Supplemental Data

Supplemental Methods

Study samples

Twenty-eight patients (age 1-21 years-old) with either relapsed CD22-positive B-ALL, relapse after HCT, first or greater relapse for Down syndrome, or patients refractory to two prior induction regimens were enrolled in the COG AALL1621 single arm phase 2 trial. CD22 positivity was requirement for enrollment with no threshold applied. Patients received one cycle of InO at the FDA-approved adult dose of 1.8mg/m2 (0.8mg/m2 on day 1, 0.5mg/m2 on days 8 and 15). Based on response at day 28 (cycle 1), patients with at least stable disease (SD) could receive a second cycle; those with CR/CR with incomplete count recovery (CRi) received InO 0.5mg/m2 on days 1, 8, and 15 in cycle 2, while those without CR/CRi received the same fractionated dose as cycle 1. Patients with CR/CRi after 2 cycles were eligible to receive up to 6 total cycles. Samples for this study were collected before treatment with InO, at end of cycle 1 and end of cycle 2[1]. A total of 68 samples from 28 patients were analyzed (Supplemental Table 1).

Response rates (Supplemental Table 2): CR was defined as an M1 marrow (<5%) without circulating blasts or persistent extramedullary disease and with recovery of ANC to > 500/μL and platelet count >50,000/μl without transfusion for 7 days. CRi was defined as an M1 marrow without count recovery. Partial response (PR) included those with an M2 marrow with ≥5-25% blasts if originally diagnosed with an M3 marrow (>25% blasts), with or without resolution of extramedullary disease. Progressive disease (PD) was defined as an increase in marrow blast percentage or the appearance of new sites of leukemia during therapy. Note: Clinical MRD was determined by COG-approved laboratories at each treating institution and was not performed centrally.

CyTOF marker labeling and detection

Peripheral blood mononuclear cells (PBMCs) or marrow cells were Ficoll-isolated and cryopreserved. To identify live cells, thawed cells were labeled with 0.5 μM cisplatin (Cell-ID Cisplatin 198, Fluidigm) for 5 min at room temperature (RT) and quenched with 5x volume autoMACS buffer (Miltenyi Biotec). Next, cells were fixed in 1.6% paraformaldehyde-PBS (PFA; Electron Microscopy Sciences, Cat. #15710). After fixation, each sample was barcoded with individual triplets of 15 μM Paladium 20-plex barcodes in 0.02% Saponin-PBS (UCSF Flow Core). Cells were washed 3x using autoMACS buffer before pooling. Pooled samples were sequentially stained with three antibody cocktails: surface marker antibodies (cocktail A) and intracellular proteins (cocktails B and C). Heavy-metal isotope-conjugated antibodies are listed in Supplemental Table 3. Cells were incubated with each cocktail for 30 min at RT. Before staining with antibody cocktails B and C, cells were permeabilized with MaxPar Perm-S buffer (Fluidigm; 20 min incubation on ice) and methanol (Electron Microscopy Sciences, Cat. #18510; 15 min incubation on ice), respectively. Finally, total cells were identified by DNA intercalation (0.125 μM MaxPar Intercalator-Ir, Fluidigm) in 1.6% PFA at 4°C overnight and labeled cells were recorded on the Helios CyTOF2 instrument using an event rate of ~400-600 events/sec run with normalization beads (EQ Four Element Calibration Beads #201078; Fluidigm).

Tumor cell identification by conventional gating strategy

Mass cytometry files were normalized through a data normalization algorithm[2]. Intensity values of a sliding window of bead standards corrected for instrument fluctuations and between samples. Following normalization and debarcoding, data were analyzed using FlowJo 10.7.1 (FlowJo LLC, Ashland, OR). In short, singlets were gated by event length and DNA; live cells were identified as cisplatin-negative cells. Tumor cells found in CD3-/CD235-/CD16-/CD33- or CD33dim cells were further identified by B cell lineage markers and CD34 (Supplemental Figure 12).

High-dimensional analysis of training cohort

Clinical outcomes of nine randomly selected patients were unblinded and used as the training cohort to develop the predictor algorithm (Supplemental Table 4). COG outcomes CR and CRi were grouped as 'CR', whereas patients with PR, SD, and PD were labeled as 'non-CR/CRi'. After identification of tumor cells from patients in the training cohort (Supplemental Figure 12), cell-community clustering (CCC) analysis was performed using an R-based workflow[3]. Briefly, the training cohort samples were processed to generate cluster statistics and memberships for every cell event from the grappolo package[4], and a clustering plot was generated using CD22 and intracellular molecules to calculate similarities between nodes from the vite package[5]. Grouping was based on known InO outcome (CR/CRi vs. non-CR/CRi), individual identification or timepoint to identify leukemic cells clusters corresponding to indicated outcomes. For single cell phenotyping and intramolecular characterization, following t-SNE analysis of live cells based on the cytofkit workflow[6], single cell matrix data of tumor cells identified by surface markers were extracted (Supplemental Figure 3), and used for further analysis.

Validation cohort analysis

The predictive algorithm from high-dimensional analysis of the training cohort was applied to the validation cohort (19 patients; Supplemental Table 5) in a blinded fashion. Collective results are displayed for all 28 patients.

Statistical analysis

Statistical analyses were performed using R software (R version 3.6.1). Application of predictive values was determined using Fisher's exact test. Comparison of size of cluster nodes and leukemic cell fraction frequency was performed using Student t-test. Correlation of cell marker expression was performed using the Pearson product-moment correlation coefficient. Area under the curve (AUC) of receiving operating characteristic (ROC) curve was tested using Delong's test. Statistical significance was defined as $P < 0.05$ with a two-tailed test.

Supplemental References

1. O'Brien MM, Ji L, Shah NN, et al. A Phase 2 Trial of Inotuzumab Ozogamicin (InO) in Children and Young Adults with Relapsed or Refractory (R/R) CD22+ B-Acute Lymphoblastic Leukemia (B-ALL): Results from Children's Oncology Group Protocol AALL1621. J Clin Oncol. 2022;Jan 10:JCO2101693.
2. Finck R, Simonds EF, Jager A, et al. Normalization of mass cytometry data with bead standards. Cytom. Part A. 2013;83A(5):483–494.
3. https://github.com/ParkerICI/flow-analysis-tutorial.
4. https://github.com/ParkerICI/grappolo.
5. https://github.com/ParkerICI/vite.
6. Chen H, Lau MC, Wong MT, et al. Cytofkit: A Bioconductor Package for an Integrated Mass Cytometry Data Analysis Pipeline. PLoS Comput. Biol. 2016;12(9):e1005112–e1005112.

Supplemental Table 1: Clinical characteristics of 48 patients enrolled in the COG AALL1621 single arm phase 2 trial including the 28 patients analyzed in this study

