Supplemental Methods

Flow cytometry

For immunophenotyping, cells were pre-incubated with Aqua Blue dead cell exclusion dye (Invitrogen), followed by surface staining. Flow buffer was Dulbecco's phosphate-buffered saline, 1% FBS, 0.02% sodium azide. Cells were fixed using 0.5% paraformaldehyde. T cells were subdivided into CD8+ or CD4+ subsets. Absolute CD4 and CD8 T-cell counts were determined using AccuCount particles (Spherotech). T-cell differentiation and Th1, Th2 polarization of CD4+ T cells were assessed based on CD45RO, CCR7, CCR6, and CXCR3 expression as previously described.[24] Intracellular staining for granzyme B, Ki-67 and CTLA-4 expression was performed using Foxp3/Transcription factor staining buffer set (Invitrogen). All antibodies were titrated prior to use and fluorescence minus one controls were used.[24] Samples were acquired on a LSRFortessa (BD Biosciences), and data were analyzed with FlowJo (Version 10, TreeStar).

Luminex cytokine immunoassay

Supernatants were analyzed using Milliplex MAP Human High Sensitivity T Cell Magnetic 13-plex Bead Panel, (GM-CSF; IFN_{γ}; IL-10; MIP-3α; MIP-1β IL-1 β; IL-2; IL-4; IL-5; IL-6; IL-7; IL-8; TNFα) (Millipore Corporation). A 96-well microplate was washed for 10 minutes with the provided 1X wash buffer containing 0.05% ProClin. Standard and quality B12 controls were reconstituted in culture media; standard curves were generated by serial 1:4 dilutions. Supernatant was diluted two-fold in assay buffer, mixed with 25 µl of bead mixture, and incubated for 18 hours at 4°C on a plate shaker. Detection antibodies were incubated at 20°C for 1 hour, followed by Streptavidin-Phycoerythrin for 30 minutes. Analysis was performed with Luminex 200 software using a 5-parametric curve-fitting method to calculate analyte concentrations.

In vitro treatment with epcoritamab and venetoclax

PBMCs from CLL patients were isolated by density-gradient centrifugation (Ficoll Lymphocyte Separation Media; ICN Biomedicals) and viably frozen in 90% fetal bovine serum (FBS), 10% dimethyl sulfoxide (DMSO) (Sigma) in liquid nitrogen. Cryopreserved PBMCs were thawed and plated at 3 x 10^6 cells/ml in 24-well plates in RPMI 1640 medium (Gibco) containing 10% FBS, 1% penicillin-streptomycin, and 50 µM β-mercaptoethanol (Sigma). For combination with venetoclax, PBMCs were plated in 96-well plates, and treated with epcoritamab, or non-targeting B12 isotype, with the addition of 5nM venetoclax (ABT-199, Selleckchem) or DMSO, simultaneously. When indicated, CLL cells were mixed with healthy donor PBMCs prior culture. CLL PBMCs with > 95% CLL or with < 95% CLL cells that were depleted of autologous CD3+ T cells using CD3 microbeads (Miltenyi Biotech) were co-cultured with healthy donor PBMCs (source of T cells) to have an effector to target (T:CLL) ratio of 1:3. Mixed cells were then cultured

at 3 x 10^6 cells/ml in 96-well plates. Epcoritamab, or non-targeting B12 isotype, were added simultaneously with the addition of 5nM venetoclax (ABT-199, Selleckchem) or DMSO. CLL cell viability was measured by flow cytometry every 24 hours for 7 days.

