MolecularMedicine

Supplemental Data

Novel Targeted Therapy for Precursor B-Cell Acute Lymphoblastic Leukemia: Anti-CD22 Antibody-MXD3 Antisense Oligonucleotide Conjugate

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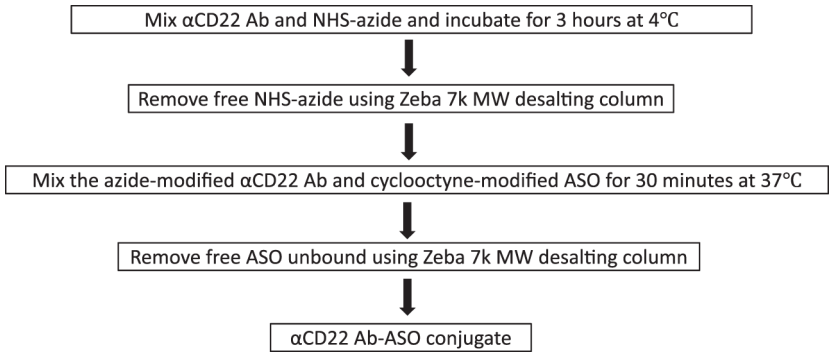
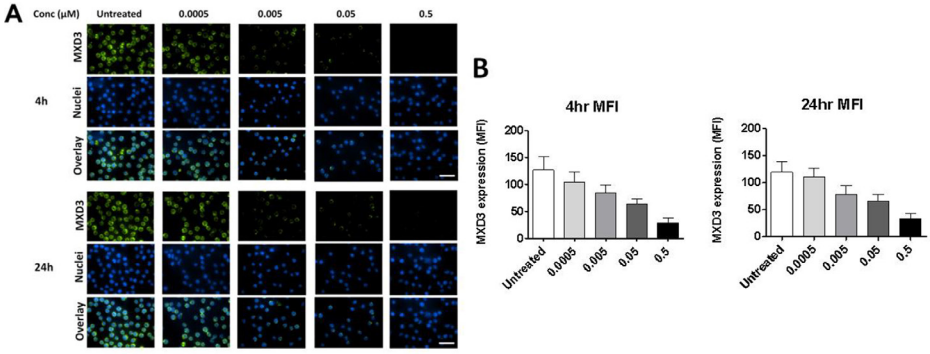
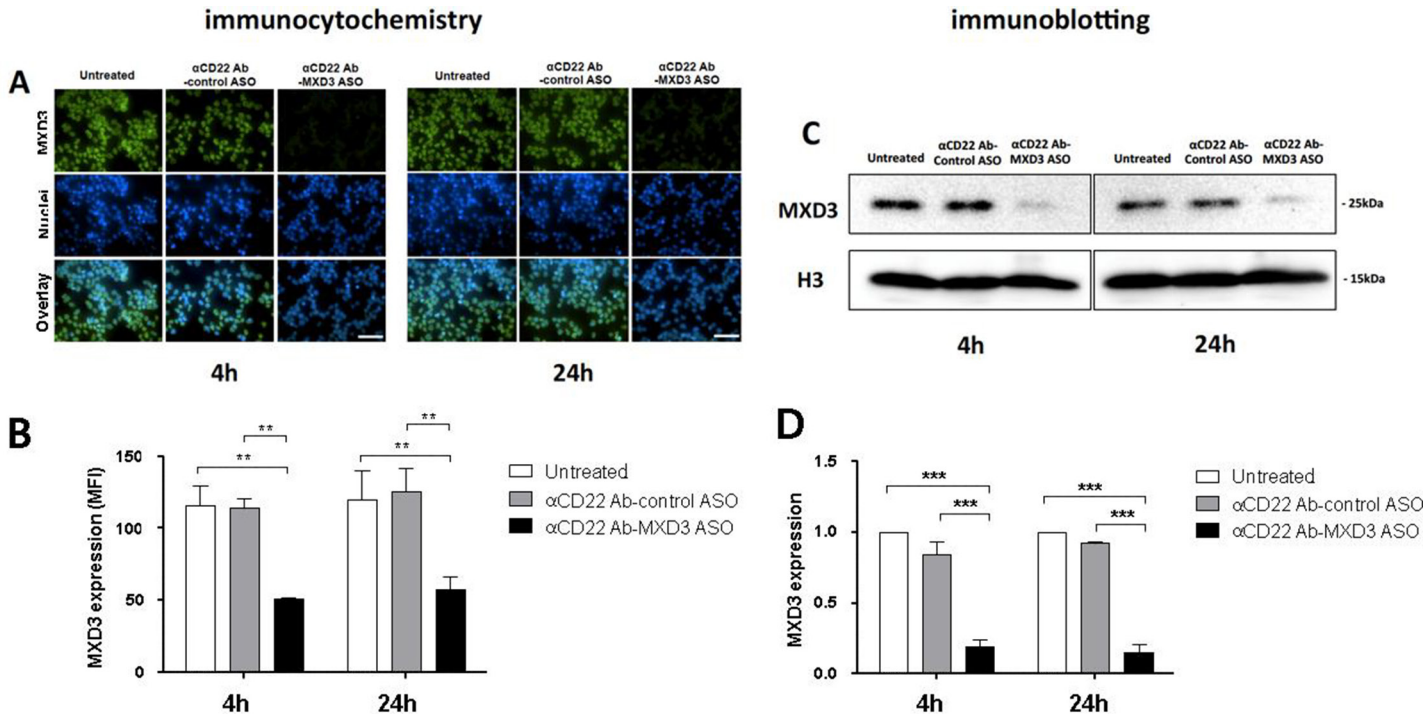