Patient Characteristic	(n = 48)	Analyzed (n = 28)	Not-analyzed (n = 20)	P value
Age at Enrollment (years)				0.66
Median (range)	9 (1, 21)	9 (1, 20)	12.5 (1, 21)	
1-9	25 (52%)	16 (57%)	9 (45%)	
10-12	4 (8%)	3 (11%)	1 (5%)	
13-17	11 (23%)	5 (18%)	6 (30%)	
18-21	8 (17%)	4 (14%)	4 (20%)	
Sex				0.21
Female	19 (40%)	9 (32%)	10 (50%)	
Male	29 (60%)	19 (68%)	10 (50%)	
Self-Reported Race				0.21
Asian	2 (5%)	0	2 (12%)	
Black or African American	5 (12%)	2 (8%)	3 (18%)	
White	34 (81%)	22 (88%)	12 (71%)	
Multiple Races	1 (2%)	1 (4%)	0	
Unknown	6	3	3	
Self-Identified Ethnicity				1.00
Hispanic or Latino	11 (26%)	7 (28%)	4 (24%)	
Not Hispanic or Latino	31 (74%)	18 (72%)	13 (76%)	
Unknown	6	3	3	
Type of Relapse/Refractory				0.59
Second or greater relapsen	32 (67%)	19 (68%)	13 (65%)	
First relapse refractory to at least one	10 (21%)	4 (14%)	6 (30%)	
Primary refractory disease with at least 2	2 (4%)	2 (7%)	0	
Any relapse after HSCT	3 (6%)	2 (7%)	1 (5%)	
First relapse with no prior re-induction	1 (2%)	1 (4%)	0	
CNS Status at Enrollment				1.00
CNS 1	44 (92%)	26 (93%)	18 (90%)	
CNS 2	0	0	0	
CNS 3	4 (8%)	2 (7%)	2 (10%)	
Down Syndrome				0.26
No	45 (94%)	25 (89%)	20 (100%)	
Yes	3 (6%)	3 (11%)	0	
Prior HSCT				1.00
No	37 (77%)	22 (79%)	15 (75%)	
Yes	11 (23%)	6 (21%)	5 (25%)	
Prior CAR T-cell Therapy				0.74
No	37 (77%)	21 (75%)	16 (80%)	
Yes	11 (23%)[#]	7 (25%)	4 (20%)	
CD19 product	10	7	3	
Prior Blinatumomab Therapy				0.45
No	34 (71%)	21 (75%)	13 (65%)	
Yes	14 (29%)	7 (25%)	7 (35%)	
WBC (1000/mL) at Study Entry				
Median (range)	5.75 (0.3, 547)	7.45 (0.8, 547)	3.05 (0.3, 386)	
Marrow Blasts (%) at Study Entry				0.46
Median (range)	81 (6, 100)	84.5 (6, 99)	75.5 (8, 100)	
M2 (5%-25%)	9 (19%)	4 (14%)	5 (25%)	
M3 (>25%)	39 (81%)	24 (86%)	15 (75%)	
Cytogenetics/FISH				
Trisomy 4 & 10	0	0	0	1.00
ETV6-RUNX1 t(12;21)(p13;q22)	5 (12%)	4 (16%)	1 (6%)	0.63
iAMP21	2 (5%)	1 (4%)	1 (6%)	1.00
KMT2A rearrangement	6 (14%)	3 (12%)	3 (18%)	0.67
BCR-ABL1 t(9;22)	2 (5%)	1 (4%)	1 (6%)	1.00
Other	14 (33%)	9 (36%)	5 (29%)	0.75
PBX1-TCF3 t(1;19)	4	2	2	
Not available	6 133%)	3 (11%)	3 (15%)	

#one patient had prior CD19 and CD22 CAR-T cell therapy

Abbreviations: CAR, chimeric antigen receptor; FISH, fluprescence in situ hybridization; HSCT, hematopoietic stem cell transplantation; WBC, white blood cell count

Supplemental Table 2: Morphologic and minimal residual disease response to two cycles of Inotuzumab ozogamicin for 28 analyzed patients

Cycle 1 Response	Number of patients (total N = 28)	% among 28 patients	Number of patients who received cycle 2 (total n=18)	Cycle 2 Response	Assigned category in this manuscript
CR/CRi, MRD <0.01%	13	46.4%	10	• 10 CR/CRi, MRD <0.01%	• CR/CRi
CR/CRi, MRD ≥ 0.01%	3	10.7%	1	• 1 PD	• non-CR/CRi
PR	2	7.1%	2	• 1 CRi, MRD ≥0.01% • 1 PD	• CR/CRi • non-CR/CRi
SD	8	28.6%	5	• 1 CR, MRD ≥0.01% • 2 PR • 1 SD • 1 PD	• CR/CRi • non-CR/CRi • non-CR/CRi • non-CR/CRi
PD	2	7.1%	0 (not eligible for further therapy after cycle 1)		• non-CR/CRi

Abbreviations: CR; complete response; CRi, complete response with incomplete count recovery, MRD, minimal residual disease; PD, progressive disease; PR, partial response; SD, stable disease.

Supplemental Table 3. Mass cytometry panel

Antigen target	Metal tag	Supplier	Clone	Catalog #
BAD	151 Eu	BioLegend	W16055A	795502
Bcl-2	153 Eu	Bio-Rad	100	MCA1550
Bcl-xL	147Sm	proteintech	4C12A6	66020-1-Ig
Bim	145 Nd	Abclonal	N.D	A19702
CD10	156 Gd	Fluidigm	HI10A	3156001B
CD117 (ckit)	143 Nd	Fluidigm	104D2	3143001B
CD16	209 Bi	Fluidigm	3G8	3144001B
CD179a (VpreB)	143 Sm	Fluidigm	HSL96	3149012B
CD19	142 Nd	Fluidigm	HIB19	3142001B
CD20	171 Yb	Fluidigm	2H7	3171012B
CD22	159 Tb	Fluidigm	HIB22	3159005B
CD235a/b	141 Pr	Fluidigm	HIR2	3141001B
CD24	169 Tm	Fluidigm	ML5	3169004B
CD3	170 Er	Fluidigm	UCHT1	3170001B
CD33	163Dy	Fluidigm	WM53	3169010B
CD34	166 Er	Fluidigm	581	3166012B
CD45	89 Y	Fluidigm	Hi30	3089003B
CD79B (Ig-Beta)	162 Dy	Fluidigm	CB3-1	3162008B
c-Parp	154 Sm	BD Biosciences	4C10-5	7153897
HLA-DR	173 Yb	Fluidigm	L243	3173005B
IgD	146 Nd	Fluidigm	IA6-2	3146005B
IgM (Surface)	172 Yb	Fluidigm	MHM-88	3172004B
Ki-67	168 Er	Fluidigm	B56	3168007B
Mcl-1	174 Yb	Bio-Rad	polyclonal	AHP998
MRP1/2	148 Nd	BioLegend	QCRL3	312102
p21	160 Gd	Abclonal	N.D	A19094
p4E-BP1 [Thr37/46]	176 Yb	BioLegend	A18010A	619602
p53	175 Lu	Bio-Rad	DO-1	MCA 1701
pCHK1 [Ser345]	165 Ho	Abclonal	N.D	AP0578
pERK 1/2 [T202/Y204]	167 Er	Fluidigm	D1314.4E	3167005A
p-GP/MDR1	158 Gd	BioLegend	C494	907001
pLyn [Y418]	144 Nd	abcam	EP503Y	ab239824
pNF-kB p65 [S529’	155 Gd	BioLegend	Poly6226	622602
pStat5 [Y694]	150 Nd	Fluidigm	47	3150005A
TdT	164 Dy	Fluidigm	E17-1519	3164015B

Note: Antibodies that were not purchased from Fluidigm were custom-conjugated as described in Methods.

N.D: not described.