Supplemental Tables

Table S1. Individual patient characteristics and sample usage

Patient ID	Sample ID	Treated	Age	Sex	Time on T_x (mo)	Rai stage	TN/RR	Cytogenetics	IGHV Status	ALC, k/μL	E:T	Figure
CLL-01	4086	N	53	M	0	2	TN	ND	U	94.6	0.03	6
CLL-02	4973	N	50	M	0	4	TN	del13q, del17p	M	68.97	0.02	1, 2, 5, 6
CLL-03	5407	N	61	M	0	4	TN	ND	U	10.23	0.03	6
CLL-04	5411	N	48	F	0	1	TN	del13q	M	226.01	0.01	6
CLL-05	5508	N	77	M	0	3	TN	Normal	U	37.04	0.04	1, 2, 6
CLL-06	5539	N	58	F	0	1	TN	del13q, del17p, del6q	ND	139	0.03	1, 2, 5, 6
CLL-07	5582	N	67	F	0	1	TN	del13q	ND	170.94	0.03	1, 2, 5, 6
CLL-08	5652	N	61	F	0	2	TN	del13q14	M	175.66	0.03	6
CLL-09	6606	ibrutinib	67	F	5.5	1	TN	del13q	U	32.1	0.09	1, 2, 3, 4
CLL-10	7469	ibrutinib	53	F	12.4	2	TN	del17p	M	65.6	0.12	1, 2, 3, 4, 6
CLL-11	**7557**	ibrutinib	57	M	15	4	TN	del17p	U	23.37	0.50	5
CLL-12	7791	ibrutinib	71	M	5.5	4	TN	del13q, del17p	M	65.77	0.04	1, 2, 3, 4
CLL-13	8041*	ibrutinib	69	F	5.9	ND	RR	del13q, del11q	ND	26.8	0.05	1, 2
CLL-14	8289	N	63	F	0	1	TN	del13q	M	73.15	0.03	6
CLL-15	8302*	ibrutinib	67	F	15	4	RR	del13q, del11q	U	6.71	0.17	1, 2
CLL-16	**8527***	ibrutinib	76	M	38.8	4	RR	del13q, del11q, del17p, Tri12	U	31.2	0.13	1, 2, 3, 4, 6
CLL-17	8701	acalabrutinib	68	F	5.5	3	RR	del 13q	M	54.53	0.04	6
CLL-18	8942	acalabrutinib	59	F	5.5	3	TN	Tri12, NOTCH1	U	7.77	0.20	1, 2, 3, 4, 6
CLL-19	8943*	acalabrutinib	54	F	11.8	3	RR	del13q	M	30.85	0.07	1, 2, 6
CLL-20	9013	acalabrutinib	50	M	5.5	1	RR	Normal	M	9.65	0.18	1, 2, 3, 4, 6
CLL-21	9153*	ibrutinib	68	M	24.2	1	RR	del13q, Tri12	ND	5.34	0.22	1, 2, 3, 4
CLL-22	9205*	acalabrutinib	55	M	5.5	4	RR	del13q14	U	41.74	0.04	1, 2, 3, 4
CLL-23	9778*	acalabrutinib	65	M	5.5	4	RR	del14q, del11q	U	129.83	0.04	1, 2, 3, 4
CLL-24	9939	acalabrutinib	68	F	5.2	3	TN	del13q, del17p	U	36.62	0.04	1, 2
CLL-25	**9979**	ibrutinib	63	M	45.8	3	TN	del17p	U	6.22	0.45	1, 2, 3, 4, 5, 6
CLL-26	10030	N	71	M	0	2	TN	t(14,19)	U	69.39	0.03	6
CLL-27	10621	N	56	M	0	2	TN	del13q, NOTCH1	U	25.26	0.03	1, 2
CLL-28	10743	acalabrutinib	53	M	5.7	2	TN	Normal	U	26.53	0.21	1, 2, 3, 4
CLL-29	**10812**	ibrutinib	74	M	54.7	1	TN	del13q, del11q, del17p	U	15.45	0.44	1, 2, 3, 4, 5, 6
CLL-30	10872*	acalabrutinib	54	M	5.5	4	RR	Normal	U	145.5	0.01	1, 2
CLL-31	10989*	acalabrutinib	67	M	5.5	1	RR	Normal	U	106.46	0.10	1, 2, 3, 4
CLL-32	**11052**	ibrutinib	39	M	72	2	TN	Normal	U	8.29	0.29	1, 2, 3, 4, 6
CLL-33	11120	N	75	F	0	0	TN	Normal	M	262.43	0.03	1, 2, 3, 4, 6
CLL-34	**11182**	ibrutinib	65	M	59.6	3	TN	del13q, del11q, del17p	U	8.83	0.64	1, 2, 3, 4, 6
CLL-35	11213*	acalabrutinib	68	F	5.4	1	RR	del13q	M	30.55	0.05	1, 2, 3, 4
CLL-36	11294*	ibrutinib	65	M	6.5	1	RR	del17p	U	42.48	0.02	1, 2, 3, 4, 6
CLL-37	11597	N	64	F	0	3	TN	del13q	U	327.68	0.01	6
CLL-38	11682	N	55	F	0	1	TN	Normal	U	150.38	0.03	1, 2, 3, 4, 6
CLL-39	**11841**	ibrutinib	65	M	67	4	TN	del13q, del17p	U	9.64	0.32	6
CLL-40	11913*	acalabrutinib	57	F	47.7	3	RR	del13q14	M	7.63	0.25	1, 2, 3, 4, 6
CLL-41	11940	N	57	M	0	2	TN	del13q	U	41.37	0.04	1, 2, 3, 4, 6
CLL-42	11944	N	53	M	0	2	TN	Tri12	U	278.35	0.02	1, 2, 3, 4
CLL-43	11983	acalabrutinib	61	M	20.1	3	TN	del13q14	U	3.55	0.56	1, 2, 3, 4, 6
CLL-44	12007	N	53	F	0	1	TN	Tri12	U	62.87	0.05	1, 2, 3, 4
CLL-45	12021	N	66	M	0	1	TN	del13q	M	42.45	0.08	1, 2, 3, 4
CLL-46	12025*	ibrutinib	75	F	6	1	RR	Normal	U	54.39	0.05	1, 2, 3, 4
CLL-47	12032	acalabrutinib	67	M	28.6	3	TN	del13q14, NOTCH1	U	3.57	0.32	1, 2, 3, 4, 6
CLL-48	12101*	acalabrutinib	74	M	26.3	1	RR	del13q14, del11q	U	7.37	0.32	1, 2, 3, 4
CLL-49	12128	N	55	F	0	1	TN	del13q	M	126.38	0.03	1, 2
CLL-50	12253*	ibrutinib	78	F	57	0	RR	del13q, del11q	U	11.34	0.05	1, 2, 3, 4, 6
CLL-51	12624	N	60	M	0	4	TN	del13q	M	184.83	0.02	1, 2, 3, 4, 6
CLL-52	12650	ibrutinib	56	M	9.8	2	TN	del13q, del17p	M	56.36	0.06	1, 2, 3, 4, 6
CLL-53	12798	ibrutinib	57	M	7.8	1	TN	del13q, Tri12	M	317.2	0.01	1, 2, 3, 4
CLL-54	**13100**	acalabrutinib	62	M	26.4	3	TN	Tri12, NOTCH1	U	9.08	0.69	1, 2, 3, 4, 5, 6
CLL-55	13256	ibrutinib	64	M	12.9	2	TN	del13q	M	7.93	0.40	1, 2, 3, 4
CLL-56	**13788***	acalabrutinib	69	M	35.8	4	RR	Tri12, del13q, NOTCH1	U	27.47	0.08	1, 2, 3, 4, 6
CLL-57	**13959**	acalabrutinib	50	M	60.4	2	TN	del6q, del11q	U	14.2	0.12	6

Patients on BTKi were responding to therapy at time of sample collection or progressing on BTKi (bold). TN, treatment-naïve or RR, relapsed-refractory disease prior to enrollment on BTKi therapy or sample collection. Effector:Target (E:T) ratio was determined by flow cytometry using the formula: (%CD8 + %CD4)/%CLL. F = female; M = male; IGHV = Immunoglobulin heavy chain mutation status, where M = mutated, U = unmutated (≥98% sequence homology to germline), ND = not determined; ALC = absolute lymphocyte count at time of sample collection; del = deletion of indicated chromosomal region as determined by FISH.
* Patients who had prior chemoimmunotherapy.