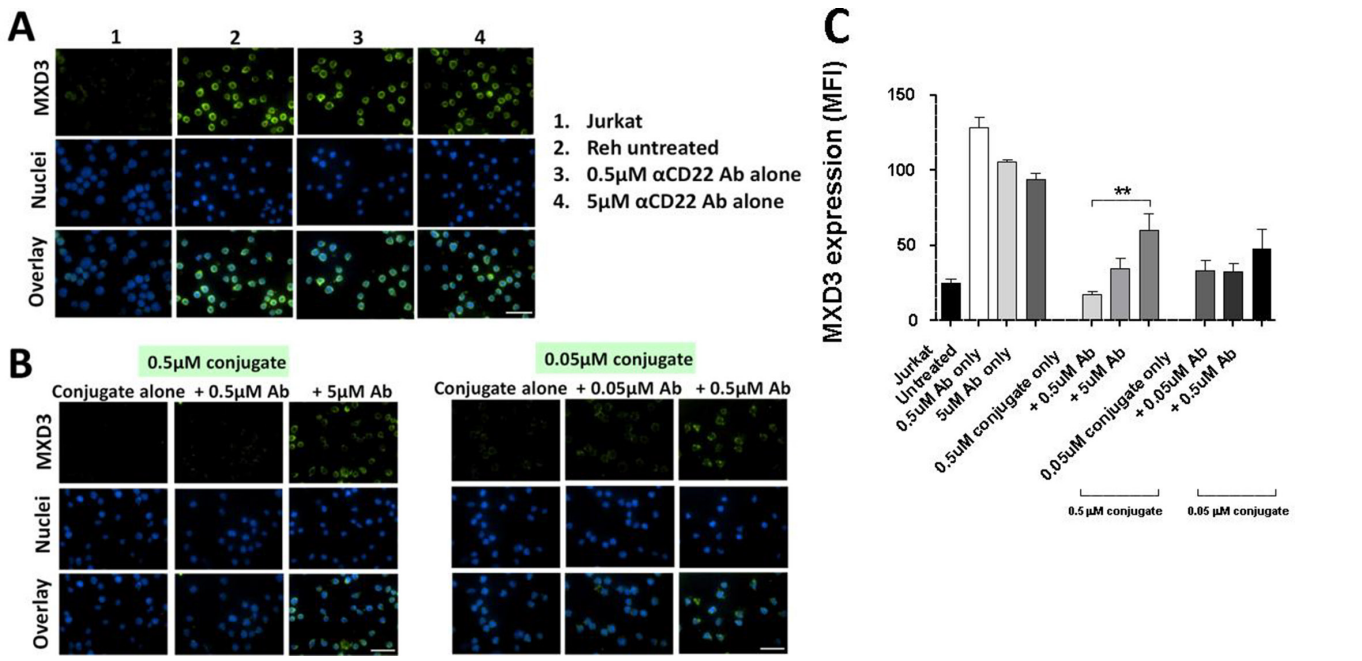
Supplementary Figure S1. Flow chart of the synthesis of the αCD22 Ab-ASO conjugate.



