Supplemental materials and methods.

Flow cytometry(details) :

For surface staining, cells were stained with various markers (supplemental table S1) at room temperature for 15 minutes, washed with PBS and then re-suspended in PBS containing 1:200 dilution of LIVE/DEAD® Fixable Near-IR stain. Cells were then incubate for 15 minutes under room temperature and were fixed with 1.5% formaldehyde for 20 minutes under room temperature, washed one time and resuspended in FACS buffer (PBS with 5% fetal calf serum) before analysis on flow cytometer.

For intracellular cytokine staining, PBMC were stimulated with PMA (25ng/ml, Sigma-Aldrich) and Ionomycin (500ng/ml, Sigma-Aldrich) in the presence of monensin (2mM; eBioscience) for 5 hours, or as described below under B10/B10 Pro conditions. Cells were then harvested and were stained with surface markers and then LIVE/DEAD® Fixable Near-IR stain (Thermo Fisher Scientific) as described above, with the exception that monensin was added to all the staining buffers. Cells were then fixed with 1.5% formaldehyde for 20 minutes under room temperature, and were then washed twice with permeablization buffer (FACS buffer containing 0.25% Saponin, from Sigma-Aldrich), stained with appropriate cytokine antibodies(supplemental table 1), washed again with permeablization buffer, and were then analyzed by flow cytometer.

For intracellular/intranuclear staining of Foxp3 and CTLA4, cells were first stained with surface maker and then labeled with LIVE/DEAD® Fixable Near-IR stain (Thermo Fisher Scientific) as described above. Cells were then fixed/permeablized using the Foxp3 / Transcription Factor Staining Buffer Set (eBioscience) according to manufacturer's protocol and were stained with Foxp3 and Foxp3 antibodies (supplemental table S1).

All the samples were analyzed using Beckman Coulter Gallios™ Flow Cytometer, which can detect up to 10 different fluorochrome conjugated antibodies simultaneously.

Analysis of IL-10 production by CLL cells.

PBMC cells were resuspended (2×10^6 cells/mL) in in Iscove's Modified Dulbecco's Media (IMDM) containing 10% fetal bovine serum (FBS), 200 µg/mL penicillin, 200 U/mL streptomycin, and 4mM L-glutamine (all from Gibco™ Thermo Fisher) and stimulated with CpG (ODN 2006, 10 µg/mL; Invivogen), CD40L (1 µg/mL; R&D Systems), PMA (50 ng/mL; Sigma-Aldrich), Ionomycin (1 µg/mL; Sigma-Aldrich), monensin (2mM; eBioscience), as indicated in 48-well flat-bottom plates before staining and flow cytometry analysis. For "B10" condition, cells were stimulated with CpG, PMA and Ionomycin in the presence of monensin for 5 hours. For "B10 Pro" condition, cells were stimulated with CpG/CD40L for 48 hours, with PMA/Ionomycin/ monensin added for the last 5 hours.

After stimulation, cells were stained for surface markers, including CD19, CD5, CD3, CD4, and CD8. PECF-594 labeled CD14, CD11b, CD16, CD56 and CD123 were added as a "dump channel" to gate out corresponding cell types (supplemental table S1). After surface staining, cells were labeled with LIVE/DEAD® Fixable Dead Cell Stains from ThermoFisher before being fixed with 1.5% Formaldehyde. Fixed cells were then permeablized with FACS buffer containing 0.25% Saponin and were stained with IL-10 antibody (supplemental table S1).

Activation induced cell death in human T cells. :

T cells were isolated from healthy human donors using EasySep™ Human T Cell Isolation Kit. Isolated T cells were stimulated in vitro with plate bound CD3/CD28 for 3 days. Cells were then rested in complete medium containing 50IU/ml IL-2 for additional 7-11 days before they were treated with vehicle, Ibrutinib or acalabrutinib for 30 minutes. Cells were then plated on to 48 well

plates coated with CD3; incubate for 6 hours (for flow cytometry based apoptosis assay) or 3 hours (to isolate mRNA for qPCR to quantify FAS-L expression.) in the presence of IL2 to induce AICD.

For AICD analysis, cells were stained with annexin-V fitc and Propidium Iodide (PI) using the BD biosciences 10X staining buffer according to the manufacturer's protocol before being analyzed on flow cytometer.