Supplemental Table 4. Patients used for training cohort

COG	Patient ID	End cycle 1 MRD		End cycle 2 MRD		Clinical Response, CIcle 1	Clinical Response, CIcle 2
		Clinical	CyTOF	Clinical	CyTOF		
PATGUY	B	≤0.01%	0%	≤0.01%	0%	CR	CR
PAWLZW	O	≤0.01%	0.50%	≤0.01%	1.31%	CR	CR
PAYLUG	H	≤0.01%	0.28%	≤0.01%	0.02%	CR	CR
PAYRKA	L	≤0.01%	0.48%	≤0.01%	0.52%	CRi	CR
PAYRLM	M	≤0.01%	0.05%	≤0.01%	0.16%	CRi	CR
PAVDPU	K	at least 1.0%	6.28%	at least 1.0%	32.96%	PR	CRi
PAVDRV	C	at least 1.0%	31.57%	at least 1.0%	70.66%	SD	PD
PAWZIV	P	0.1-0.99%	3.57%	at least 1.0%	11.58%	CRi	PD
PAWUXD	Z	at least 1.0%	68.75%	at least 1.0%	17.08%	SD	PR

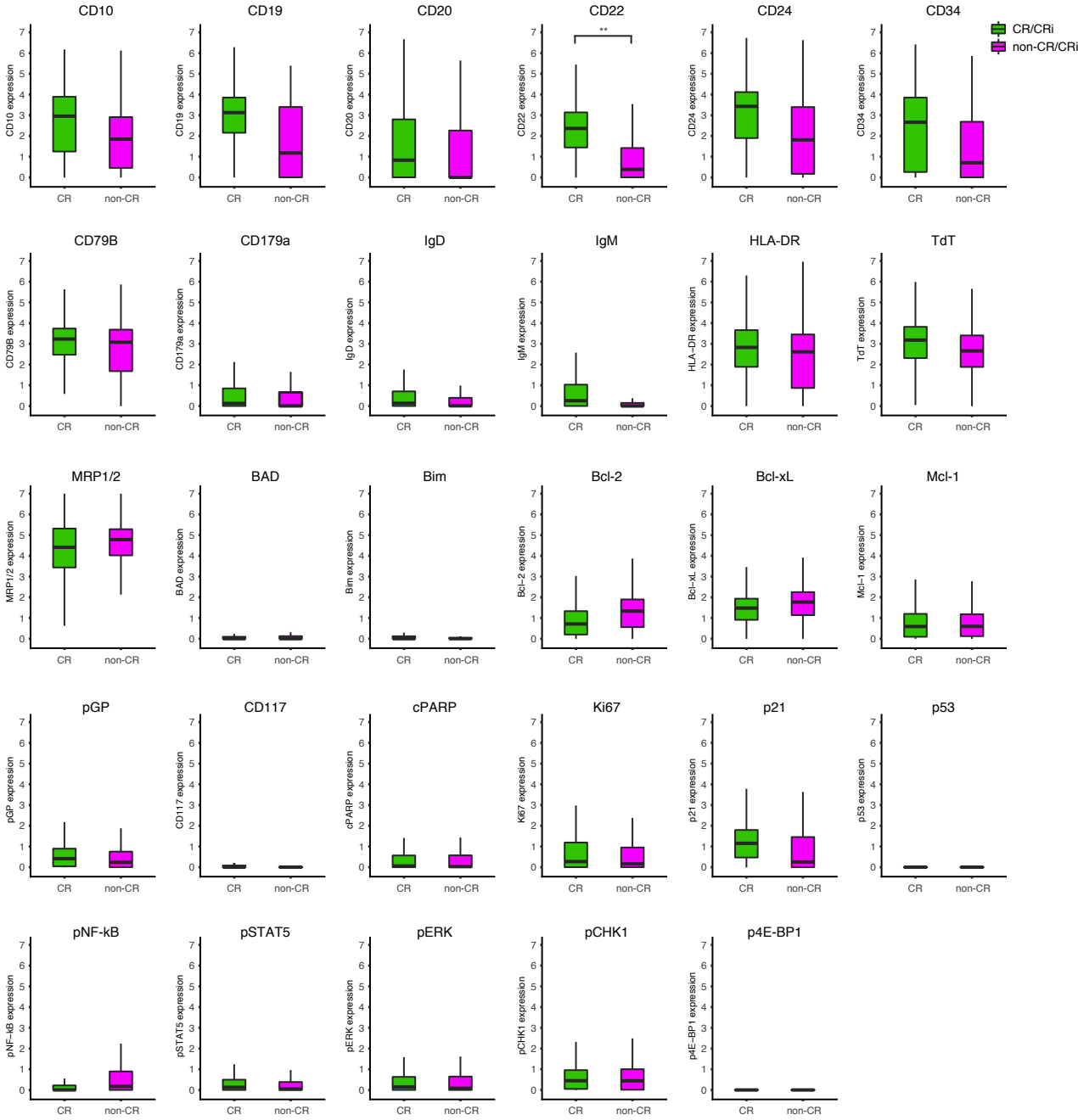
Note: Clinical MRD was determined by each treating institution and was not performed centrally. N/A: not applicable.

Supplemental Table 5. Patients used for validation cohort

COG	Patient ID	End cycle 1 MRD		End cycle 2 MRD		Clinical Response ,CIcle 1	Clinical Response ,CIcle 2
		Clinical	CyTOF	Clinical	CyTOF		
PAZJDW	AA	≤0.01%	0.06%		N/A	CR	
PAXXVC	I	≤0.01%	0.57%		N/A	CR	
PAXXFC	J	≤0.01%	0.46%		N/A	CR	
PAZKZH	U	0.1-0.99%	0%		N/A	CR	
PAXFBW	W	≤0.01%	0.10%	≤0.01%	N/A	CR	CR
PAZNCH	BB	≤0.01%	0.19%	≤0.01%	0.28%	CRi	CR
PAXWZM	D	0.1-0.99%	0.51%		N/A	CRi	
PATCPU	DD	≤0.01%	3.75%	≤0.01%	N/A	CR	CR
PATZSN	N	≤0.01%	0.02%	≤0.01%	0.10%	CR	CR
PAZITY	R	at least 1.0%	6.66%	0.01-0.099%	1.48%	SD	CR
PAVHEV	Y	≤0.01%	1.71%	≤0.01%	0.66%	CR	CR
PAYIVS	F	at least 1.0%	42.25%		N/A	SD	
PAWMIM	S	at least 1.0%	6.74%		N/A	SD	
PAYNHJ	X	at least 1.0%	4.56%	at least 1.0%	N/A	SD	SD
PAYYZW	CC		20.83%		N/A	PD	
PAUBRC	G	at least 1.0%	11.90%	at least 1.0%	3.01%	SD	PR
PAVPXP	V	at least 1.0%	59.28%		N/A	SD	
PAYGAE	A	at least 1.0%	N/A		N/A	PR	PD
PAYMXC	E		N/A		N/A	PD	

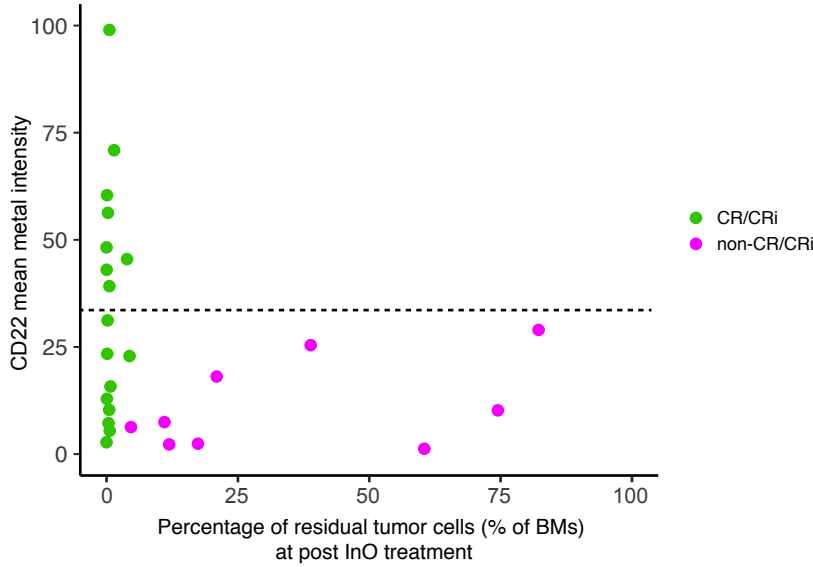
Note: Clinical MRD was determined by each treating institution and was not performed centrally. N/A: not applicable.

Figure S1.

