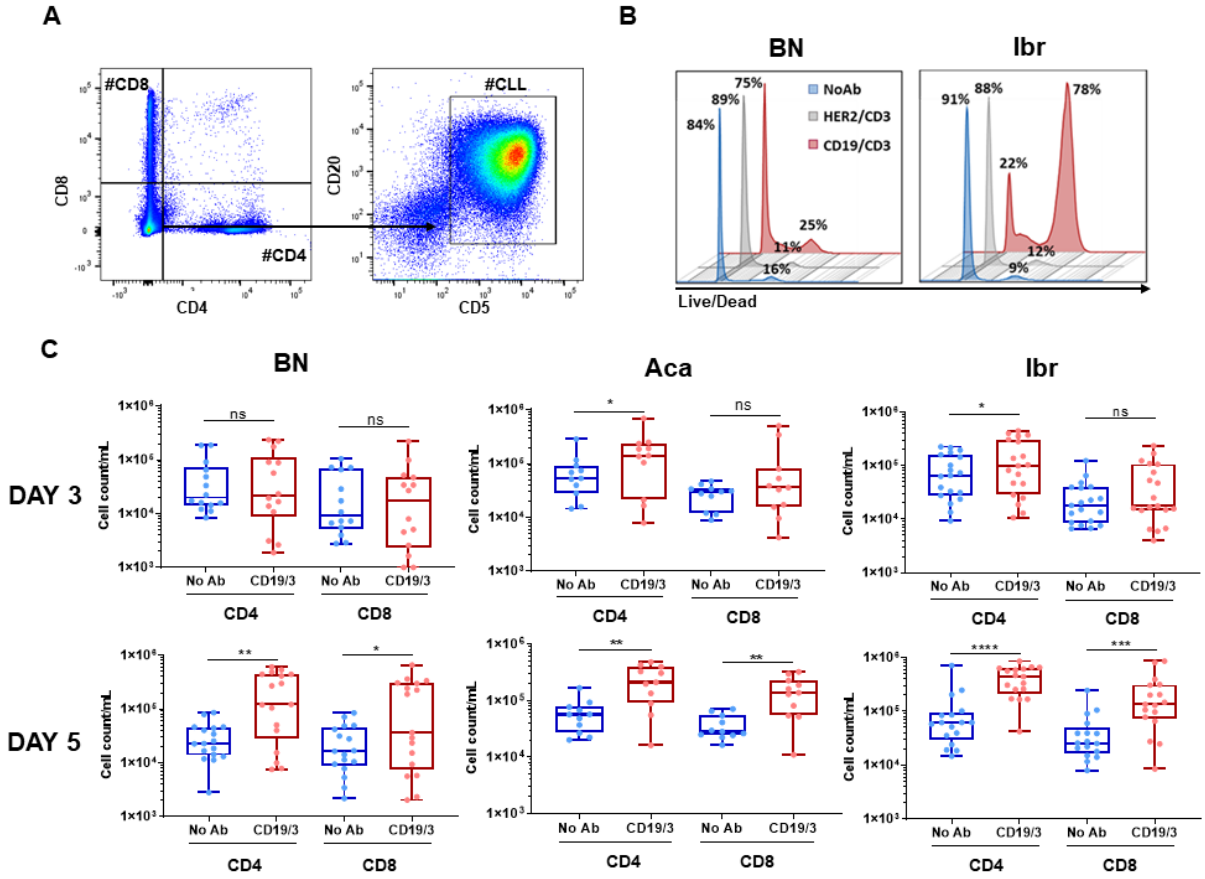