For FAS Ligand mRNA quantification, mRNA were extracted from T cells after 3 hours of re-stimulation using QIAGEN "RNeasy Mini"RNA Isolation Kit. mRNA was then reverse transcribed to cDNA using the M-MLV Reverse Transcriptase from Thermo Fisher. Quantitative PCR for FAS-L were performed using the Taqman probe/primer mix (FAM labeled) from Thermo Fisher using GAPDH as internal control.

Activation induced cell death in human NK cells

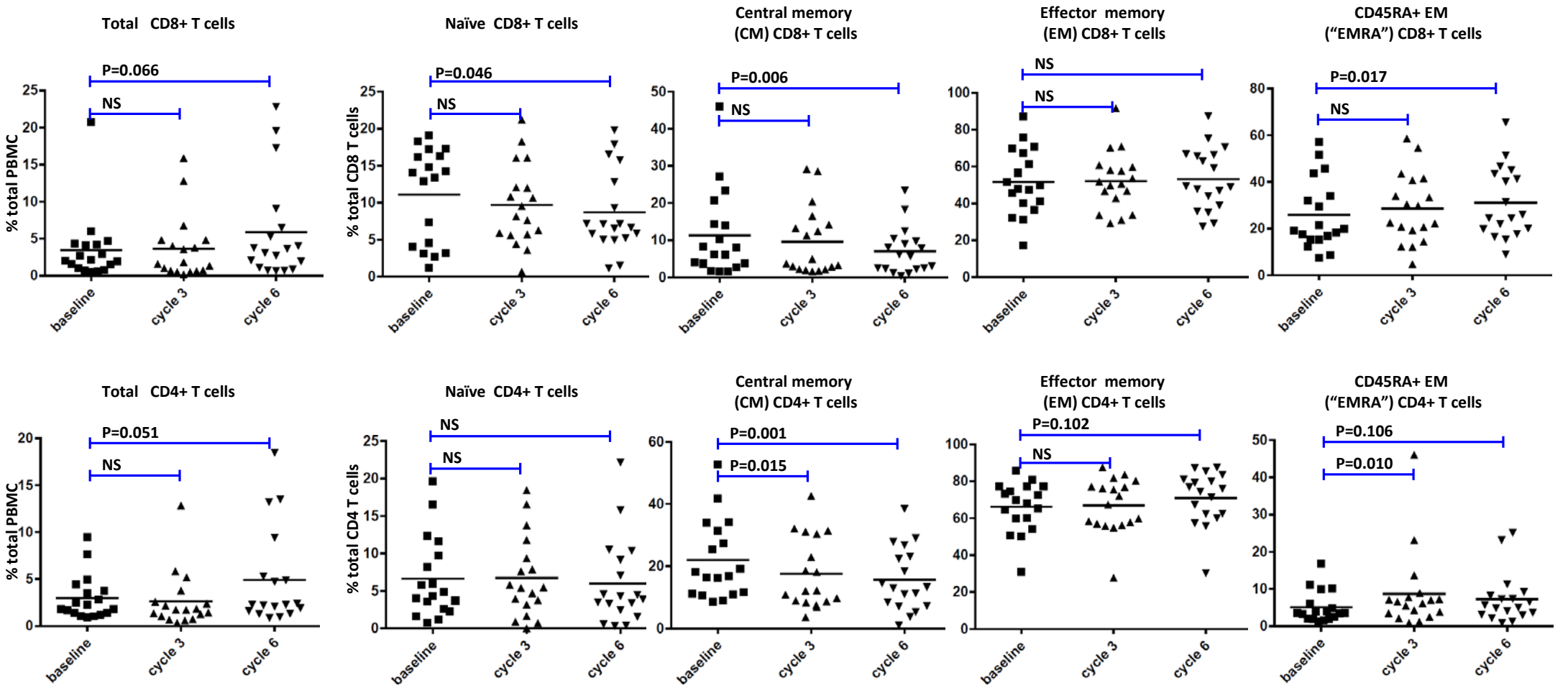
Human $CD56^{+}/CD3^{-}/14^{-}/20^{-}$ NK cells were isolated from peripheral blood leuko-Paks from normal donors (American Red Cross) by incubation with an NK cell RosetteSep negative enrichment cocktail (Stem Cell Technology), followed by Ficoll-Hypaque density gradient centrifugation as previously described(96). NK cells were then sorted to greater than 99% purity with a FACSAria II cell sorter (BD Biosciences). Purified NK cells were plated at $5x10^{4}$ cells/well in a 96-well round bottom plate and cultured for three days at 37°C. Medium consisted of RPMI 1640 supplemented with 10% fetal bovine serum (FBS), and 1% antibiotic/antimycotic (Life Technologies). The cytokines IL-2 (Peprotech) and IL-15 (National Cancer Institute) were supplemented as indicated for a final concentration of 10ng/mL. IL-12 (Miltenyi Biotec) was added where indicated at a concentration of 10ng/mL to induce activation induced cell death.