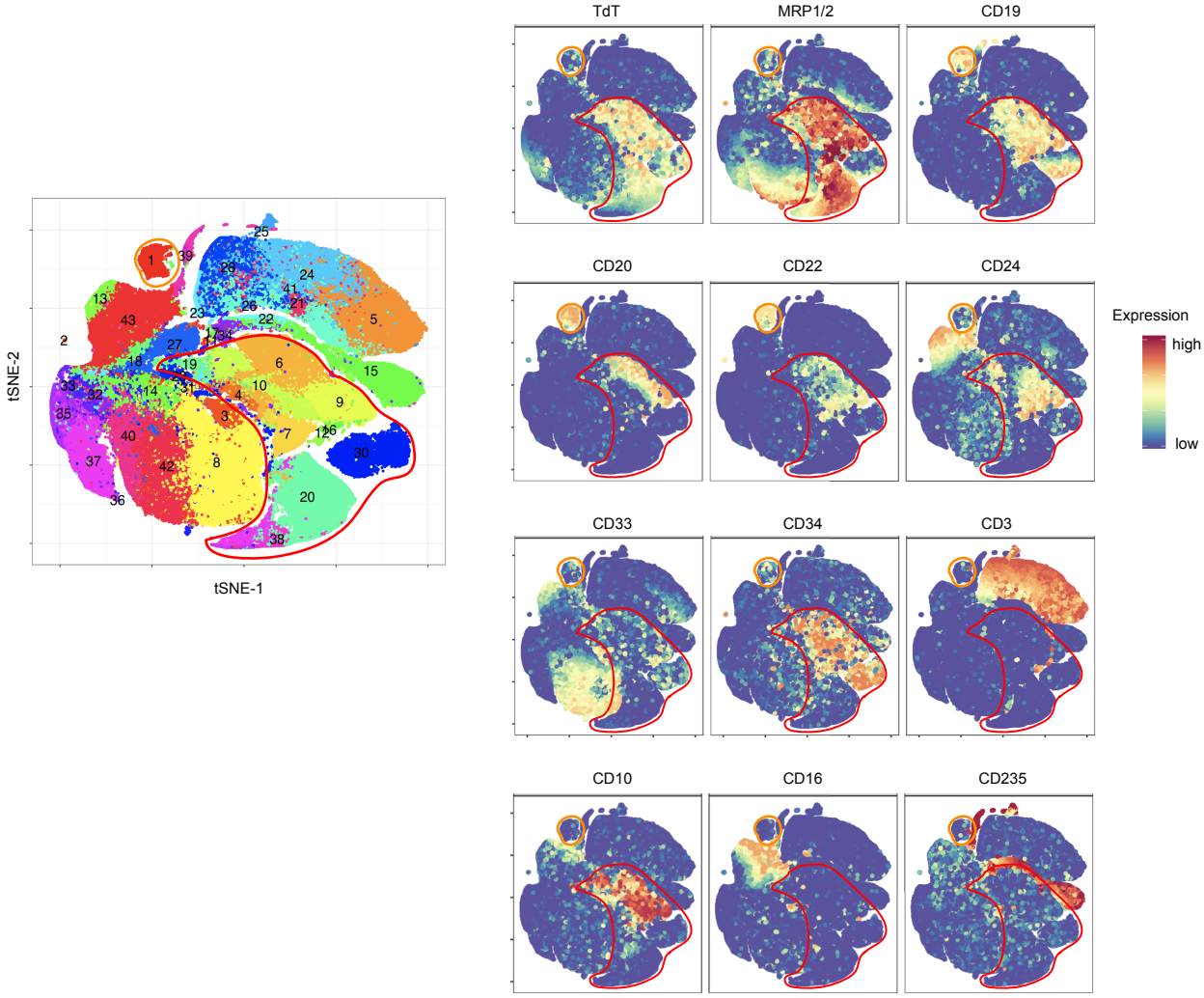


Supplemental Figure 1. Expresson of individual markers on tumor cells in CR/CRi and non-CR/CRi patients. Boxplots show marker expression of tumor cells. Data represent CR/CRi patients (n = 6) and non-CR/CRi patients (n = 3) from the training cohort. Statistical significance for average of marker expression was analyzed using the Student *t* test. ***P* <0.01.

Figure S2.

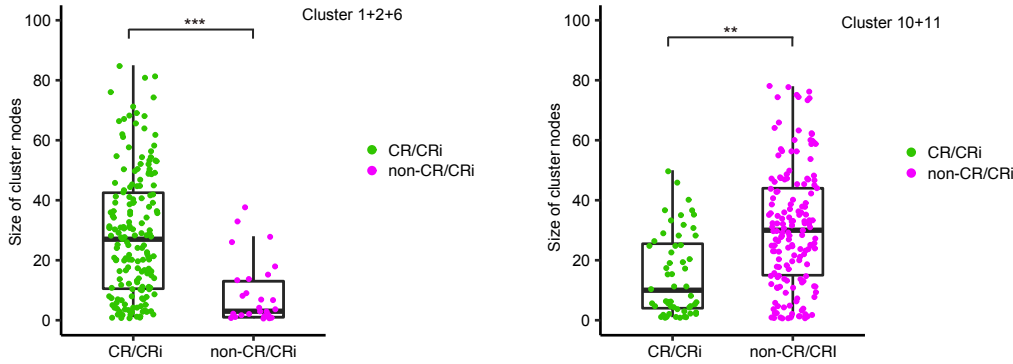


Supplemental Figure 2. Association between CD22 expression (MMI) pretreatment and residual tumor burden post treatment. The y-axis indicates CD22 expression as measured by CyTOF mean metal intensity (MMI) prior to Ino treatment. The x-axis indicates percentage of residual tumor cells at post InO treatment. Data represent CR/CRi patients (green, n = 17) and non-CR/CRi patients (pink, n = 9). BM, bone marrow cells.



Supplemental Figure 3. t-SNE analysis of the training cohort.