Supplementary Figure S2. αCD22 Ab-MXD3 ASO conjugate demonstrates MXD3 knockdown in preB ALL cells in a concentration-dependent manner. (A) Reh cells were treated with the αCD22 Ab-MXD3 ASO conjugate at four different concentrations (0.0005, 0.005, 0.05 and 0.5 μmol/L of the Ab in the conjugate). MXD3 protein expression was measured at 4 and 24 h after the treatment. The overlay pictures show a composite image of both MXD3 protein (with Alexa488) and nuclei (with DAPI). Images were acquired at 40× magnification/1.4 numerical aperture at room temperature using a Nikon Ti-U inverted microscope and NIS-Elements BR software. Scale bar indicates 50 μm. The images shown are from one representative experiment out of three experiments. (B) Mean fluorescence intensity (MFI) was used to quantify MXD3 protein expression from (A). Each bar represents the average MFI of all measured cells per treatment type from three independent experiments. Error bars represent SEM (n = 3). (Overall ANOVA $p < 0.001$ for both A and B).

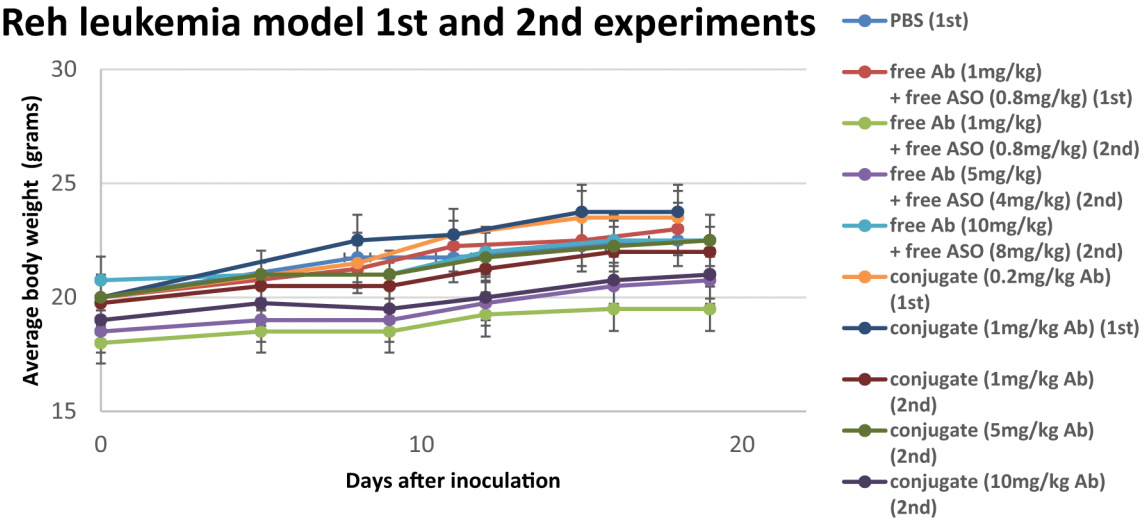


Supplementary Figure S3. MXD3 knockdown in Reh cells is confirmed by immunocytochemistry and immunoblotting. Reh cells were treated with the αCD22 Ab-MXD3 or -control ASO conjugate (0.5 μmol/L of the Ab in the conjugate). MXD3 protein expression was measured on the same samples at 4 and 24 h after the treatment. Significant MXD3 protein knockdown was observed in the cells treated with the αCD22 Ab-MXD3 conjugate compared with untreated or cells treated with αCD22 Ab-control ASO conjugate. (A) Immunocytochemistry results are shown. The overlay pictures show a composite image of both MXD3 protein (with Alexa488) and nuclei (with DAPI). Images were acquired at 40× magnification/1.4 numerical aperture at room temperature using a Nikon Ti-U inverted microscope and NIS-Elements BR software. Scale bar indicates 50 μm. The images shown are from one representative experiment out of three experiments. (B) Mean fluorescence intensity (MFI) was used to quantify MXD3 protein expression from (A). Each bar represents the average MFI of all measured cells per treatment type from three independent experiments. Error bars represent SEM (n = 3). Average MXD3 knockdown in the cells treated with αCD22 Ab-MXD3 ASO conjugate, compared with untreated or αCD22 Ab-control ASO conjugate, was 56% and 55% at 4 h and 52% and 54% at 24 h, respectively (** ANOVA $p < 0.001$, significant differences from multiple comparisons shown). (C) Immunoblotting results from the same experiment in (A) are shown. Histone H3 was used as an internal control. The images shown are from one representative experiment out of three experiments. (D) MXD3 expression levels relative to those in untreated cells (after corrected with Histone H3 expression levels) are shown. Error bars represent SEM (n = 3). The results are average data from three independent experiments (same as in B). MXD3 knockdown in the cells treated with αCD22 Ab-MXD3 ASO conjugate, compared with untreated or αCD22 Ab-control ASO conjugate, were 81% and 77% at 4 h and 85% and 84% at 24 h, respectively (Overall ANOVA ***$p < 0.001$, significant differences from multiple comparisons shown).