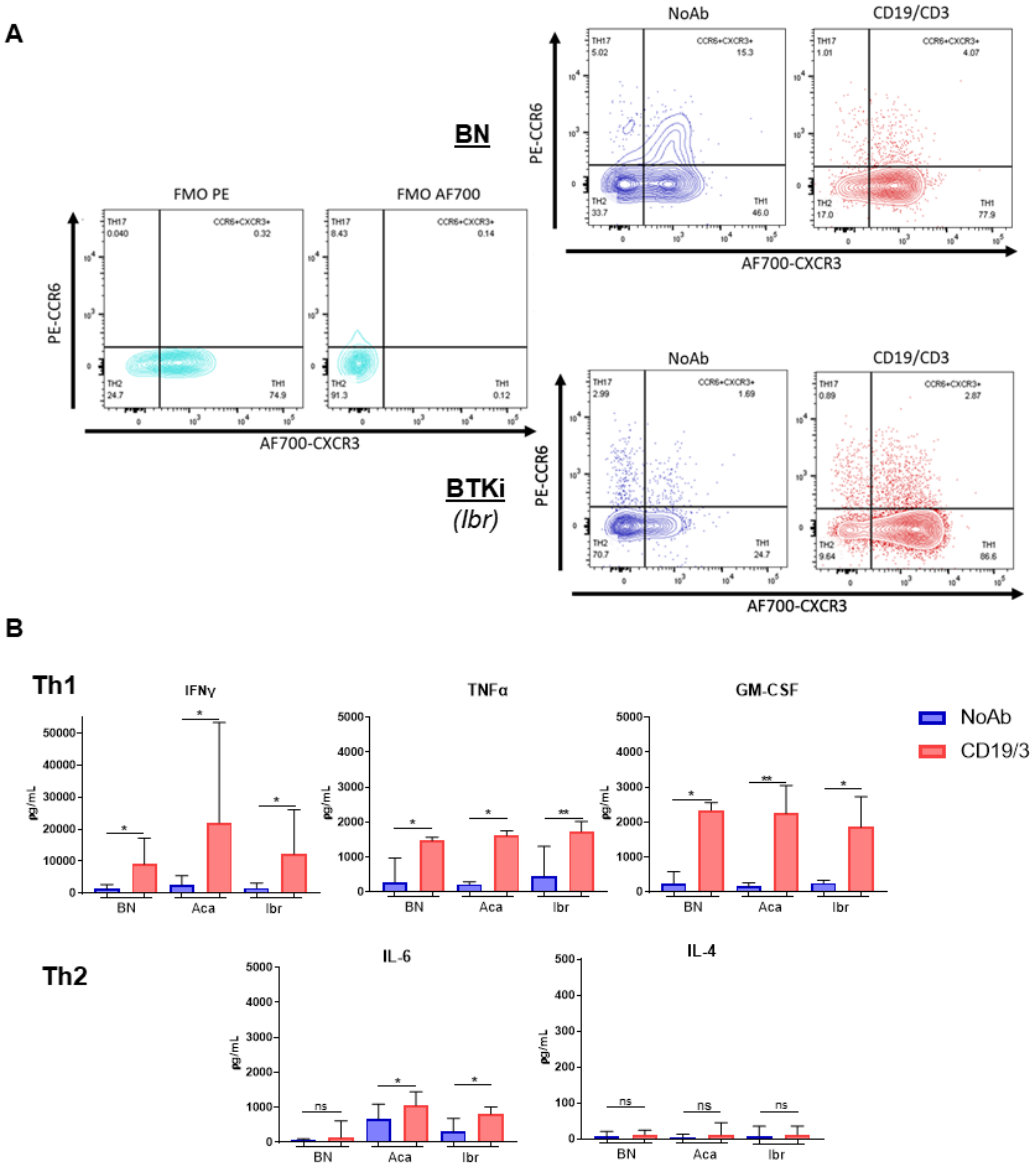