Table S2. Characteristics of progressive disease in patients on BTKi

Patient ID[$]	**BTKi Time from start**	**Resistance Mutations (VAF)[*]**
CLL-11	Ibrutinib 15 months	ND
CLL-16	Ibrutinib 38.8 months	BTK C481S (33%)
CLL-25	Ibrutinib 45 months	BTK C481S (15%) BTK C481S (9%)
CLL-29	Ibrutinib 54 months	BTK C481S (53%) BTK C481Y (12%)
CLL-32	Ibrutinib 72 months	BTK C481S (15%)
CLL-34	Ibrutinib 59 months	BTK C481S (49%) BTK C481S (35%) PLCG2 R665W (5%)
CLL-39	Ibrutinib 67 months	BTK C481S (82%)
CLL-54	Acalabrutinib 26 months	PLCG2 R665W (<10%)
CLL-56	Acalabrutinib 35 months	BTK C481T (<10%)
CLL-57	Acalabrutinib 60 months	BTK C481T (ND)

[$]Patient ID links to patient characteristics in Table S1

[*] VAF – variant allele frequency, only mutations with VAF ≥ 5% are shown

Table S3. Flow cytometry reagents

Specificity	Conjugate	Clone	Supplier
CD3	APC-H7	Clone SK7	BD Pharmingen
CD3	BUV496	UCHT1	BD Pharmingen
CD4	APC	RPA-T4	BD Pharmingen
CD4	BV786	Clone SK3	BD Pharmingen
CD5	PECy7	L17F12	BD Pharmingen
CD8	FITC	HIT8a	BD Pharmingen
CD8	BV650	Clone RPA-T8 (RUO)	BD Pharmingen
CD14	AmyCyan/V500	Clone M5E2 (RUO)	BD Pharmingen
CD19	AmyCyan/V500	Clone HIB19 (RUO)	BD Pharmingen
CD20	PE	L27	BD Pharmingen
CD45RO	APC	UCHL1 (RUO)	BD Pharmingen
CCR7	mCherry/PE-Dazzle 594	Clone 150503 (RUO)	BD Pharmingen
CCR6	PE	11A9 (RUO)	BD Pharmingen
CXCR3	AF700	Clone 1C6/CXCR3	BD Pharmingen
HLADR	FITC	G46-6 (RUO)	BD Pharmingen
TIM3 (CD366)	PE	clone 7D3 (RUO)	BD Pharmingen
CTLA4 (CD152)	PECy5	BNI3	BD Pharmingen
LAG3 (CD223)	PECy7	11C3C65	Biolegend
Granzyme B	AF 700	GB11	BD Pharmingen
PD-1 (CD279)	BV421	EH12.1	BD Pharmingen
Ki67	BV605	Ki67	Biolegend
EOMES	FITC	WD1928	eBioscience
LIVE/DEAD™	AmyCyan/V500	Aqua Dead Cell Stain Kit	Invitrogen
LIVE/DEAD™	Pacific Blue/BV421	Violet Dead Cell Stain Kit	Invitrogen

Supplemental Figures

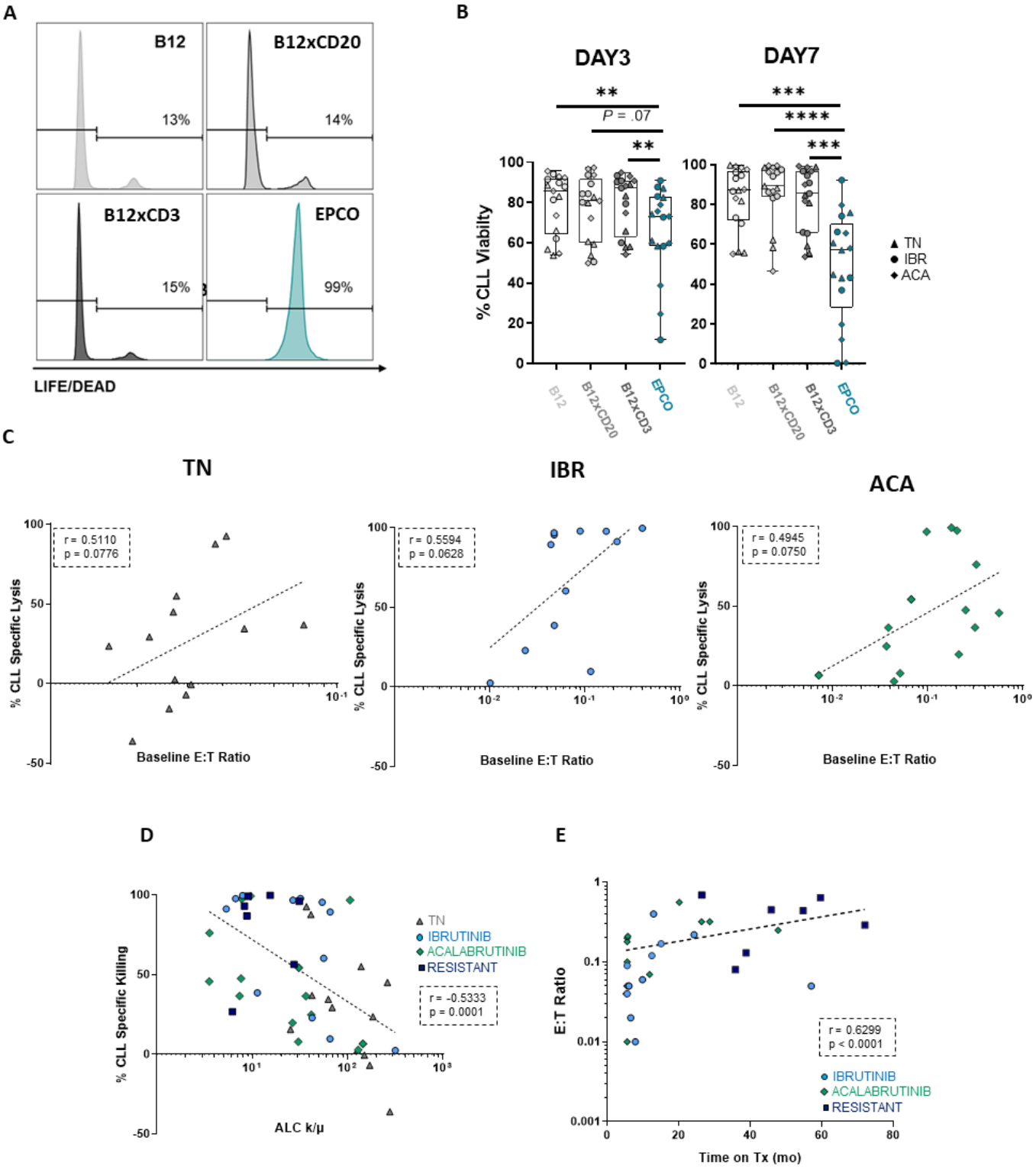


Figure S1. Epcoritamab induced cytotoxicity in CLL PBMCs correlates with the patients' absolute lymphocyte counts.