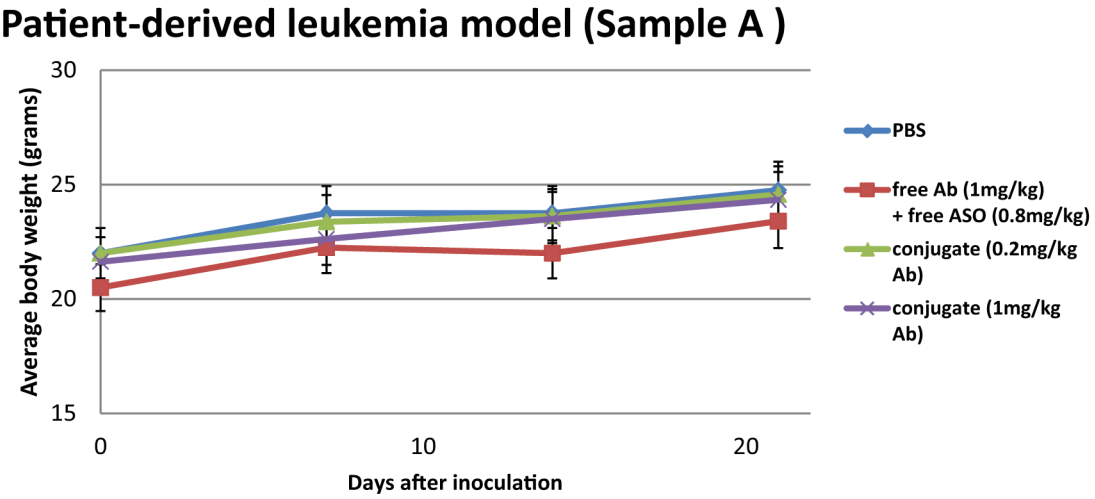


Supplementary Figure S4. The αCD22 Ab-MXD3 ASO conjugate, but not naked αCD22 Ab, shows MXD3 knockdown. (A) Treatment of Reh cells with the αCD22 Ab only at 0.5 μmol/L or 5 μmol/L did not show significant MXD3 knockdown. Jurkat cells were used as a negative control for MXD3 expression. (B) Reh cells were treated with the αCD22 Ab-MXD3 ASO conjugate (0.5 μmol/L or 0.05 μmol/L) with or without cold αCD22 Ab at indicated concentrations above. MXD3 knockdown by the conjugate treatment was partially inhibited when cold αCD22 Ab was added simultaneously at the concentrations of equal or 10 times higher than the Ab concentration in the conjugate. Images were acquired at 40× magnification/1.4 numerical aperture at room temperature using a Nikon Ti-U inverted microscope and NIS-Elements BR software. For both (A) and (B) the images shown are from one representative experiment out of three experiments. (C) MFI from (A) and (B). For 0.5 μmol/L of the conjugate, the percentage knockdown was 86% of untreated. Addition of the Ab, at 0.5 μmol/L or 5 μmol/L, reduced the percentage MXD3 knockdown to 73% and 53%, respectively. For 0.05 μmol/L of the conjugate, the percentage knockdown was 74% of untreated. Addition of the Ab, at 0.05 μmol/L or 0.5 μmol/L, reduced the percentage knockdown to 75% and 63%, respectively. MXD3 protein expression were measured at 4 h after treatment. Scale bar indicates 50 μm. Error bars represent SEM (n = 3). Each bar represents the average MFI of all measured cells per treatment type from three independent experiments. MXD3 expression in cells treated with 0.5 μmol/L conjugate only versus addition of 5 μmol/L Ab (Overall ANOVA $^{**}p < 0.001$, based on ln MFI, multiple comparison results shown).