Cell viability and apoptosis were assessed after three days in culture by annexin V (BD Biosciences) apoptotic and TO-PRO-3 (Molecular Probes) viability flow cytometric analysis(97). NK cells were harvested and stained with annexin V per manufacturer's instructions (BD

Biosciences). TO-PRO-3 was added immediately prior to acquisition, and all samples were analyzed with a LSRII cytometer (BD Biosciences) within one hour of annexin V staining. Analysis of dual staining of annexin V and TO-PRO-3 was analyzed using FlowJo (TreeStar).

Figure S1

A. Patients treated with Ibrutinib



B. Patients treated with acalabrutinib

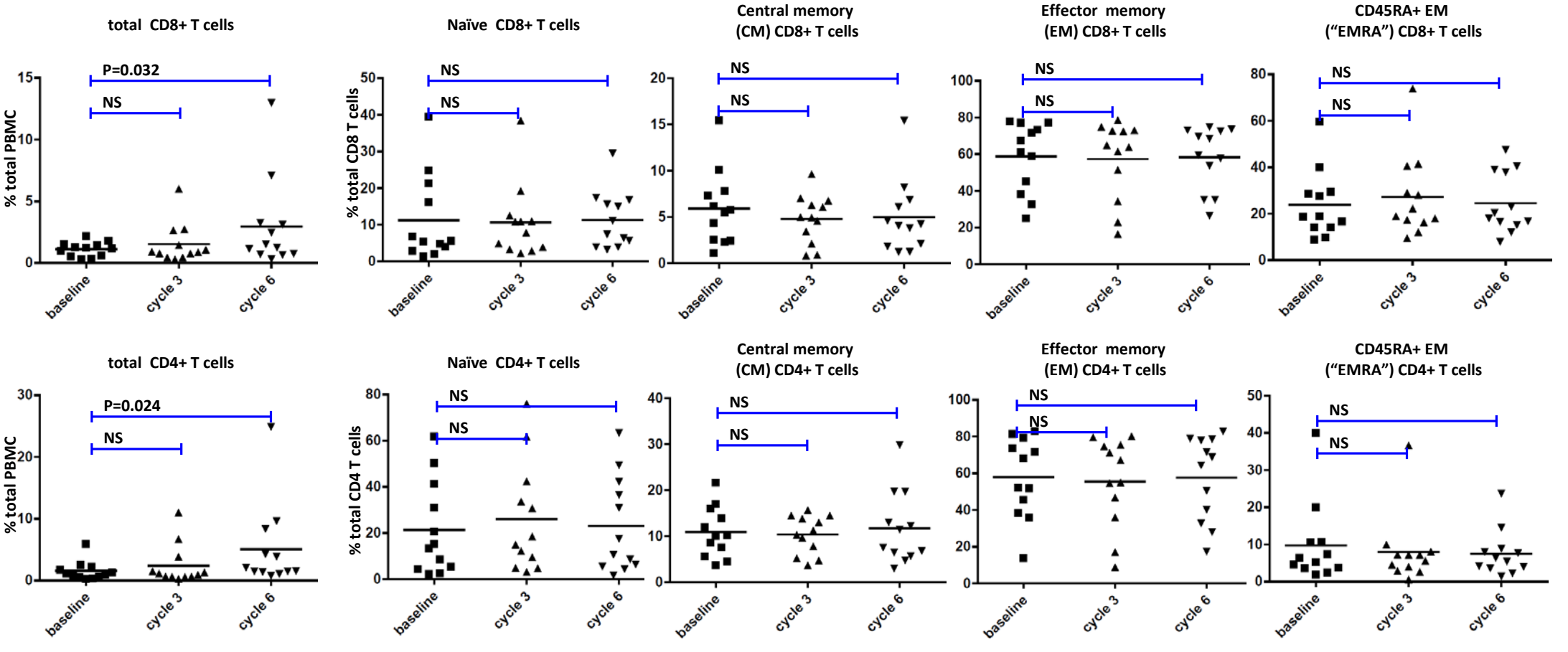