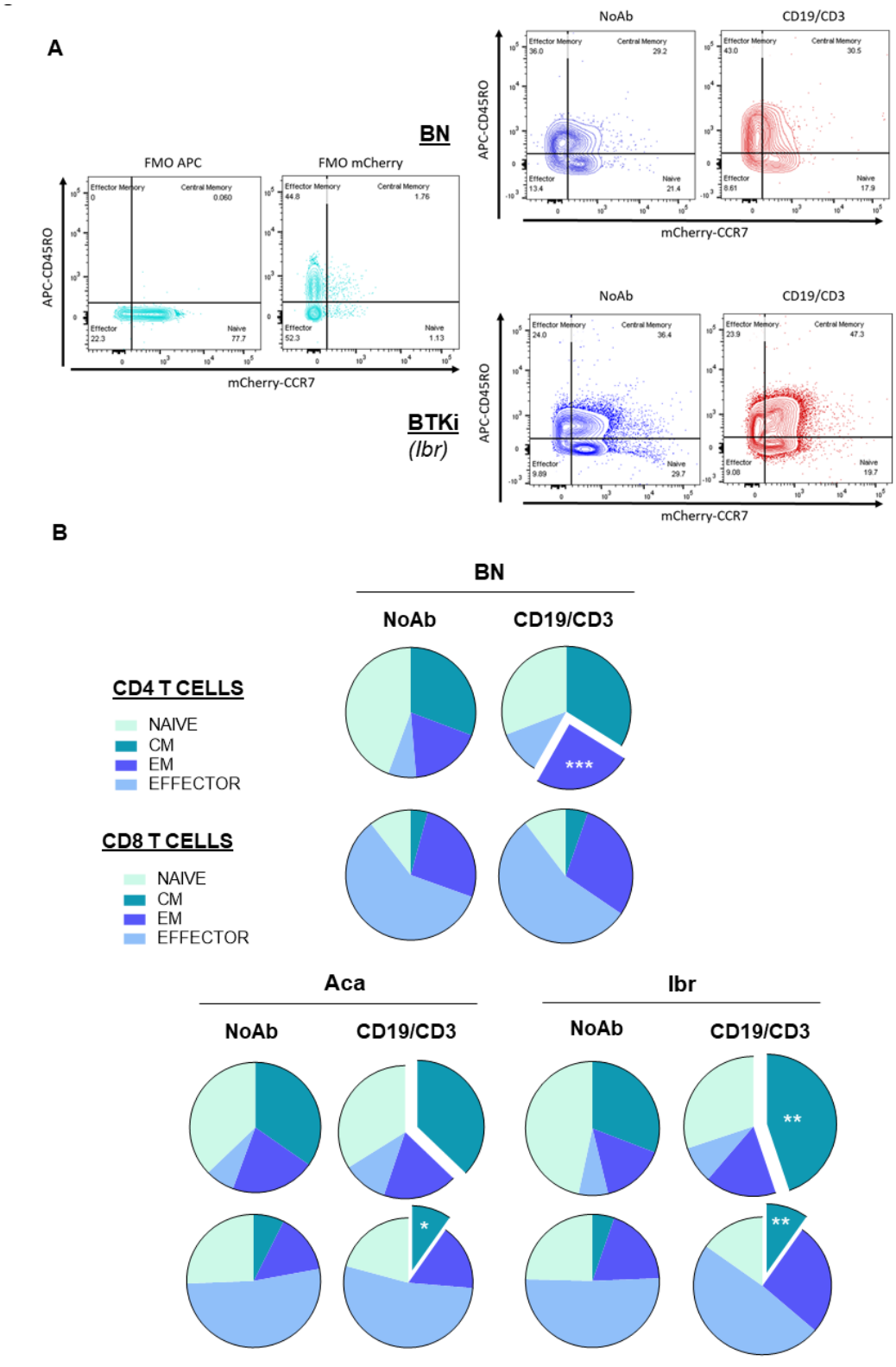