A



B



Supplementary Figure S5. Mice show stable weight gain during treatment. Average body weight of the mice during treatment in the Reh (A) and the patient sample A (B) experiments. None of the mice lost weight during the treatments.

Supplementary Table S1. HLA and CD22 expression of the cells harvested from leukemia xenograft models. Reh (A) and patient-derived leukemic cells (B and C) engrafted in the mice, respectively, showed high expression of HLA and CD22 as quantified by flow cytometry. *indicates the samples which were fixed for subsequent analyses.

A

	Mouse	HLA%	CD22%	CD22/HLA
PBS	1	72.5	70.4	1.0
	2	74.8	70.8	0.9
	3	62.7	57.6	0.9
	4	63.3	58.7	0.9
Free Ab (1mg/kg) + free ASO (0.8mg/kg)	1	73.6	52.4	0.7
	2	82.7	72.7	0.9
	3	90.0	77.0	0.9
	4	80.0	93.2	1.2
	5	92.4	79.9	0.9
	6	92.6	77.4	0.8
	7	90.5	74.7	0.8
	8	92.8	79.1	0.9
Free Ab (5mg/kg) + free ASO (4mg/kg)	1	86.3	83.8	1.0
	2	90.7	84.1	0.9
	3	91.3	82.2	0.9
	4	91.2	82.3	0.9
Free Ab (10mg/kg) + free ASO (8mg/kg)	1	91.9	71.0	0.8
	2	90.1	82.4	0.9
	3	91.0	87.6	1.0
	4	78.0	60.2	0.8
Conjugate (0.2mg/kg Ab)	1	94.9	79.4	0.6
	2	90.6	39.3	0.4
	3	94.3	83.5	0.9
	4	93.0	91.2	1.0
Conjugate (1mg/kg Ab)	1	96.2	83.2	0.9
	2	84.2	72.3	0.9
	3	79.4	69.3	0.9
	4	82.6	59.2	0.7
	5	91.2	77.5	0.8
	6	89.7	77.3	0.9
	7	93.6	88.9	0.9
	8	93.7	77.9	0.8
Conjugate (5mg/kg Ab)	1	85.2	68.1	0.8
	2	89.6	74.0	0.8
	3	89.5	74.3	0.8
	4	93.4	77.3	0.8
Conjugate (10mg/kg Ab)	1	89.8	78.8	0.9
	2	89.9	80.2	0.9
	3	83.1	68.7	0.8
	4	90.0	75.3	0.8

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Supplementary Table S1. *Continued.*

