 40 µg/kg.
‡Patients who were evaluated after each cycle; only these patients evaluated for number of cycles prior to neutralization.‡
Abbreviations: CR, complete remission; HCL, hairy cell leukemia; MRD, minimal residual disease.

Table S12. Soluble CD22 Levels and Change in Levels With Moxetumomab Pasudotox at Moxetumomab Pasudotox 50-μg/kg Dose Level

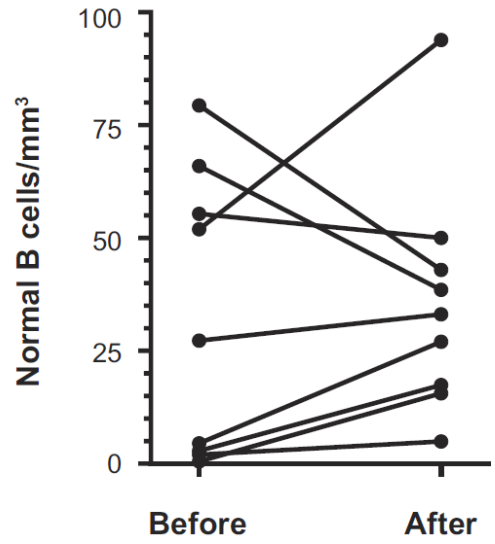
Soluble CD22 prior to cycle 1 — median (range), ng/mL		**P value**
CR	15 (4–125); n = 19	0.02
No CR	50 (6–353); n = 10	
CR without MRD	12 (4–23); n = 10	0.15
CR with MRD	23 (5–125); n = 8	
Soluble CD22 prior to cycle 2 (ng/mL) — median (range), ng/mL		**P value**
CR	3 (0–38.0); n = 13	0.07
No CR	10 (2–91); n = 7	
CR without MRD	1 (0–2); n = 5	0.02
CR with MRD	5 (1–38); n = 7	
Percent change in soluble CD22 from before cycle 2 to before cycle 1 — median (range)		**P value**
CR	-83 (-96 to -43); n = 12	0.08
No CR	-69 (-81 to 12); n = 6	
CR without MRD	-91 (-96 to -77); n = 5	0.13
CR with MRD	-77 (-90 to -66); n = 6	
Soluble CD22 at the time of restaging — median (range), ng/mL		**P value**
CR	0 (0–3); n = 10	0.18
No CR	13 (NA); n = 1	
CR without MRD	0 (0–1); n = 6	0.05
CR with MRD	2 (1–3); n = 3	

Abbreviations: CR, complete remission; MRD, minimal residual disease.

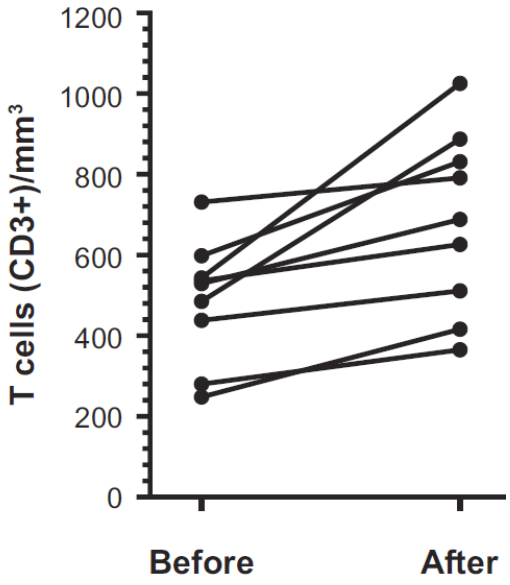
Figure S1. Effect of moxetumomab pasudotox on normal lymphocytes. Flow cytometric analysis of lymphocytes in posttreatment samples, obtained after one or two cycles of moxetumomab pasudotox, contained no detectable residual HCL cells.

Abbreviations: HCL, hairy cell leukemia.

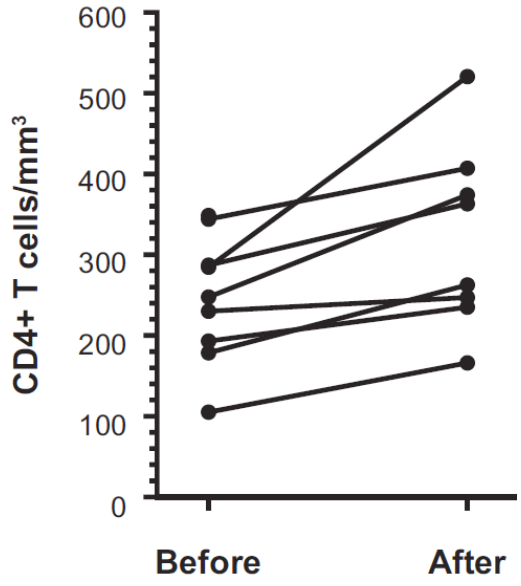
A.



B.



C.


600
500
400
300
200
100
0
CD4+ T cells/mm3
Before
After

D.