Supplemental Figure S1. CD19/CD3 bsAb promotes rapid expansion of autologous T cells in BTKi-treated patient samples. PBMCs from BTKi-naïve (BN) and BTKi-treated (BTKi) patients were cultured with either CD19/CD3 bsAb, or medium only. (A) Representative gating strategy shown for a BTKi-naïve patient sample. (B) Percent dead CLL cells (LIVE/DEAD positive) after 3 days in culture with either CD19/CD3 bsAb, HER2/CD3 bsAb, or medium only (No Ab) shown for a BTKi-naïve (BN) and an ibrutinib-treated (Ibr) patient sample. CD4+ and CD8+ T cell counts were quantified after 3 and 5 days by flow cytometry. (C) T cell counts/mL for BTKi-naïve (BN, n = 17), acalabrutinib-treated (n = 11), and ibrutinib-treated (n = 19) samples after culture with CD19/CD3 bsAb (CD19/CD3, red circles) or medium only (NoAb, blue circles). Each symbol represents one patient sample. Wilcoxon matched-pair signed-rank test for comparison of different treatments applied to individual patient samples and Mann-Whitney test for comparison of different patient groups. *, $P <$ .05; **, $P <$ .001; ***, $P <$ .001; ****, $P <$ .0001.

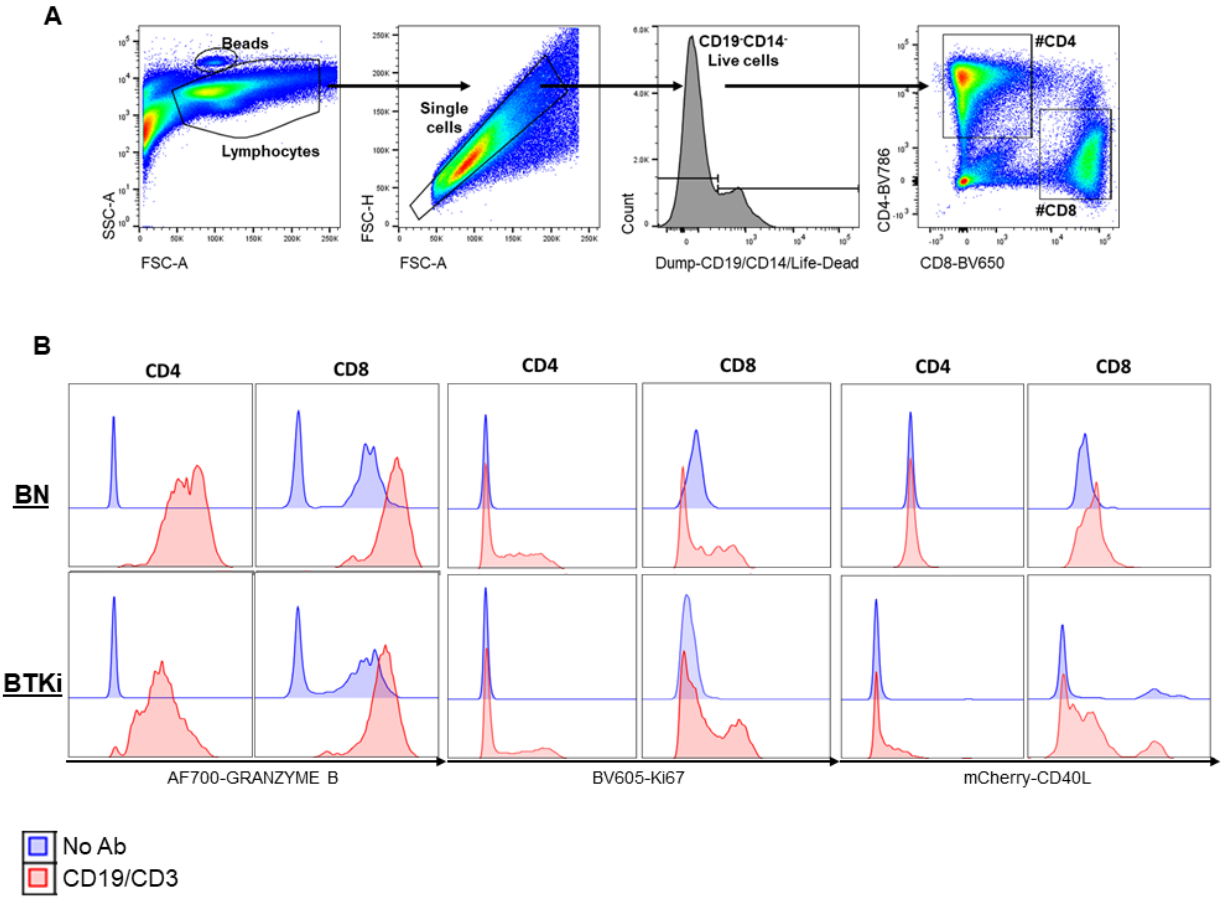


Supplemental Figure S2. Strategy to identify Th1 or Th2 polarization of CD4 T cells. PBMCs from BTKi-naïve (BN, n = 14) and BTKi-treated (BTKi, n = 30) patients were cultured with either CD19/CD3 bsAb, or medium only. (A) Gating on CD4+ T cells, flow cytometry was performed to analyze T cell polarization into Th1, Th2, Th17 T helper subsets based on CCR6 and CXCR3 expression. Representative contour plot layout of a BTKi-naïve (BN) and an ibrutinib-treated (BTKi, Ibr) patient after 3 days of culture with CD19/CD3 bsAb (CD19/3, red) or medium only (No Ab, blue) are shown. Fluorescence minus one (FMO) controls were used to set the gates. (B) Th1 (IFN_{γ}, TNFα, GM-CSF) and Th2 (IL-6, IL-4) cytokine levels were measured by Luminex cytokine assay in cell supernatants harvested after 5 days of exposure to CD19/CD3 bsAb (CD19/CD3, red bars) or medium only (NoAb, blue bars) for BTKi-naïve (BN, n = 7), acalabrutinib-treated (Aca, n = 8), and ibrutinib-treated (Ibr, n = 8) patients. Asterisks indicate statistical significance using Wilcoxon's signed rank test. *, *P*< .05; **, *P*< .01.