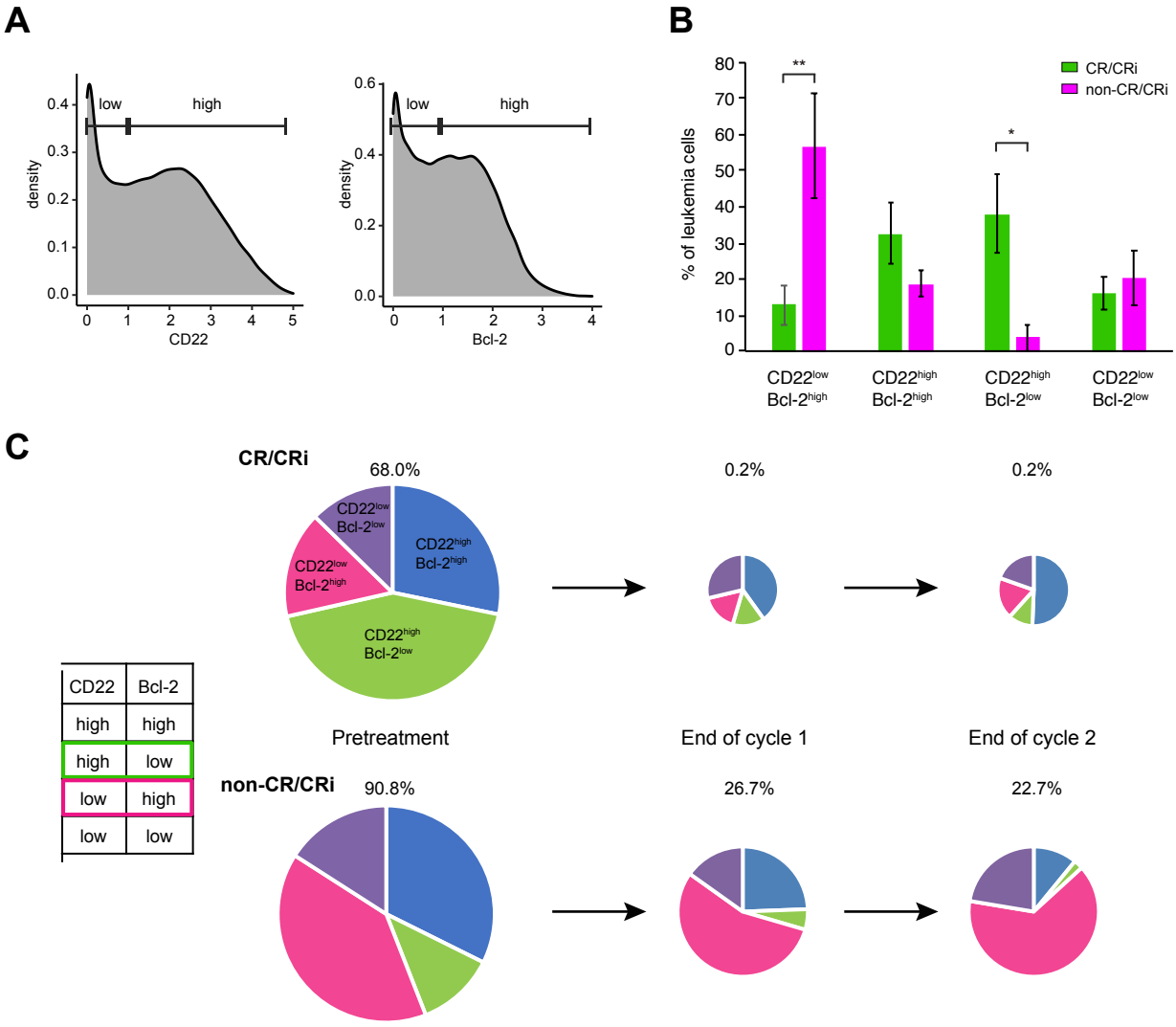
t-SNE analysis was performed using live cells as described in Supplemental figure 1 and generated 43 clusters. Tumor cells clustered with B-cell lineage markers, CD34 and MRP1/2 were identified as red-shaped clusters based on the distribution of each marker. Normal B cells were identified as orange-shaped clusters. Single cell matrix data of tumor cells were extracted for single cell phenotyping. Data represent CR/CRi patients (n = 6) and non-CR/CRi patients (n = 3) from the training cohort.



Supplemental Figure 4. Comparison of cluster size on cell comunity clustering analysis

Boxplots showing the size of cluster nodes indicative of good and poor response predictors in the training cohort. Statistical significance was analyzed using Student *t* test. ***P* <0.01, ****P* <0.001.

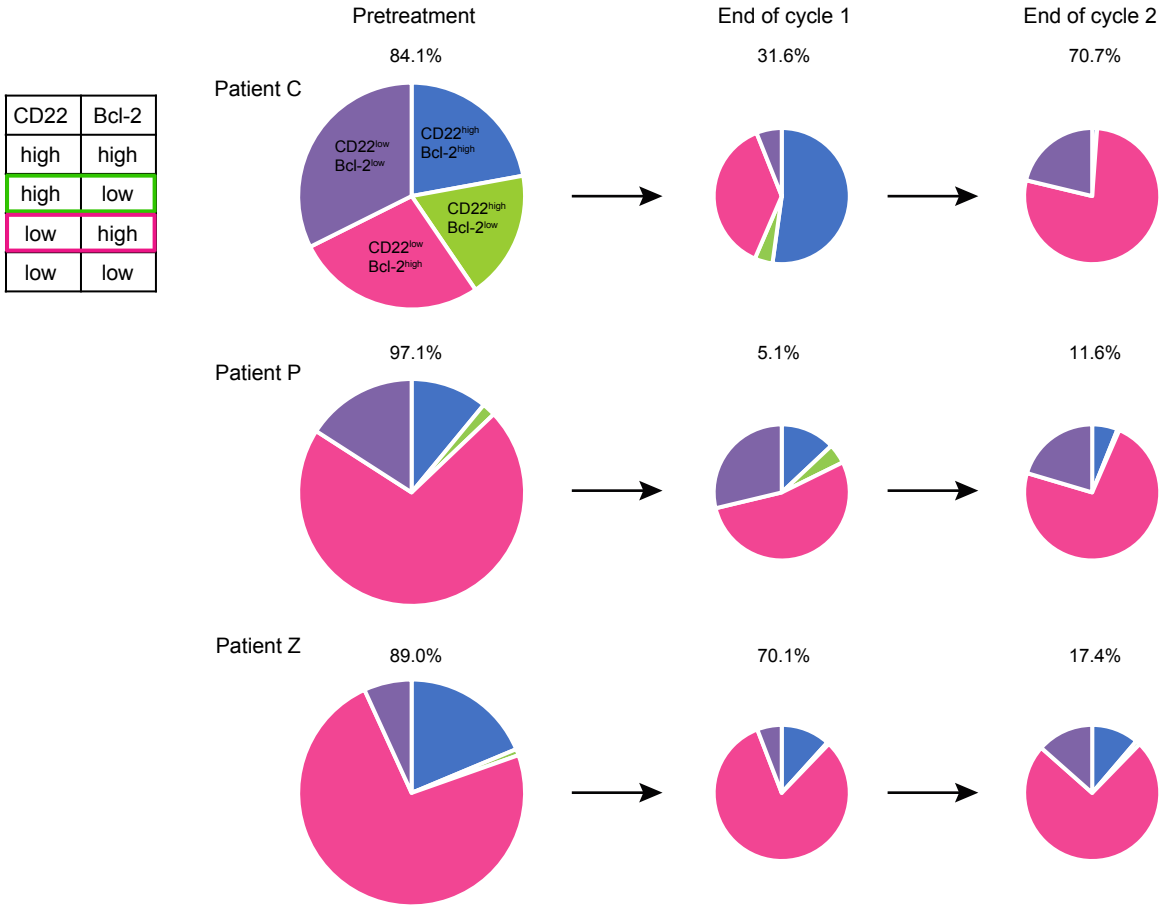
Figure S5.



Supplemental Figure 5. Leukemia cell fractions by CD22 and Bcl-2 expression, and longitudinal analysis of leukemia cell composition over InO treatment

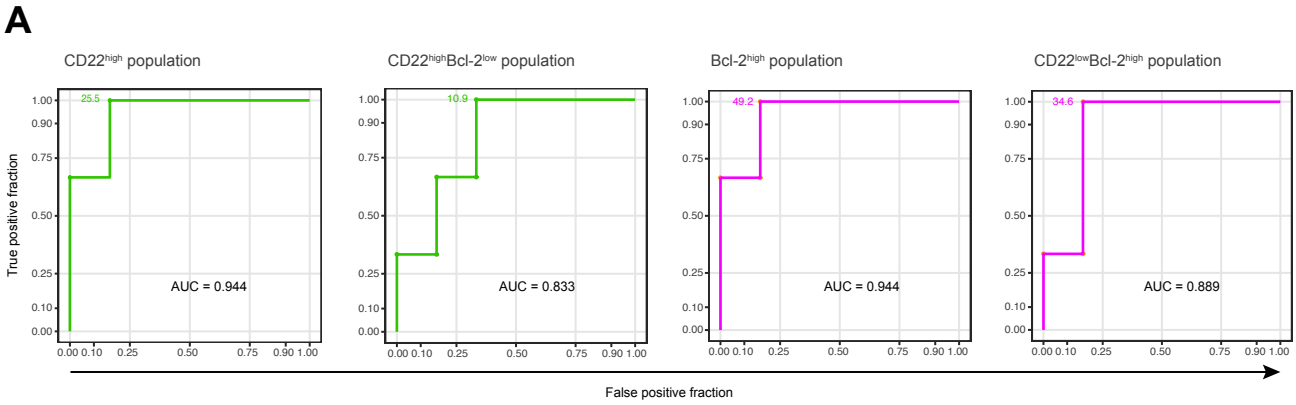
(A) Cell density plots shown for bimodal expression of CD22 and Bcl-2 expression by CyTOF. Data represent CR/CRi patients (n = 6) and non-CR/CRi patients (n = 3) from the training cohort. (B) Frequencies of four leukemia cell fractions divided by CD22 and Bcl-2 expression. Data represent the average percentage ± SEM from CR/CRi patients (n = 6) and non-CR/CRi patients (n = 3). Statistical significance was analyzed using Student t test. *P <0.05, **P <0.01. (C) Leukemia cell composition of four fractions by CD22 and Bcl-2 expression at pretreatment, end of 1 cycle, and end of 2 cycle. The values above the pie chart represent the average percentage of leukemia cells in all nucleated cells at each time point. Data represent the average from CR/CRi patients (n = 6) and non-CR/CRi patients (n = 3). Statistical significance was analyzed using Student *t* test. **P <0.01, ***P <0.001.