B

	Mouse	HLA%	CD22%	CD22/HLA	
PBS	1	99.0	93.1	0.9	*
	2	93.0	93.0	1.0	*
	3	96.4	77.5	0.8	*
	4	96.6	22.9	0.4	*
	5	94.5	79.8	0.8	*
	6	90.1	58.9	0.7	*
	7	94.5	62.8	0.7	*
	8	92.0	65.5	0.7	*
Free Ab (1mg/kg) + free ASO (0.8mg/kg)	1	93.6	97.2	1.0	*
	2	92.7	76.1	0.8	*
	3	92.7	47.4	0.5	*
	4	89.0	59.3	0.7	*
	5	96.5	68.6	0.7	*
	6	99.4	94.6	1.0	*
	7	92.1	90.3	1.0	*
	8	85.8	55.2	0.6	*
Conjugate (0.2mg/kg Ab)	1	90.0	95.3	1.1	
	2	94.3	83.4	0.9	
	3	94.2	93.9	1.0	
	4	94.4	84.4	0.9	
	5	93.8	86.1	0.9	
	6	92.0	87.8	1.0	
	7	96.0	94.2	1.0	
	8	97.6	96.5	1.0	
Conjugate (1mg/kg Ab)	1	91.6	93.5	1.0	
	2	93.1	93.9	1.0	
	3	91.4	93.6	1.0	
	4	91.1	80.3	0.9	
	5	94.8	89.7	0.9	
	6	90.8	84.8	0.9	
	7	92.6	88.0	1.0	
	8	86.6	76.0	0.9	

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Supplementary Table S1. ***Continued.***

C

	Mouse	HLA%	CD22%	CD22/HLA
PBS	1	84.3	73.7	0.9
	2	75.4	73.4	1.0
	3	73.9	70.5	1.0
	4	65.6	66.9	1.0
	5	88.8	88.1	1.0
	6	69.0	67.1	1.0
Free Ab (1mg/kg) + free ASO (0.8mg/kg)	1	87.8	78.9	0.9
	2	87.6	78.5	0.9
	3	90.9	84	0.9
	4	88.8	81.9	0.9
	5	82.5	76.6	0.9
	6	90.3	74.6	0.8
Conjugate (0.2mg/kg Ab)	1	76.3	75.1	1.0
	2	73.2	66.6	0.9
	3	94.2	88.2	0.9
	4	91.6	79.8	0.9
	5	88.6	83	0.9
	6	87.6	82.5	0.9
Conjugate (1mg/kg Ab)	1	77.5	73.3	0.9
	2	74.5	69.8	0.9
	3	80.2	73.2	0.9
	4	87.1	82.3	0.9
	5	85.6	74.7	0.9
	6	87.9	79.3	0.9

Supplementary Table S2. Characteristics of the two primary leukemia samples used in the study. WBC: white blood cells, CNS: central nervous system, MRD: minimal residual disease, CD22 expression: the numbers indicate % relative to HLA expression on the cells at inoculation and at harvest (in parenthesis) for the xenograft models. MXD3 expression was tested on the primary cells (before xenograft).

	Sample A	Sample B
initial WBC (×10e3/μL)	439.6	2.2
Age (year old) / sex	4 / male	16 / male
CNS disease	Negative	Negative
Cytogenetics	47,XY,+mar(13)/46,XY(7)	No metaphase cells
MRD on day 29 bone marrow	Negative	Negative
Morphology	Lymphoblast	Lymphoblast
Phenotype	CD10, 19, 20, TdT, cCD79a positive	CD10, 19, 20, TdT, cCD79a positive
CD22 expression* %	92 (87)	92 (93)
MXD3 expression (relative to Reh) %	148	246

Supplementary Table S3. Blood test results of the mice from the patient-derived leukemia xenograft model. (A) CBC and (B) chemistry panels for the patient-derived leukemia xenograft mice during treatment. One representative mouse from each treatment arm was tested weekly.