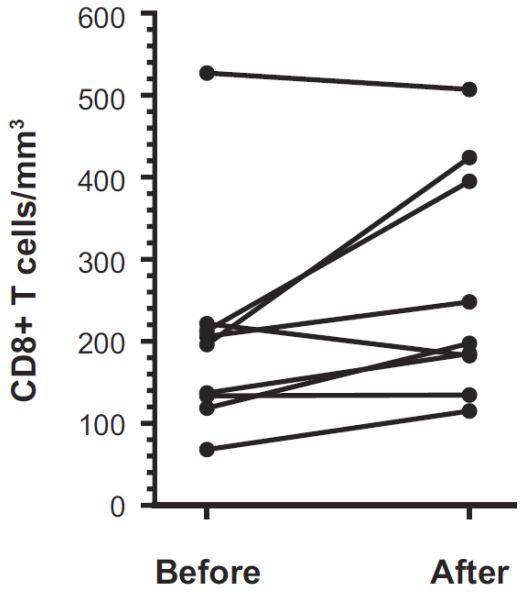

600
500
400
300
200
100
0
CD8+ T cells/mm3
Before
After

Figure S2. Time to Response (safety population). Achievement of objective response or CR is shown from the time of beginning treatment. Points represent patients treated at 5 (□), 10 (Δ), 20 (◊), 30 (□), 40 (○) and 50 (unlabeled) μg/kg every other day × three doses. For CR, since a bone marrow negative for HCL was required, in addition to resolved cytopenias for at least 4 weeks, bone marrows were not undertaken until the latter criterion was fulfilled.

Abbreviations: CR, complete remission; HCL, hairy cell leukemia.

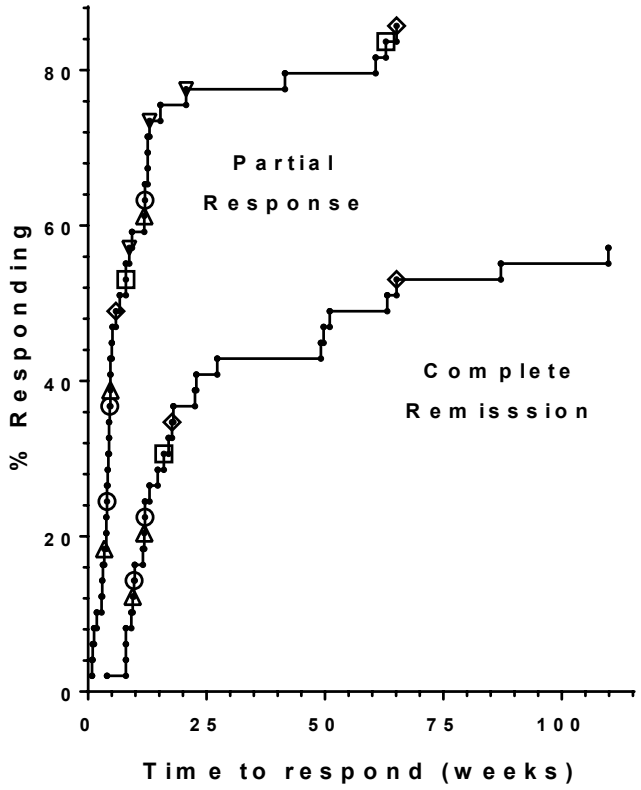
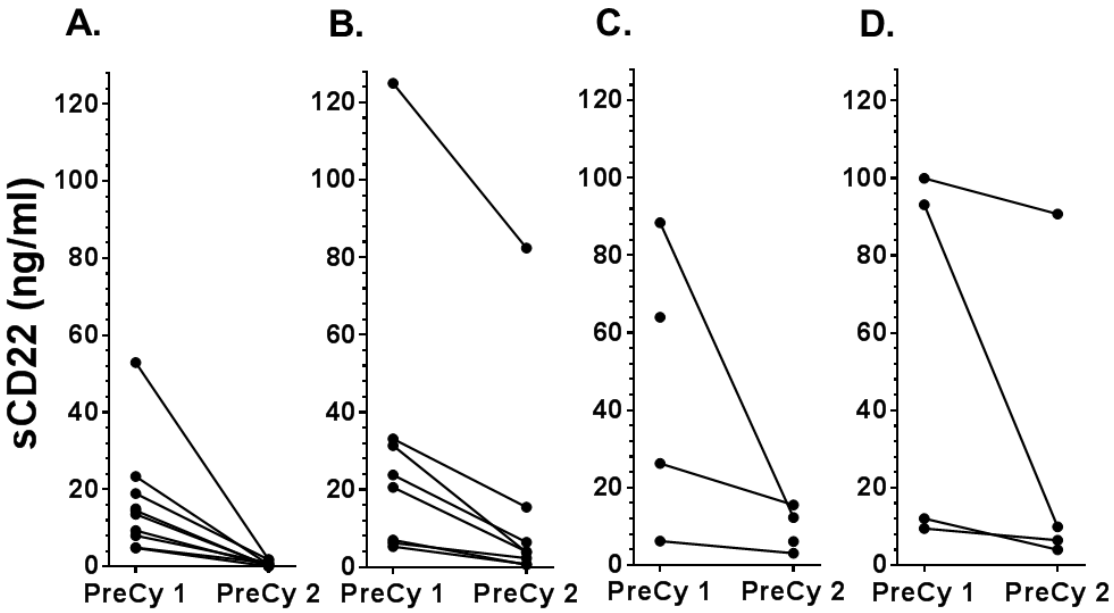


Figure S3. Soluble CD22 after 1 cycle of moxetumomab pasudotox. Concentrations before cycle 1 and before cycle 2 for patients at 50 μg/kg who achieved CR with negative (A) or positive (B) MRD, partial response (C), or stable disease (D).

Abbreviations: CR, complete remission; Cy, cycle; MRD, minimal residual disease.



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3. Bottcher S, Ritgen M, Kneba M. Flow cytometric MRD detection in selected mature B-cell malignancies. *Methods Mol Biol*. 2013;971:149-174.