Figure S1: The effect of ibrutinib or acalabrutinib treatment on the frequency of different subsets of peripheral T cells. **(A)**. Percentage of different T cell subsets among total CD8 T cells(upper panel) and CD4 T cells (lower panel) before and after ibrutinib Treatment (n=18). **(B)**. Percentage of different T cell subsets among total CD8 T cells(upper panel) and CD4 T cells (lower panel) before and after acalabrutinib treatment (n=12). T cells are differentiated into subsets based on their expression of CCR7 and CD45RA: naïve T cells (CCR7+CD45RA+), central memory T cells (CCR7+CD45RA-), effector memory T cells (CCR7-CD45RA-), and most differentiated effector memory T cells (T-EMRA, CCR7-CD45RA+).

Figure S2

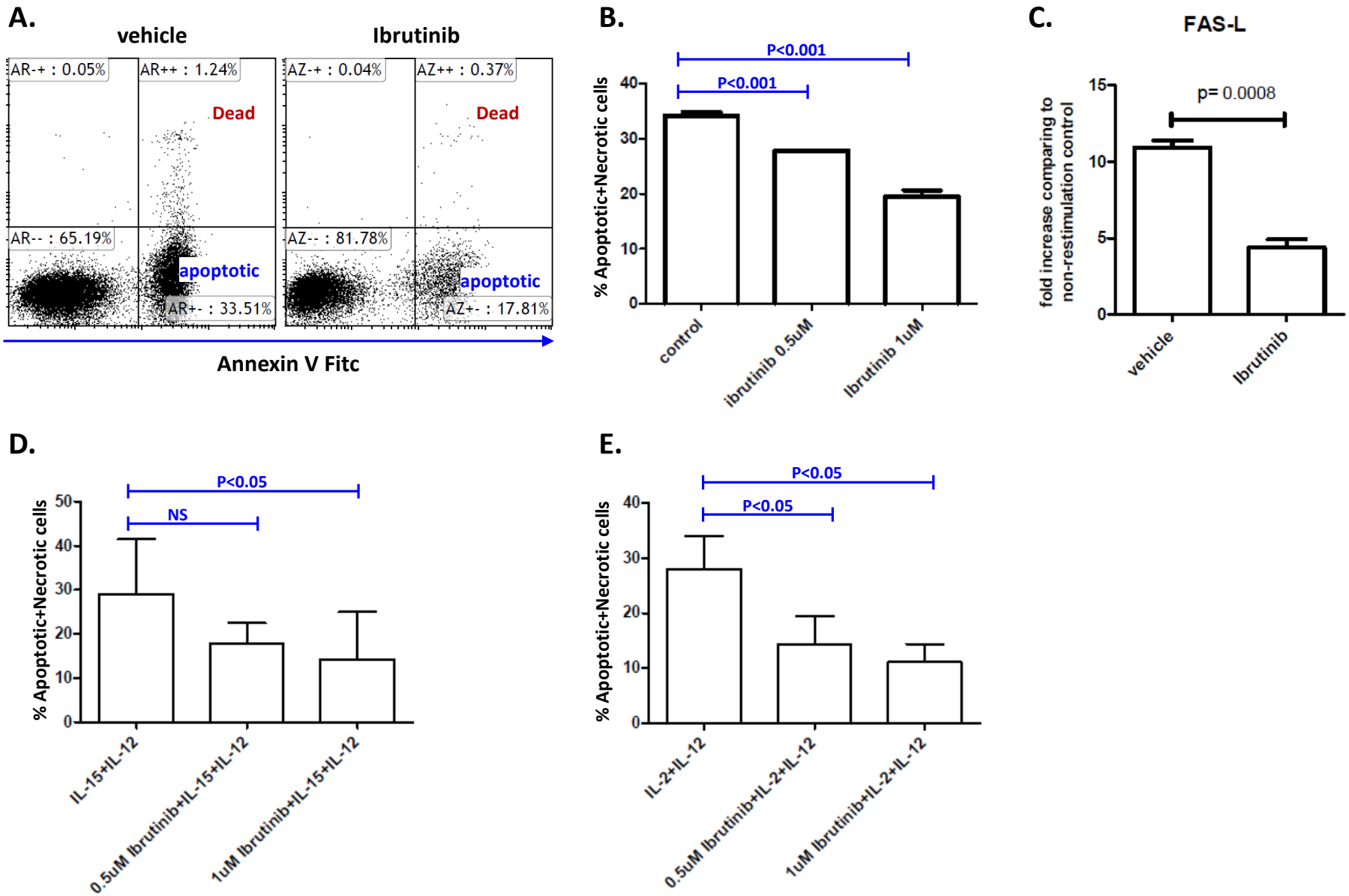
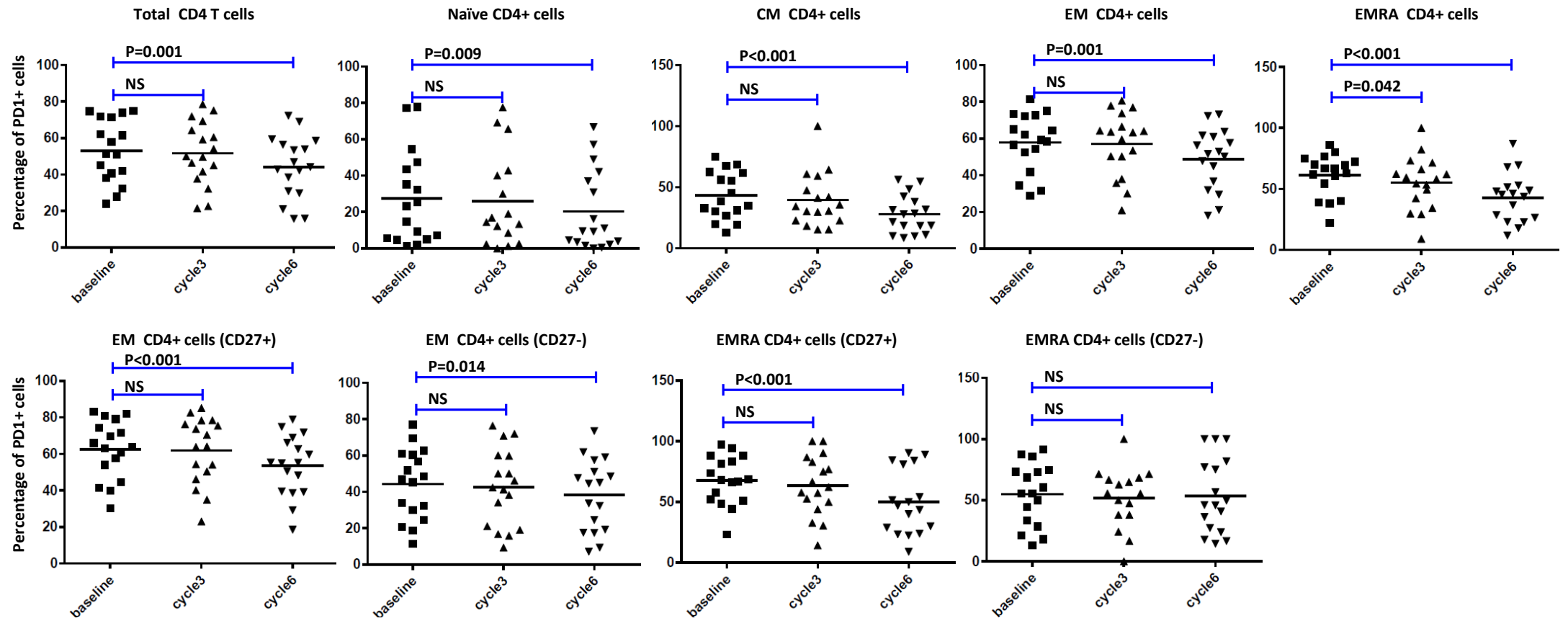