A

	Days after leukemia inoculation			
PBS	0	7	14	28
WBC (K/ul)	1.3	0.8	1.2	6.2
Absolute Neutrophil cells (K/ul)	0.7	0.4	0.9	1.3
Absolute Lymphocyte cells (K/ul)	0.3	0.2	0.2	2.7
Absolute Monocyte cells (K/ul)	0.3	0.2	0.1	2.1
Absolute Eosinophil cells (K/ul)	0.0	0.0	0.0	0.0
Absolute Basophil cells (K/ul)	0.0	0.0	0.0	0.0
Neutrophil %	52.2	42.9	74.1	21.4
Lymphocyte %	22.7	28.2	14.7	43.3
Monocyte %	23.3	24.4	8.3	34.6
Eosinophil %	1.3	4.3	3.0	0.6
Basophil %	0.5	0.2	0.0	0.2
RBC (M/ul)	8.5	9.8	10.3	9.9
Hemoglobin (g/dL)	13.4	13.4	15.0	13.3
Hematocrit %	36.3	43.8	51.9	43.3
MCV (fL)	42.8	44.8	50.4	43.8
MCH (pg)	15.8	13.7	14.6	13.4
MCHC (g/dL)	36.9	30.6	28.9	30.7
RDW %	18.2	18.1	19.1	19.0
Platelets (K/uL)	784.0	632.0	990.0	317.0
MPV (fL)	5.7	5.5	5.0	5.4
Presence of clots	None	None	None	None

	Days after leukemia inoculation			
free Ab (1mg/kg) + free ASO (0.8mg/kg)	0	7	14	28
WBC (K/ul)	1.1	0.8	2.6	1.5
Absolute Neutrophil cells (K/ul)	0.5	0.2	0.4	0.1
Absolute Lymphocyte cells (K/ul)	0.3	0.3	0.6	1.1
Absolute Monocyte cells (K/ul)	0.3	0.2	1.5	0.3
Absolute Eosinophil cells (K/ul)	0.0	0.0	0.1	0.0
Absolute Basophil cells (K/ul)	0.0	0.0	0.0	0.0
Neutrophil %	44.4	26.8	16.8	5.7
Lymphocyte %	25.2	40.8	22.3	75.3
Monocyte %	27.8	30.4	57.9	17.7
Eosinophil %	2.5	0.8	2.4	1.1
Basophil %	0.2	1.2	0.6	0.2
RBC (M/ul)	9.8	8.3	10.2	6.6
Hemoglobin (g/dL)	14.1	11.7	14.8	9.4
Hematocrit %	41.7	36.9	51.9	29.1
MCV (fL)	42.6	44.2	50.7	43.8
MCH (pg)	14.4	14.0	14.5	14.2
MCHC (g/dL)	33.8	31.7	28.5	32.3
RDW %	17.9	18.2	18.7	17.7
Platelets (K/uL)	832.0	494.0	620.0	432.0
MPV (fL)	5.6	5.5	5.1	5.2
Presence of clots	None	None	None	None

	Days after leukemia inoculation			
conjugate (0.2mg/kg Ab)	0	7	14	28
WBC (K/ul)	0.7	1.0	1.0	1.0
Absolute Neutrophil cells (K/ul)	0.2	0.6	0.2	0.1
Absolute Lymphocyte cells (K/ul)	0.3	0.2	0.3	0.4
Absolute Monocyte cells (K/ul)	0.3	0.2	0.5	0.4
Absolute Eosinophil cells (K/ul)	0.0	0.0	0.0	0.0
Absolute Basophil cells (K/ul)	0.0	0.0	0.0	0.0
Neutrophil %	28.6	60.3	23.5	14.2
Lymphocyte %	35.6	22.5	28.6	44.3
Monocyte %	33.5	14.5	44.8	39.1
Eosinophil %	1.8	2.3	3.0	2.0
Basophil %	0.5	0.3	0.2	0.3
RBC (M/ul)	8.6	10.4	9.5	9.2
Hemoglobin (g/dL)	12.3	14.5	14.1	12.7
Hematocrit %	37.1	47.6	49.0	40.6
MCV (fL)	43.1	46.0	51.5	44.2
MCH (pg)	14.3	14.0	14.8	13.8
MCHC (g/dL)	33.2	30.5	28.8	31.3
RDW %	17.8	18.5	19.1	17.6
Platelets (K/uL)	449.0	146.0	758.0	458.0
MPV (fL)	5.7	6.1	5.2	5.0
Presence of clots	clot	None	None	None