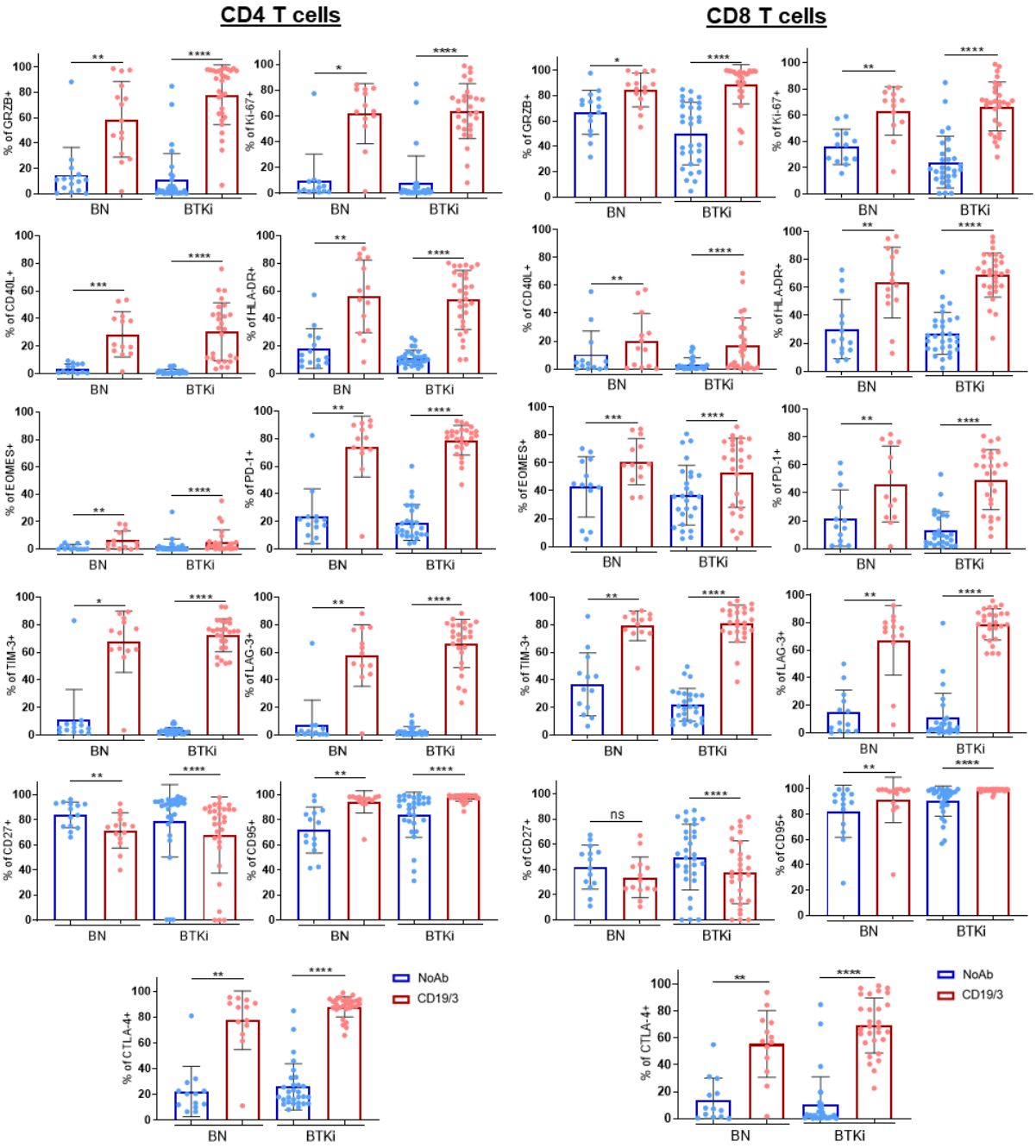


Supplemental Figure S3. T-cell differentiation in response to CD19/CD3 bsAb. PBMCs from BTKi-naïve (BN, n = 14) and BTKi-treated (BTKi, n = 30) patients were cultured with either

CD19/CD3 bsAb, or medium only. Gating on CD4+ or CD8+ subset, flow cytometry was performed to analyze T-cell differentiation, into naïve T cells, central memory T cells (CM), effector memory T cells (EM), and effector T cells, based on CCR7 and CD45RO expression. (A) Representative contour plot layout of a BTKi-naïve (BN) and an ibrutinib-treated (BTKi, Ibr) patient after 3 days of culture with CD19/CD3 bsAb (CD19/3, red) or medium only (No Ab, blue) are shown. Fluorescence minus one (FMO) controls were used to set the gates. (B) The median proportions of naïve (light green), central memory (CM, green), effector memory (EM, blue) and effector cells (light blue) within CD4+ and CD8+ T cells for BTKi-naïve (BN, n = 14), acalabrutinib-treated (Aca, n = 11), and ibrutinib-treated (Ibr, n = 19) samples patients are represented in pie charts. Asterisks indicate statistical significance using Wilcoxon matched-pair signed-rank test for comparison of different treatments applied to individual patient samples. *, $P < .05$; **, $P < .01$; ***, $P < .001$.