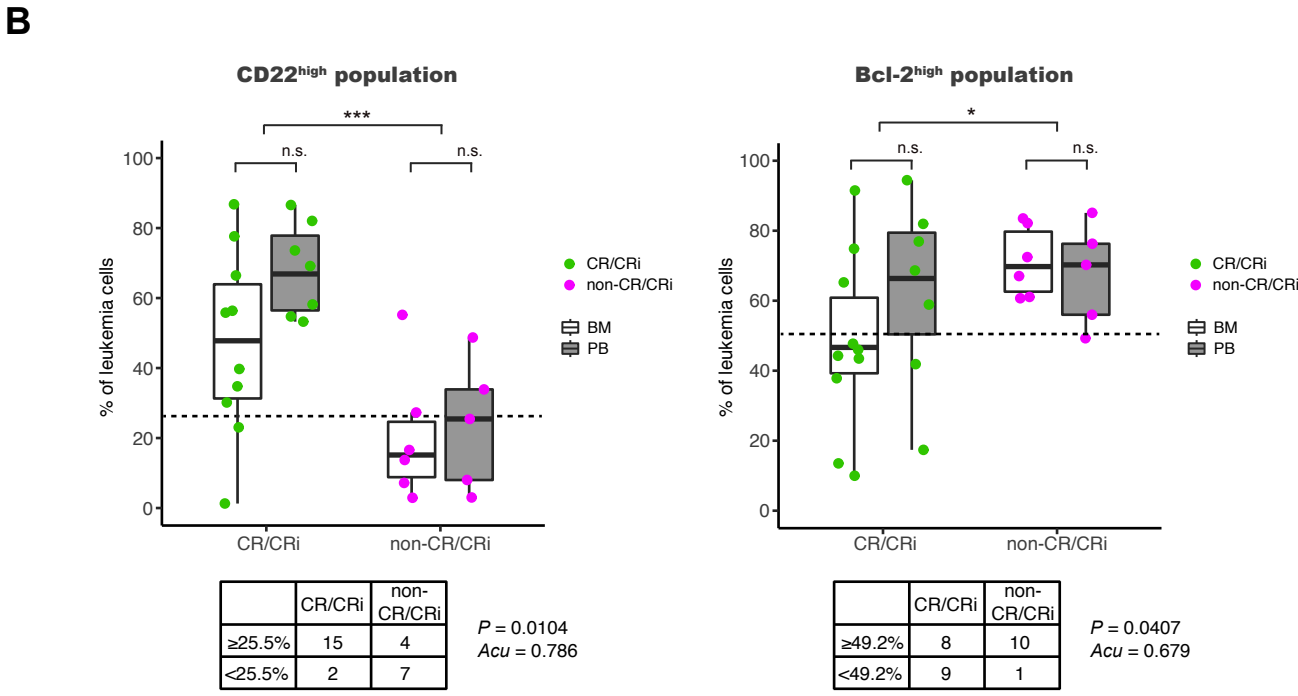
Figure S6.



Supplemental Figure 6. Leukemia cell composition in individual non-CR/CRi patients from the training cohort.

Pie charts display frequencies of leukemia cell fractions characterized by CD22 and Bcl-2 expression at pretreatment, end of cycle 1, and end of cycle 2. The values above the pie chart are leukemia cell percentages of all nucleated cells at respective time points. Colored boxes indicate the good response marker ($CD22^{high}Bcl\text{-}2^{low}$, green) and the poor response marker ($CD22^{low}Bcl\text{-}2^{high}$, pink).





	CR/CRi	non-CR/CRi
≥25.5%	15	4
<25.5%	2	7

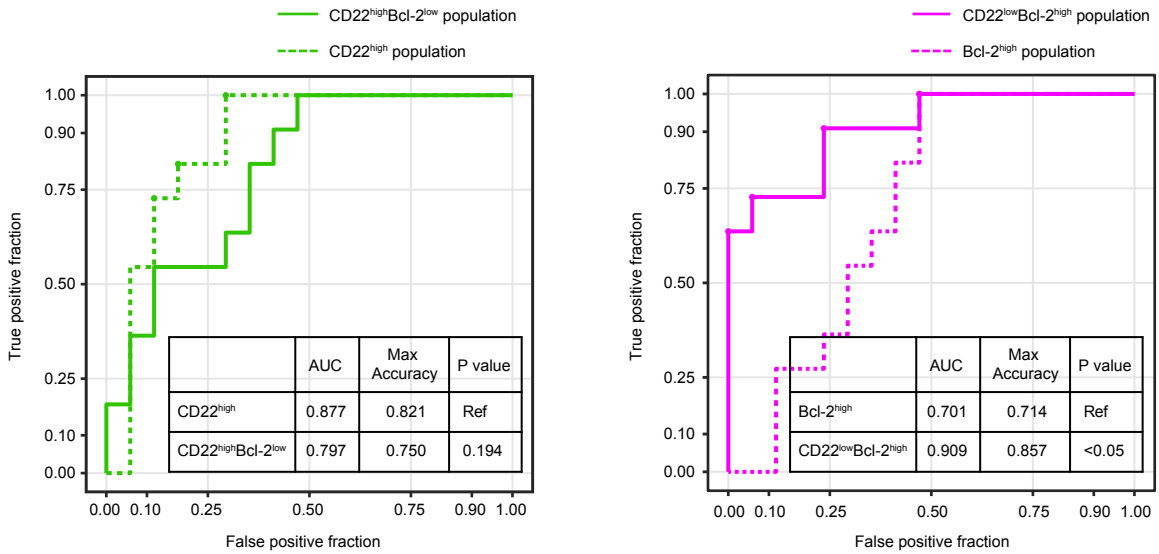
P = 0.0104
Acu = 0.786

	CR/CRi	non-CR/CRi
≥49.2%	8	10
<49.2%	9	1

P = 0.0407
Acu = 0.679

Supplemental Figure 7. Application of predictive values from training cohort to validation cohort.