Cell viability in PBMCs from patients with CLL was measured after in vitro culture with antibodies as indicated. (A) Representative example of cell viability in PBMCs cultured with B12, B12xCD20, B12xCD3, or epcoritamab (EPCO). (B) Summary of viability measurements for the three different controls and epcoritamab treated samples from treatment-naïve (TN, n = 5), ibrutinib-treated (IBR, n = 6), or acalabrutinib-treated (ACA, n = 6) patients after 3 and 7 days. (C) Spearman's correlation of baseline E:T ratios and percent of CLL cells specific lysis in samples from treatment-naïve (TN, n = 13, grey triangles), ibrutinib-treated (IBR, n = 12, blue circles), and acalabrutinib-treated (ACA, n = 14, green diamonds) cultured with epcoritamab for 7 days. (D) Spearman's correlation of patients' absolute lymphocyte count at the time of sample collection and percent specific killing of CLL cells in samples from all three groups, and progressing patients (RES, n = 7, blue squares) cultured with epcoritamab for 7 days. (E) Spearman's correlation of baseline E:T ratios and time on BTKi therapy in months (Time on Tx) in samples from ibrutinib-treated, acalabrutinib-treated, and progressing patients. Each symbol represents one patient sample. Asterisks indicate statistical significance using Wilcoxon matched-pair signed-rank test for comparison of different treatments applied to individual patient samples. **, $P < .01$; ***, $P<.001$; ****, $P<.0001$.

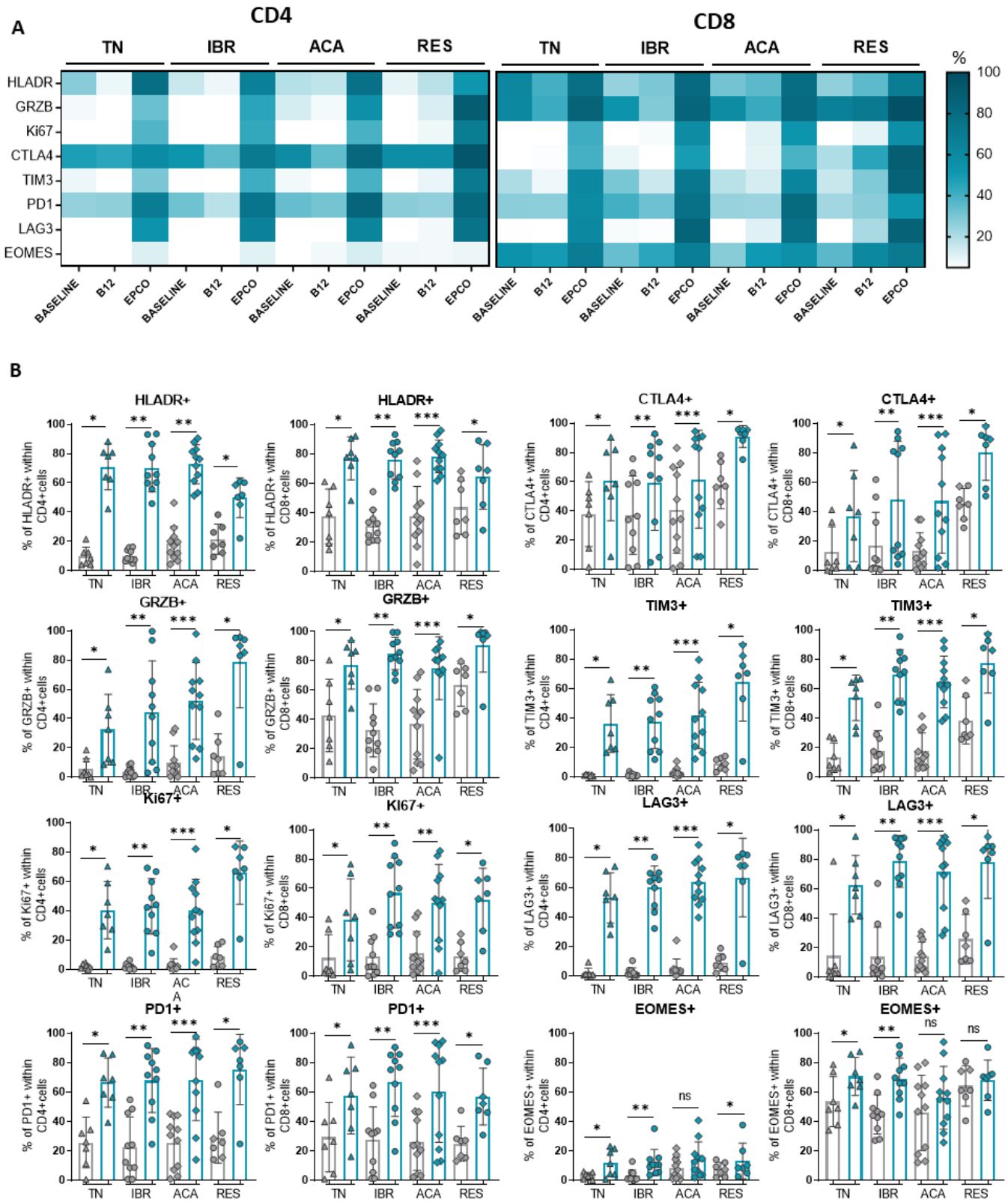


Figure S2. T-cell activation at baseline and after 3 days of exposure to epcoritamab. Markers of CD4+ and CD8+ T-cell activation and cytotoxic potential were assessed by flow cytometry in treatment-naïve (TN, n = 7), ibrutinib-treated (IBR, n = 10), acalabrutinib-treated

(ACA, n = 11), and BTKi-resistant (RES, n = 7) patient samples. (A) Heat map depicts median frequencies of CD4 and CD8 T-cells staining for HLA-DR, CTLA-4, granzyme B (GRZB), TIM-3, Ki-67, LAG-3, PD-1, and EOMES, at baseline and after 3 days of culture with epcoritamab (EPCO) or B12 control (B12). (B) Frequencies of CD4 and CD8 T-cells staining for indicated markers after 3 days of culture with epcoritamab (green bars) or B12 control (grey bars). Patient groups as in A. Asterisks indicate statistical significance using Wilcoxon's signed rank test. *, *P*< .05; **, *P*< .01; ***, P<.001.