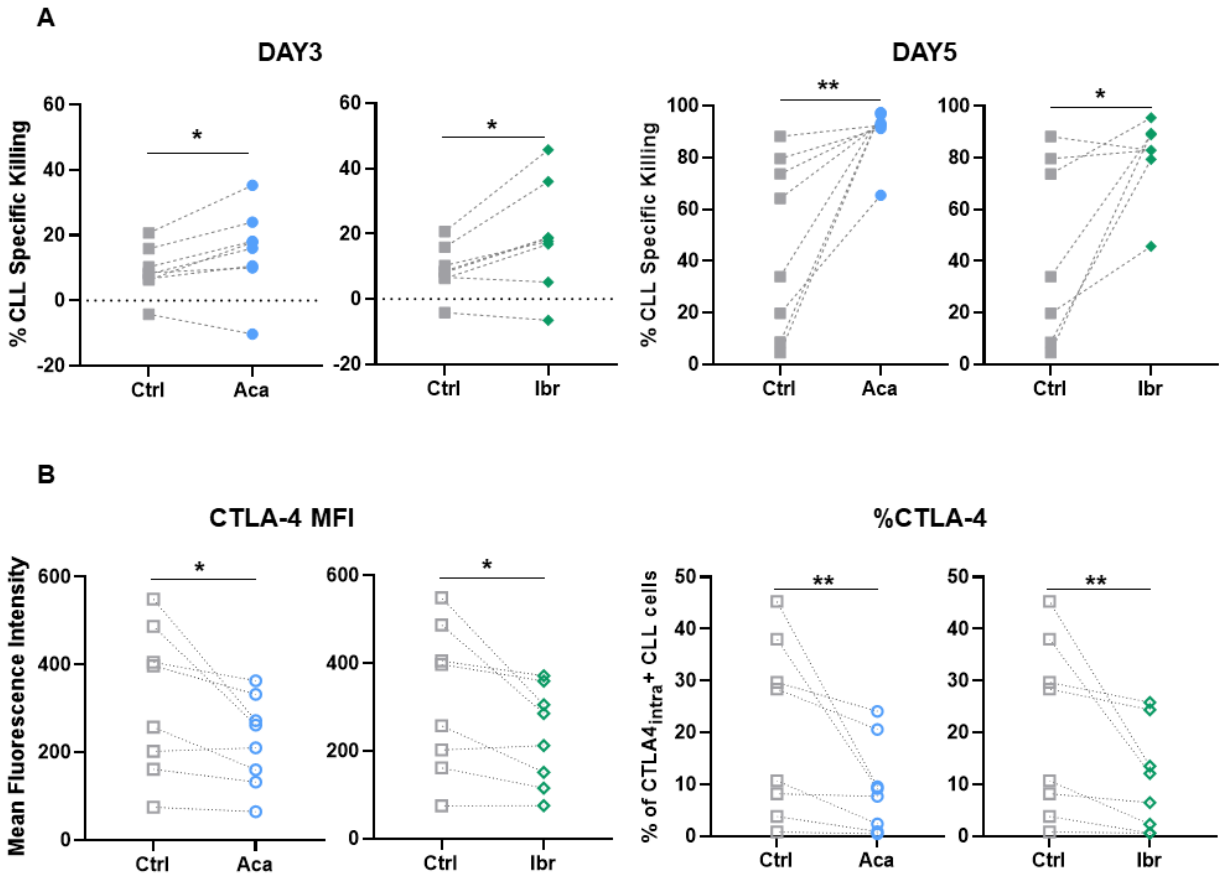


Supplemental Figure S4. Increased expression of T-cell indicated activation markers in response to CD19/CD3 bsAb. PBMCs from BTKi-naïve (BN, n = 13) and BTKi-treated (BTKi, n = 26) patients were cultured with either CD19/CD3 bsAb, or medium only. To analyze T-cell activation, activation factors and inhibitory checkpoint expression was assessed in CD4+ or CD8+ subsets by flow cytometry after 3 days of culture. (A) Representative gating strategy shown for a BTKi-treated patient sample. (B) Representative histograms overlays shown for BTKi-naïve (BN) and BTKi-treated (BTKi) patient samples of Granzyme B, Ki67 and CD40L mean fluorescence intensity on CD4+ or CD8+ T cells.



Supplemental Figure S5. % of T-cells expressing indicated activation markers in response to CD19/CD3 bsAb. PBMCs from BTKi-naïve (BN, n = 13) and BTKi-treated (BTKi, n = 26) patients were cultured with either CD19/CD3 bsAb, or medium only. Comparison of CD4+ and CD8+ T-cell frequencies staining positive for EOMES, PD-1, TIM-3, LAG-3, CD40L, HLA-DR, CD27, CD95, CTLA-4. Ki-67, and granzyme B (GRZB) between patient samples treated with CD19/CD3 bsAb (red bars) and samples with medium control (NoAb, blue bars). 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; ***, *P*< .001; ****, *P*< .0001.



Supplemental Figure S6. *In vitro* treatment with BTKis increases cytotoxicity of CD19/CD3 bsAb and downregulates CTLA-4 in CLL cells. (A) Purified CLL cells obtained from BTKi-naïve patients (BN, n = 8) were incubated with 1 µM acalabrutinib (Aca, blue circles), 1 µM ibrutinib (Ibr, green diamonds), or left untreated (Ctrl, grey squares) for 24 hours prior to addition of CD19/CD3 bsAb and healthy donor T cells at 1:10 E:T ratio. Depicted is the specific killing of CLL cells after 3 and 5 days of culture. Each dot represents one patient, lines connect samples obtained from the same patient. (B) Expression of intracellular CTLA-4 was measured by flow cytometry in CLL cells after 24 hours of culture with 1 µM acalabrutinib (Aca, blue circles), 1 µM ibrutinib (Ibr, green diamonds), or left untreated (Ctrl, grey squares). Mean Fluorescence Intensity (MFI) of the entire CLL population, and CLL-cell frequencies staining positive for CTLA-4 are shown for each patient sample. Each dot represents one patient, lines connect samples obtained from the same patient. Statistical significance by Wilcoxon matched-pair signed-rank test. *, P < .05; **, P< .01.