Figure S2: Ibrutinib treatment of human T cells or NK cells protects against activation induced cell death in a dose dependent manor. **(A)- (C),** T cells were isolated from healthy human donors blood samples, stimulated in vitro with CD3/CD28 for 3 days, rested in culture medium containing 50 IU IL-2 for 11 days, then were restimulated with plate bound CD3 for 6 hours (as in A. and B.) or 3 hours (as in C.) in the presence of IL2 to induce activation induced cell death in the presence of absence of ibrutinib. Each indicated condition. **(A)**. representative FACS dot plots of Annexin V & PI staining. **(B)**. Bar graphs that show the percentage of non-viable was done in triplicate. Data shown are representative of three independent experiments (apoptotic + necrotic, as defined by Annexin V positive and PI positive cells) cells after induction of AICD. **(C)**. FAS-L mRNA upregulation in activated T cells upon induction of AICD was impaired by ibrutinib treatment. mRNA was isolated from the T cells after induction of AICD, cDNA was synthesized and qPCR for FAS-L and GAPDH was done. Figure A, B and C represent 3 independent experiments. **(D) & (E),** Human $CD56^{+}/CD3^{-}/14^{-}/20^{-}$ NK cells were isolated from peripheral blood from normal donors (N=3) by negative enrichment, and were then sorted to greater than 99% purity by FACSAria II sorter. Purified NK cells were plated at $5x10^{4}$ cells/well and were cultured for three days. IL-15 **(D)** and IL-2 **(E)** were added as indicated for a final concentration of 10ng/mL. IL-12 (Miltenyi Biotec) was added where indicated at a concentration of 10ng/mL to induce activation induced cell death. Bar graphs that show the percentage of non-viable (apoptotic + necrotic, as defined by Annexin V positive and TO-PRO-3 positive cells) cells after induction of AICD. (N=3)