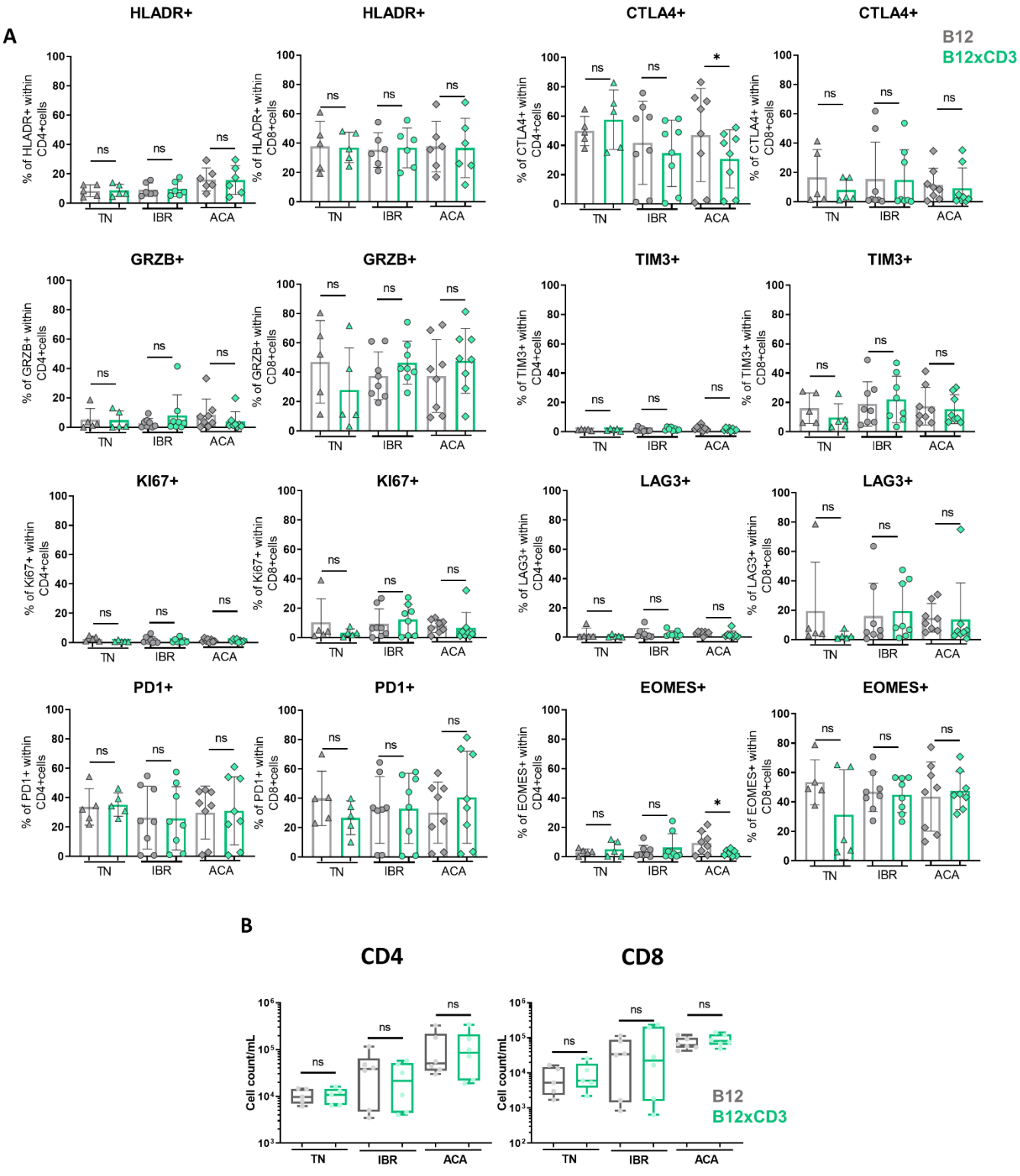
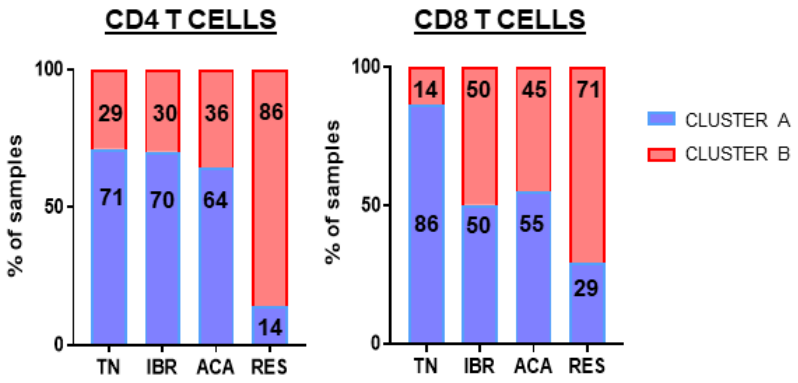


Figure S3. T-cell activation after 3 days of exposure to B12XCD3 bispecific control.

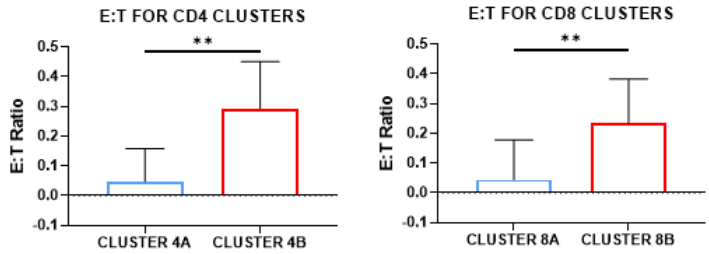
Markers of CD4+ and CD8+ T-cell activation and cytotoxic potential were assessed by flow cytometry in treatment-naïve (TN, n = 5), ibrutinib-treated (IBR, n = 8), and acalabrutinib-treated (ACA, n = 8) patient samples. (A) Frequencies of CD4 and CD8 T-cells staining for indicated markers after 3 days of culture with B12XCD3 (green bars) or B12 control (grey bars). (B) CD4+ and CD8+ T-cell counts were quantified by flow cytometry after 7 days of culture with B12XCD3 (green symbols) or B12 control (grey symbols). Each symbol represents one patient sample. Asterisks indicate statistical significance using Wilcoxon's signed rank test. *, *P*< .05.