Patient ID	Sample ID	Treatment	Age	Sex	Time on T_x (mo)	Rai stage	T_xN/RR	Cytogenetics	IGHV Status	ALC x10e3/µL	E:T	Figure
CLL-01	6125	PRE (ibrutinib)	70	F	0	4	RR	del13q	U	77.67	0.03	5,6
CLL-01	6593	ibrutinib	71	F	5.5	4	RR	del13q	U	76.45	0.02	5,6
CLL-02	6353	PRE (ibrutinib)	76	F	0	2	RR	del11q, +12	U	40.42	0.05	1,4
CLL-02	7201	ibrutinib	77	F	11.4	2	RR	del11q, +12	U	23.08	0.04	1,2,3,4
CLL-03	6811	PRE (ibrutinib)	70	M	0	4	TxN	del17p, del13q	M	100.91	0.03	4,5
CLL-03	7791	ibrutinib	71	M	5.5	4	TxN	del17p, del13q	M	65.77	0.03	4,5
CLL-04	6977	ibrutinib	68	M	11.5	3	RR	del11q	U	3.26	1.15	1,2,3
CLL-05	6988	ibrutinib	69	F	11.5	2	RR	del11q, del13q	U	11.96	0.11	1,2,3
CLL-06	6990	ibrutinib	67	F	11.8	1	RR	del13q	M	11.15	0.15	1,2,3
CLL-07	7314	ibrutinib	63	M	12.1	4	RR	del13q, del11q, del17p	M	7.77	0.35	1,2,3
CLL-08	7365	ibrutinib	70	F	11.7	2	TxN	del17p	U	14.17	0.15	1,2,3
CLL-09	7431	PRE (ibrutinib)	59	M	0	4	TxN	del17p, del13q	M	61.72	0.04	4,5
CLL-09	8097	ibrutinib	60	M	12	4	TxN	del17p, del 13q	M	63.08	0.06	1,2,4,5
CLL-10	7435	ibrutinib	73	M	11.5	1	TxN	del13q	M	26.38	0.07	1,2,3
CLL-11	7562	PRE (ibrutinib)	66	F	0	4	RR	del11q, del13q	U	155.5	0.02	1,2,3,4
CLL-11	8156	ibrutinib	67	F	11.4	4	RR	del11q, del13q	U	6.71	0.16	1,4
CLL-12	7817	ibrutinib	71	M	11.5	4	TxN	Normal	U	5.03	0.46	1,2,3
CLL-13	7832	ibrutinib	48	M	11.5	1	TxN	del17p	U	11.57	0.42	1,2
CLL-14	7848	ibrutinib	64	M	11.6	4	TxN	del17p	U	62.4	0.27	1,2
CLL-15	7852	ibrutinib	39	M	11.5	4	TxN	del17p	U	29.32	0.26	1,2

CLL-16	8184	ibrutinib	62	F	14.5	1	TxN	del17p	U	10.02	0.14	1,2,3
CLL-17	8313	PRE (acalabrutinib)	53	F	0	3	RR	del13q	M	191.09	0.01	5,6
CLL-17	8330	PRE (acalabrutinib)	53	F	0	3	RR	del13q	M	178.07	0.01	4,5
CLL-17	8943	acalabrutinib	54	F	11.9	3	RR	del13q	M	30.85	0.07	4,5,6
CLL-18	8383	PRE (acalabrutinib)	68	F	0	3	RR	del13q	M	76.51	0.07	5,6
CLL-18	8701	acalabrutinib	68	F	5.5	3	RR	del13q	M	38.3	0.07	5,6
CLL-19	8756	none	48	F	0	2	TxN	del13q	M	50.14	0.06	5
CLL-20	8787	ibrutinib	64	F	13.7	1	TxN	+12, del17p	U	2.82	1.79	1,2,3
CLL-21	9132	PRE (acalabrutinib)	65	M	0	4	RR	del13q, del11q	U	119.92	0.04	4,5
CLL-21	10116	acalabrutinib	66	M	11.4	4	RR	del11q	U	84.64	0.05	4,5
CLL-22	9141	PRE (ibrutinib)	69	M	0	4	TxN	del13q	U	64.96	0.07	5
CLL-22	9695	ibrutinib	70	M	5.4	4	TxN	del13q	U	2.15	0.33	5
CLL-23	9155	PRE (ibrutinib)	51	F	0	0	TxN	+12	U	223.17	0.03	5
CLL-23	9763	ibrutinib	52	F	5.5	0	TxN	+12	U	1.19	0.44	5
CLL-24	9253	PRE (ibrutinib)	73	M	0	3	TxN	del13q	M	75.51	0.04	5
CLL-24	9788	ibrutinib	73	M	5.5	3	TxN	del13q	M	0.99	0.32	5
CLL-25	9450	PRE (acalabrutinib)	68	F	0	3	TxN	Normal	U	225.83	0.01	4,5
CLL-25	9939	acalabrutinib	68	F	5.2	3	TxN	Normal	U	36.62	0.03	4,5
CLL-26	9746	PRE (acalabrutinib)	64	M	0	3	TxN	del13q	U	79.89	0.02	4
CLL-26	10518	acalabrutinib	65	M	11.6	3	TxN	del13q	U	17.59	0.08	1,2,3,4
CLL-27	9802	acalabrutinib	61	M	11.9	1	RR	del11q	U	9.61	0.17	1,2,3
CLL-28	10079	acalabrutinib	69	F	11.9	1	RR	del13q	U	4.45	1.83	1,2,3
CLL-29	10218	acalabrutinib	57	M	11.4	1	RR	del13q	U	11.52	0.15	1,2,3
CLL-30	10259	acalabrutinib	74	M	11.2	2	RR	+12	M	9.95	0.10	1,2