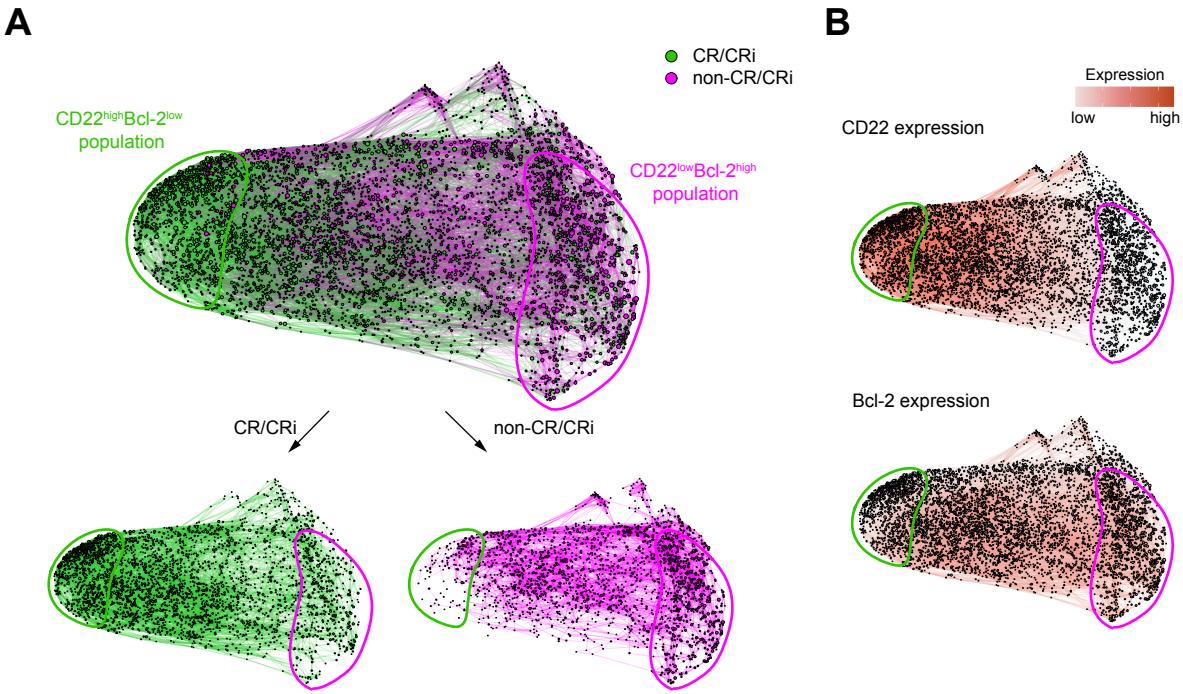
(A) ROC curves for good response predictors (green) and poor response predictors (pink) from the training cohort are shown. (B) Boxplots display the frequency of CD22high fraction and Bcl-2high fraction at pretreatment in CR/CRi (n = 17) and non-CR/CRi patients (n = 11) in either BM samples (white) or peripheral blood samples (gray). Table data insets indicate the number of patients within each therapeutic response category above or below the threshold set by the application of the prediction tool. Statistical significance of leukemia cell frequencies was analyzed using the Student *t* test. Application of predictive values was analyzed with the Fisher's exact test. BM, bone marrow sample; PB, peripheral blood sample; AUC, area under curve; Acu, accuracy. **P* <0.05, ****P* <0.001



Supplemental Figure 8. Comparison of predictive values from validation cohort.

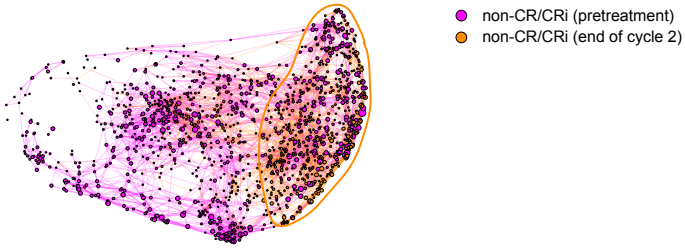
ROC curves of the validation cohort shown for good response predictors: $CD22^{high}$ cells (dotted green) and $CD22^{high}$-$Bcl\text{-}2^{low}$ cells (solid green), and poor response predictors: $Bcl\text{-}2^{high}$ cells (dotted pink) and $CD22^{low}Bcl\text{-}2^{high}$ cells (solid pink). The AUC, max accuracy and p values were described on the bottom table insets. AUC of ROC curve of $CD22^{high}Bcl\text{-}2^{low}$ cells and $CD22^{low}Bcl\text{-}2^{high}$ cells were statistically tested with DeLong's test, referring to $CD22^{high}$ cells and $Bcl\text{-}2^{high}$ cells, respectively. BM, bone marrow sample; PB, peripheral blood sample; Acu, accuracy.

$^{**}P$ <0.01, $^{***}P$ <0.001.



Supplemental Figure 9. Tumor cell-community clustering of all patients.

(A) Tumor cell-community clustering plots are shown for pretreatment data compiled from 17 CR/CRi patients (green) and 11 non-CR/CRi patients (pink) in the training and validation cohort. Bulk plots (top) and extracted plots of CR/CRi and non-CR/CRi patients (bottom) are depicted. (B) Distribution of CD22 and Bcl-2 expression on the clustering plots. For (A) and (B), colored shapes indicate $CD22^{high}Bcl\text{-}2^{low}$ population (green) and $CD22^{low}Bcl\text{-}2^{high}$ population (pink) based on the distribution of each marker.



Supplemental Figure 10. Longitudinal changes by tumor cell-community clustering analysis.

Clustering plots are shown for longitudinal changes of leukemia cell populations in non-CR/CRi patients (pink: pretreatment, orange: end of cycle 2) from the training cohort. The outlined orange ellipse delineates the leukemia cell population that is a poor response predictor with $CD22^{low}$ and $Bcl\text{-}2^{high}$ expression. The cluster profiles correspond to those in Figure 1A and 1C.

Figure S11.