Figure S4. Composition of the sample clusters based on T-cell activation state.

A



B



C

Samples within Cluster 4A and 8A

Cluster A, divided by CLL specific killing	Median CLL specific killing (range)	TN samples (%)	BTKi or RES samples (%)	IGHV UNMUTATED (%)	MEDIAN E:T RATIO (RANGE)
CD4A high	97 (90-99)	11	89	78	0.09 (0.05-0.2)
CD4A low	20 (2-25)	36	64	64	0.04 (0.02-0.05)
CD8A high	98 (89-99)	17	83	83	0.13 (0.04-0.26)
CD8A low	20 (3-26)	38	62	54	0.04 (0.02-0.09)

Treatment-naïve (TN, n = 7), ibrutinib-treated (IBR, n = 10), acalabrutinib-treated (ACA, n = 11), and BTKi-resistant (RES, n = 7) patient samples were grouped by hierarchical clustering into Cluster A (blue) and Cluster B (red) based on median centered frequencies of CD4 and CD8 T cells expressing the selected markers. (A) Bar graph showing the proportion of patient samples in Cluster A or Cluster B within each patient group. (B) Comparison of baseline E:T ratio between different clusters based on CD4 activation and CD8 activation state. (C) Proportion of TN, BTKi-treated (BTKi) or RES patient samples, proportion of samples from patients with an unmutated IGHV, and median E:T ratio in each sub-groups within Cluster 4A and 8A, divided by CLL specific killing as high (median CLL specific killing ≥ 50%) or low (median CLL specific killing ≤ 20%). Median and interquartile range (IQR) are indicated. Asterisks indicate statistical significance using Mann-Whitney U test. **, $P < .01$.

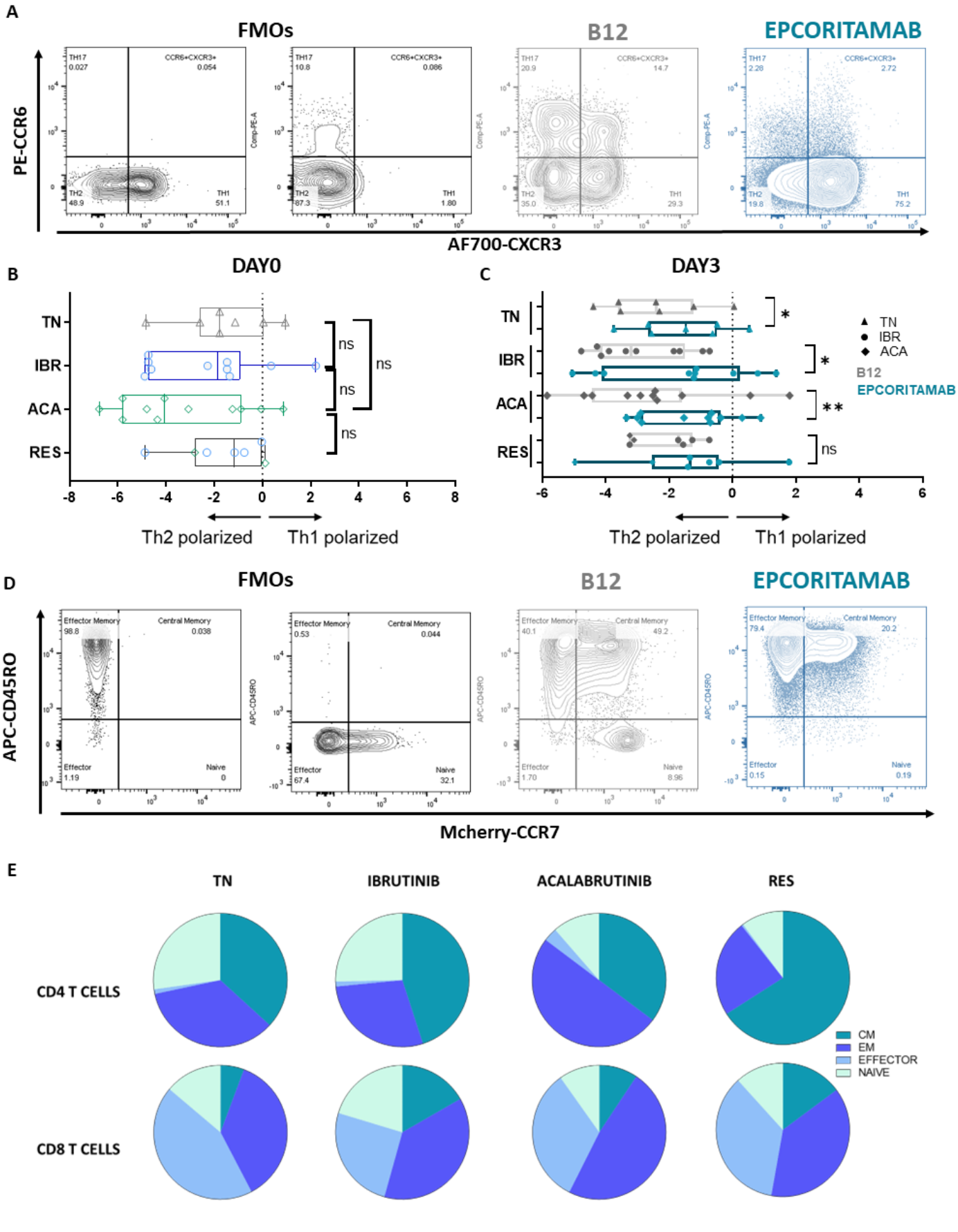

A
FMOs
B12
EPCORITAMAB
PE-CCR6
AF700-CXCR3
TH17
CCR6+CXCR3+
TH2
TH1
B
DAY0
TN
IBR
ACA
RES
ns
Th2 polarized
Th1 polarized
C
DAY3
*
**
TN
IBR
ACA
B12
EPCORITAMAB
D
FMOs
B12
EPCORITAMAB
APC-CD45RO
Mcherry-CCR7
Effector Memory
Central Memory
Effector
Naive
E
TN
IBRUTINIB
ACALABRUTINIB
RES
CD4 T CELLS
CD8 T CELLS
CM
EM
EFFECTOR
NAIVE

Figure S5. T-cell differentiation in response to epcoritamab.