CLL-31	10381	PRE (acalabrutinib)	53	M	0	4	RR	Normal	U	74.51	0.02	4,5,6
CLL-31	11707	acalabrutinib	54	M	14.9	4	RR	Normal	U	14.68	0.03	4,5,6
CLL-32	10430	PRE (ibrutinib)	67	M	0	4	TxN	del13q, del11q	M	61.4	0.03	1,2,3,4
CLL-32	12214	ibrutinib	69	M	11.9	4	TxN	del13q, del11q	M	8.99	0.03	1,2,3,4
CLL-33	10434	acalabrutinib	65	M	11.4	2	TxN	del17p	U	6.52	0.23	1,2,3
CLL-34	10515	none	66	F	0	1	TxN	del13q	M	123.11	0.03	1,2,3,6
CLL-35	10784	acalabrutinib	73	M	11.4	1	RR	del13q, del11q	U	19.77	0.12	1,2,3
CLL-36	10831	none	59	M	0	0	TxN	Normal	M	12.93	0.17	1,2,3
CLL-37	11005	PRE (ibrutinib)	71	F	0	4	TxN	del13q, del17p	U	4.9	0.14	1,2,3,4
CLL-37	12123	ibrutinib	72	F	12.6	4	TxN	del13q, del17p	U	6.14	0.08	1,2,3,4
CLL-38	11120	none	75	F	0	0	TxN	Normal	M	262.4	0.03	1,3,6
CLL-38	11454	PRE (ibrutinib)	76	F	0	0	TxN	Normal	M	253.8	0.05	5
CLL-38	12028	ibrutinib	76	F	5.5	0	TxN	Normal	M	28.38	0.06	5
CLL-39	11126	none	72	F	0	1	TxN	Normal	U	11.07	0.24	1,2,3
CLL-40	11161	none	70	F	0	1	TxN	Normal	M	8.44	0.20	1,2,3,6
CLL-41	11228	none	73	M	0	1	TxN	del13q	M	4.52	0.51	1,2,3
CLL-42	11229	none	59	M	0	0	TxN	del13q	ND	10.7	0.18	1,2,3,6
CLL-43	11278	PRE (ibrutinib)	55	F	0	1	RR	normal	U	39.26	0.03	5,6
CLL-43	12025	ibrutinib	56	F	6.1	1	RR	normal	U	54.39	0.04	5,6
CLL-44	11304	acalabrutinib	61	M	11.5	3	TxN	del13q	M	12.59	0.26	1,2,3
CLL-45	11305	none	56	F	0	1	TxN	del6q	M	26.3	0.18	1,2,6
CLL-46	11332	acalabrutinib	53	M	11.6	1	TxN	Normal	U	8.07	0.69	1,2,3
CLL-47	11508	acalabrutinib	81	M	11.9	1	TxN	del13q, del11q	M	3.17	0.73	1,2,3

CLL-48	11515	none	65	M	0	1	TxN	del13q	ND	36.51	0.14	1,2,6
CLL-49	11543	PRE (ibrutinib)	55	M	0	2	TxN	del17p, del13q	M	131.38	0.03	1,2,3,4,5,6
CLL-49	12301	ibrutinib	56	M	6.8	2	TxN	del13q	M	106.27	0.02	5,6
CLL-49	12984	ibrutinib	56	M	12.8	2	TxN	del17p, del13q	M	30.17	0.08	4,5
CLL-50	11602	acalabrutinib	61	M	11.4	3	TxN	+12	U	12.64	0.17	1,2,3
CLL-51	11610	none	66	M	0	4	TxN	Normal	M	37.57	0.05	1,2
CLL-52	11627	none	60	M	0	1	TxN	Normal	ND	5.51	1.92	1,2,3
CLL-53	11628	none	60	M	0	1	TxN	ND	ND	4.42	0.36	1,2,3
CLL-54	11670	acalabrutinib	69	F	11.2	1	RR	del13q	M	27.82	0.06	1,2,3
CLL-55	11682	none	55	F	0	1	TxN	Normal	U	150.38	0.03	1,2,3
CLL-56	11718	ibrutinib	66	M	12	1	RR	del17p, del18q	U	9.19	0.14	1,2,3
CLL-57	12038	ibrutinib	75	F	11.9	1	TxN	del17p, +12	ND	2.53	7.11	1,2,3
CLL-58	12655	ibrutinib	75	F	11.7	1	RR	Normal	U	3.17	0.78	1,2,3

Supplemental Table S1. BTKi-treatment-naïve and BTKi-treated patient characteristics.

Patients on BTKi were responding to therapy at time of sample collection. TxN, 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.

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
CD25	BUV395/6	Clone 2A3 (RUO)	BD Pharmingen
CD27	PECy7	M-T271 (RUO)	BD Pharmingen
CD95	Pacific Blue/BV421	Clone DX2 (RUO)	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
CD40L (CD154)	mCherry/PE-Dazzle 594	5C3	Biolegend
CD200	APC	325516	R&D Systems
LIVE/DEAD™	AmyCyan/V500	Aqua Dead Cell Stain Kit	Invitrogen
LIVE/DEAD™	Pacific Blue/BV421	Violet Dead Cell Stain Kit	Invitrogen

Supplemental Table S2. Antibodies used in flow cytometry experiments.