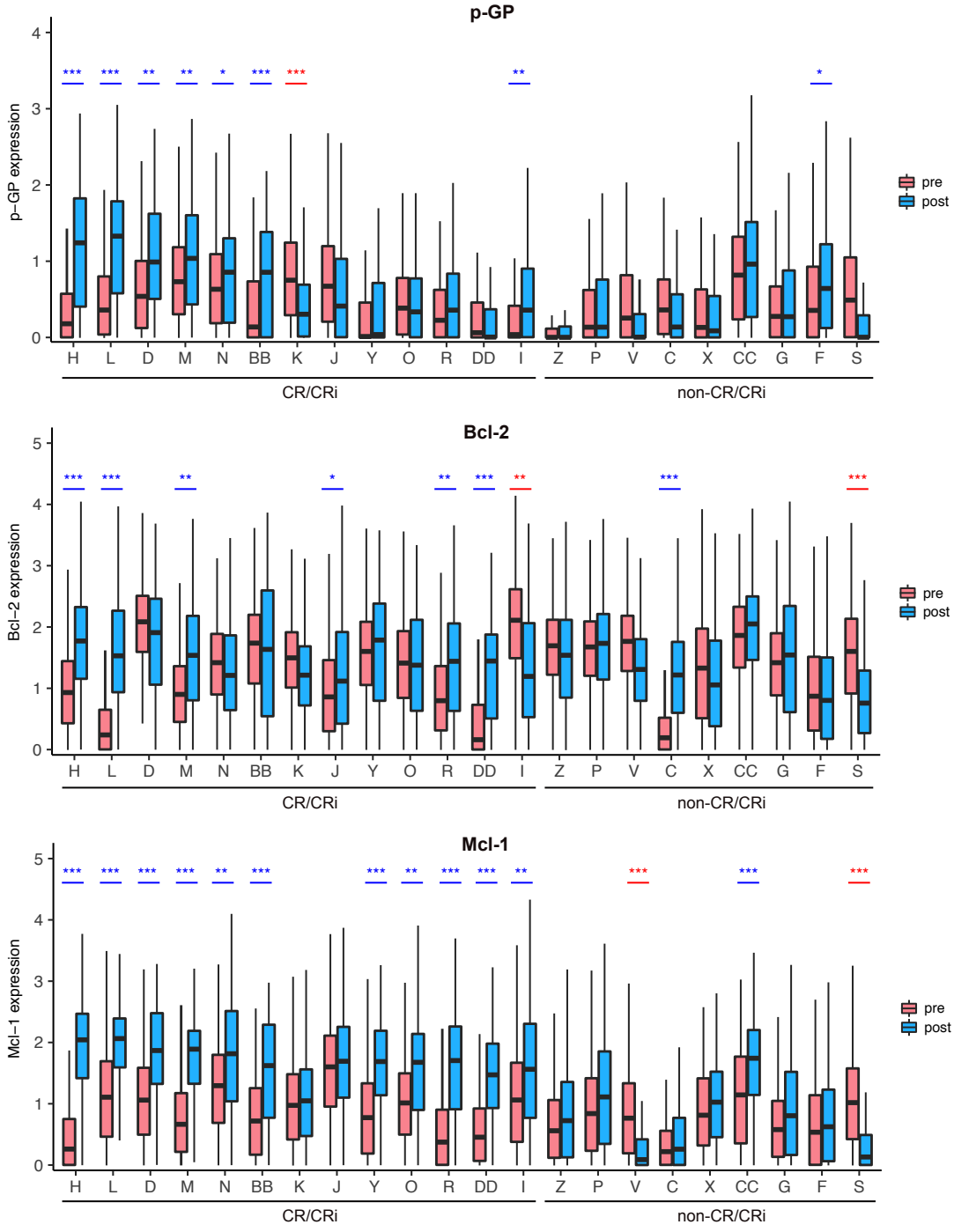
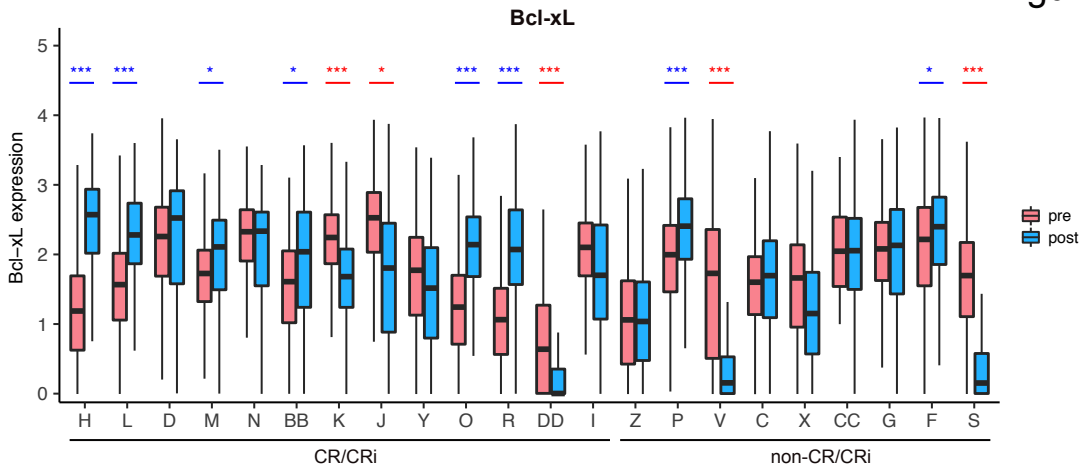
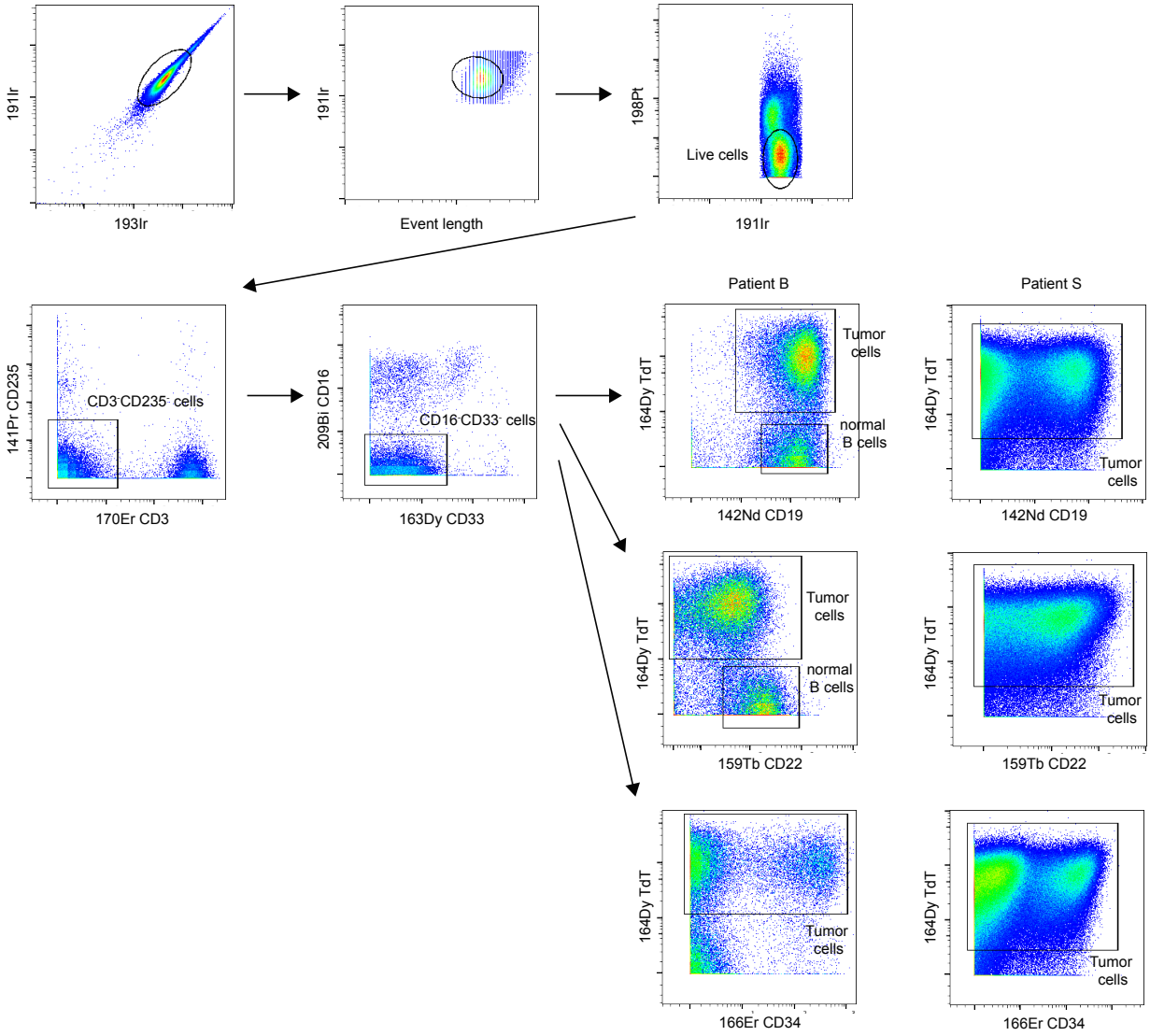

p-GP
p-GP expression
Bcl-2
Bcl-2 expression
Mcl-1
Mcl-1 expression
pre
post
H L D M N BB K J Y O R DD I
Z P V C X CC G F S
CR/CRi
non-CR/CRi

Figure S11.



Supplemental Figure 11. Longitudinal changes of individual tumor profiles.

Boxplots shown for p-GP, Bcl-2, Mcl-1, Bcl-xL expression of tumor cells at pretreatment (pink) and post treatment (blue). CR/CRi Patients (n = 13) with MRD ≥0.1% in marrow cells and non-CR/CRi patients (n = 9) are depicted. The order of patients is the same as the heatmap data in Figure 4A. Statistical significance was analyzed with the Student *t* test. Blue asterisks indicate an increase at posttreatment. Red asterisks indicate a decrease at posttreatment. **P* <0.05, ***P* <0.01, ****P* <0.001.



Supplemental Figure 12. Conventional gating strategy to identify tumor cells.

After gating for singlet, tumor cells were found in $CD3^{-}CD235^{-}CD16^{-}CD33^{-}$ or $CD33^{dim}$ cells. Then, B-lineage markers and CD34 were used to identify tumor cells. Data represent two patients.