Th1 and Th2 polarization of CD4+ T cells was assessed based on CCR6 and CXCR3 expression. (A) Representative contour plot layout of an acalabrutinib-treated patient after 7 days of culture with epcoritamab (green) or B12 control (grey). Fluorescence minus one (FMO) controls were used to set the gates. Th1/Th2 ratio (log2 transformed) was calculated from the percentage of Th1 and Th2 subsets within CD4+ T cells in PBMCs from treatment-naïve (TN, n = 7), ibrutinib-treated (IBR, n = 10), acalabrutinib-treated (ACA, n = 11), and BTKi-resistant (RES, n = 7) patients (B) at baseline, and (C) after 3 days of culture with epcoritamab (green symbols) or B12 (grey symbols). Each symbol represents one patient sample. CD4+ and CD8+ T-cell differentiation separating naïve T cells, central memory T cells (CM), effector memory T cells (EM), and effector T cells, based on expression of CCR7 and CD45RO expression. (D) Representative contour plot layout of an acalabrutinib-treated patient after 7 days of culture with epcoritamab (green) or B12 control (grey). FMO controls were used to set the gates. (E) Pie charts represent the median proportion of each subset within CD4+ and CD8+ T cells, at baseline, in each patients group. Asterisks indicate statistical significance using Wilcoxon's signed rank test for comparison between treatment groups. *, $P < .05$; **, $P < .01$.

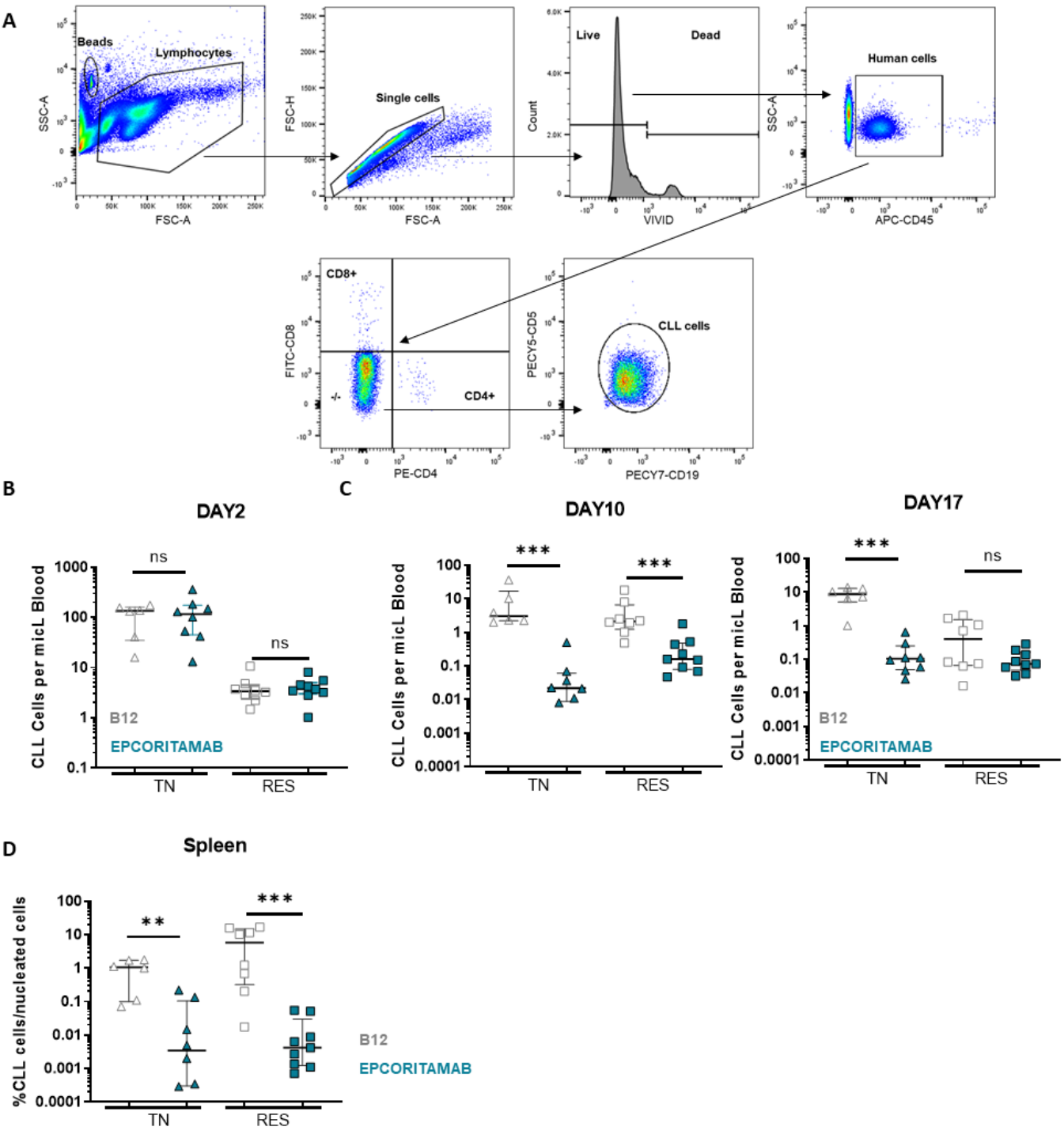


Figure S6. CLL cell engraftment in patient-derived xenografts, and elimination with epcoritamab treatment.

PBMCs from treatment naïve patients (TN, n = 3, triangles), and patients progressing on a BTKi (RES, n = 4, squares) were injected into NSG mice on experimental day 0. (A) Representative flow cytometry analysis from the peripheral blood of engrafted mice, on day 2. (B) Cell engraftment was confirmed on day 2 by flow cytometry, and equally engrafted mice were assigned to B12 control (grey) or epcoritamab (green) treatment. Mice were then treated once-weekly with either

epcoritamab or B12 control (0.5mg/kg). 5 mice were included for each patient and divided between the 2 treatment groups (B12, n = 14 , EPCORITAMAB, n = 17). (C) Comparison of the mean CLL cell count in the peripheral blood between epcoritamab-treated (green) and B12-treated (grey) groups at experimental days 10 and 17. (D) %CLL cell count among all nucleated cells in the spleen at experimental day 17. Each symbol represents one animal and symbol codes denote patient group source of xenografted cells. Median and interquartile range (IQR) are indicated. Asterisks indicate statistical significance using Mann-Whitney test. **, *P*< .01; ***, P< .001.