	Days after leukemia inoculation			
conjugate (1mg/kg Ab)	0	7	14	28
WBC (K/ul)	1.1	0.7	1.2	1.1
Absolute Neutrophil cells (K/ul)	0.4	0.3	0.1	0.3
Absolute Lymphocyte cells (K/ul)	0.3	0.2	0.5	0.2
Absolute Monocyte cells (K/ul)	0.3	0.2	0.5	0.6
Absolute Eosinophil cells (K/ul)	0.0	0.0	0.1	0.0
Absolute Basophil cells (K/ul)	0.0	0.0	0.0	0.0
Neutrophil %	38.7	40.3	11.0	22.0
Lymphocyte %	26.6	28.2	40.7	19.6
Monocyte %	28.5	29.1	42.5	56.6
Eosinophil %	3.9	2.3	5.2	1.8
Basophil %	2.3	0.2	0.7	0.0
RBC (M/ul)	9.3	9.9	8.7	9.1
Hemoglobin (g/dL)	13.5	13.9	13.0	12.9
Hematocrit %	40.0	43.9	44.7	41.2
MCV (fL)	43.2	44.2	51.7	45.1
MCH (pg)	14.6	14.0	15.0	14.1
MCHC (g/dL)	33.8	31.7	29.1	31.3
RDW %	17.5	17.7	19.2	18.0
Platelets (K/uL)	768.0	179.0	381.0	733.0
MPV (fL)	5.8	5.9	5.3	5.0
Presence of clots	None	None	None	None

B

	Days after leukemia inoculation			
PBS	0	7	14	28
Albumin g/dL	3.6	3.8	4.1	3.3
Alkaline Phosphatase U/L	41.8	0.2	73.6	68.7
Alanine transaminase U/L	32.2	67.2	36.2	37.8
Aspartate transaminase U/L	121.3	355.2	126.0	148.5
Blood Urea Nitrogen mg/dL	25.3	32.8	21.2	15.6
Creatinine mg/dL	0.1	0.1	0.2	0.2
Total Bilirubin mg/dL	0.2	0.0	0.1	0.0
Total Protein g/dL	5.5	6.8	5.8	5.3
Hemolysis	1+	2+	3+	2+

	Days after leukemia inoculation			
free Ab (1mg/kg) + free ASO (0.8mg/kg)	0	7	14	28
Albumin g/dL	3.9	4.1	3.7	3.6
Alkaline Phosphatase U/L	72.9	61.0	9.0	6.3
Alanine transaminase U/L	38.9	40.4	42.2	46.5
Aspartate transaminase U/L	154.1	170.2	203.8	213.3
Blood Urea Nitrogen mg/dL	26.0	25.8	34.0	23.7
Creatinine mg/dL	0.2	0.2	0.2	0.3
Total Bilirubin mg/dL	0.1	0.1	0.0	0.0
Total Protein g/dL	5.7	6.2	6.5	6.0
Hemolysis	1+	2+	3+	2+

	Days after leukemia inoculation			
conjugate (0.2mg/kg Ab)	0	7	14	28
Albumin g/dL	3.7	3.9	3.9	3.2
Alkaline Phosphatase U/L	42.2	56.0	15.4	<0.3
Alanine transaminase U/L	35.2	63.4	47.6	23.7
Aspartate transaminase U/L	119.4	367.4	221.8	97.5
Blood Urea Nitrogen mg/dL	30.6	21.0	26.4	21.3
Creatinine mg/dL	0.2	0.3	0.1	0.1
Total Bilirubin mg/dL	0.1	0.1	0.1	0.1
Total Protein g/dL	5.8	5.7	6.8	4.8
Hemolysis	1+	2+	3+	2+

	Days after leukemia inoculation			
conjugate (1mg/kg Ab)	0	7	14	28
Albumin g/dL	3.8	3.8	2.9	3.5
Alkaline Phosphatase U/L	62.0	<0.1	<0.1	72.0
Alanine transaminase U/L	29.5	64.8	45.1	27.0
Aspartate transaminase U/L	81.8	338.4	235.4	109.2
Blood Urea Nitrogen mg/dL	28.5	23.4	24.2	21.0
Creatinine mg/dL	0.2	0.1	0.8	0.2
Total Bilirubin mg/dL	0.1	0.0	0.1	0.1
Total Protein g/dL	5.4	7.3	5.5	5.2
Hemolysis	1+	2+	3+	2+