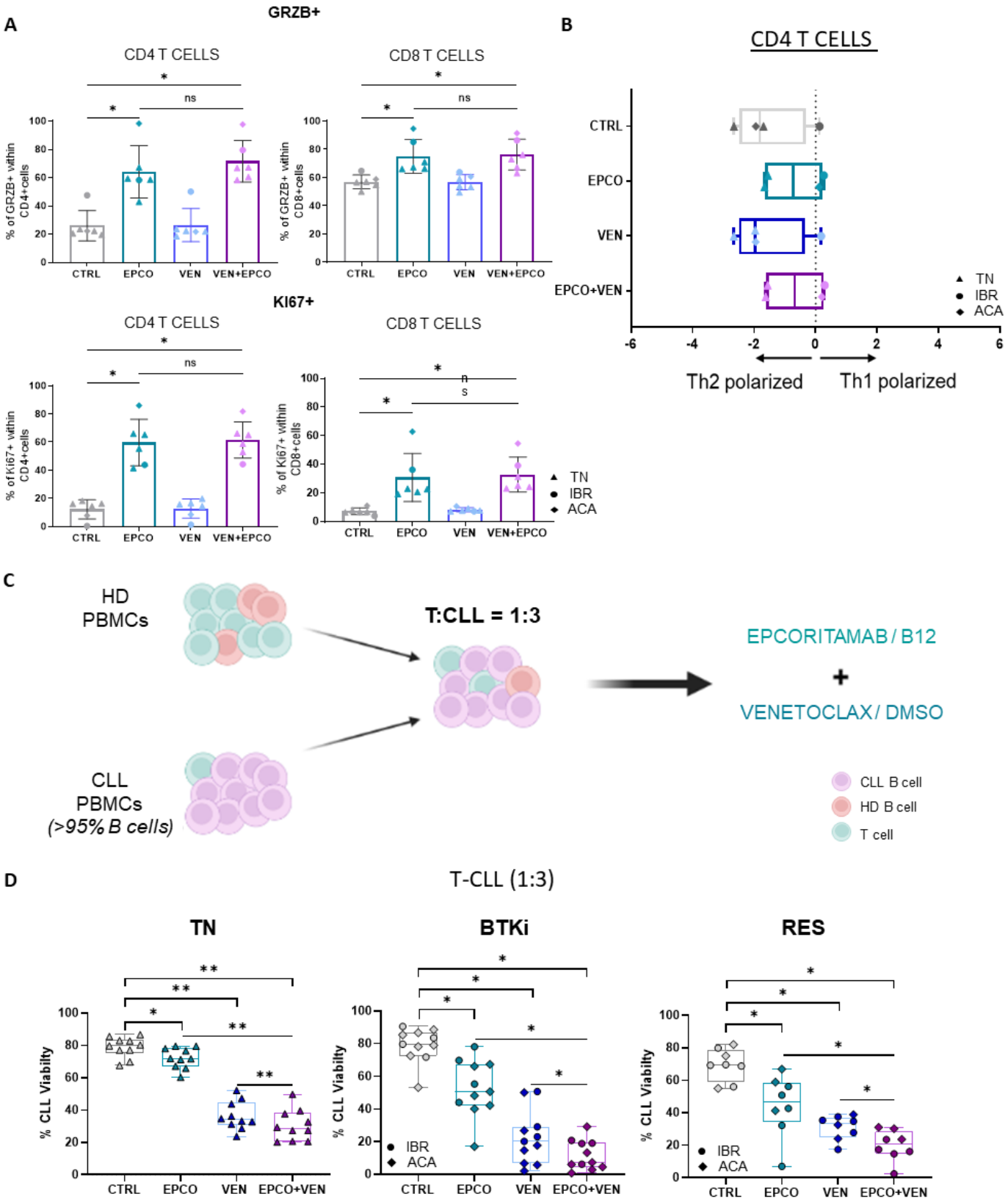

A
GRZB+
CD4 T CELLS
CD8 T CELLS
% of GRZB+ within CD4+cells
% of GRZB+ within CD8+cells
CTRL
EPCO
VEN
VEN+EPCO
KI67+
% of Ki67+ within CD4+cells
% of Ki67+ within CD8+cells
TN
IBR
ACA
B
CD4 T CELLS
EPCO+VEN
Th2 polarized
Th1 polarized
C
HD PBMCs
CLL PBMCs (>95% B cells)
T:CLL = 1:3
EPCORITAMAB / B12
+
VENETOCLAX / DMSO
CLL B cell
HD B cell
T cell
D
T-CLL (1:3)
TN
BTKi
RES
% CLL Viability

Figure S7. Testing the combination of epcoritamab and venetoclax against CLL cells using autologous or healthy allogeneic T cells as effectors.

(A) Frequencies of autologous CD4+ and CD8+ T-cell staining for granzyme B (GRZB), and Ki-67, and (B) Th1/Th2 ratio (log2 transformed) within CD4+ T cells were assessed by flow cytometry in treatment-naïve (triangles, n = 4), and BTKi-treated (n = 2) patient samples, after 3 days of culture with B12/DMSO control (CTRL), epcoritamab (EPCO), 5nM venetoclax (VEN) or combined epcoritamab and venetoclax (EPCO+VEN). (C) PBMCs from a healthy donor and CLL cells from treatment-naïve (TN, n = 10); BTKi-treated (BTKi, n = 11, 4 on ibrutinib and 7 on acalabrutinib); and patients progressing on a BTKi (RES, n = 8) were mixed to an E:T ratio of 1:3, and cultured with either B12/DMSO control (CTRL), epcoritamab (EPCO), 5nM venetoclax (VEN), or combined epcoritamab and venetoclax (EPCO+VEN). (D) CLL cell viability after 48 hours of culture. Asterisks indicate statistical significance using Wilcoxon matched-pair signed-rank test for comparison of different treatments applied to individual patient samples. *, $P < .05$; **, $P < .01$.