Figure S3

A. Patients treated with Ibrutinib



B. Patients treated with acalabrutinib

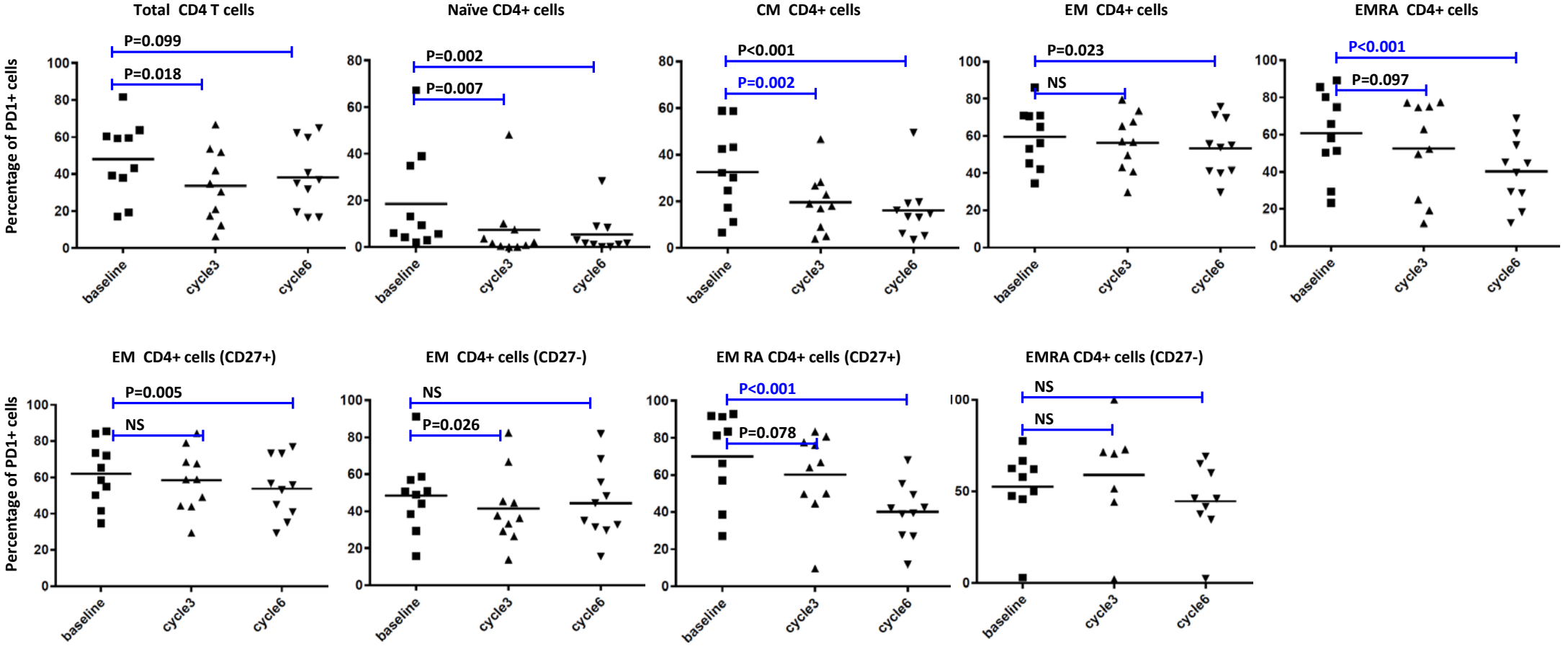
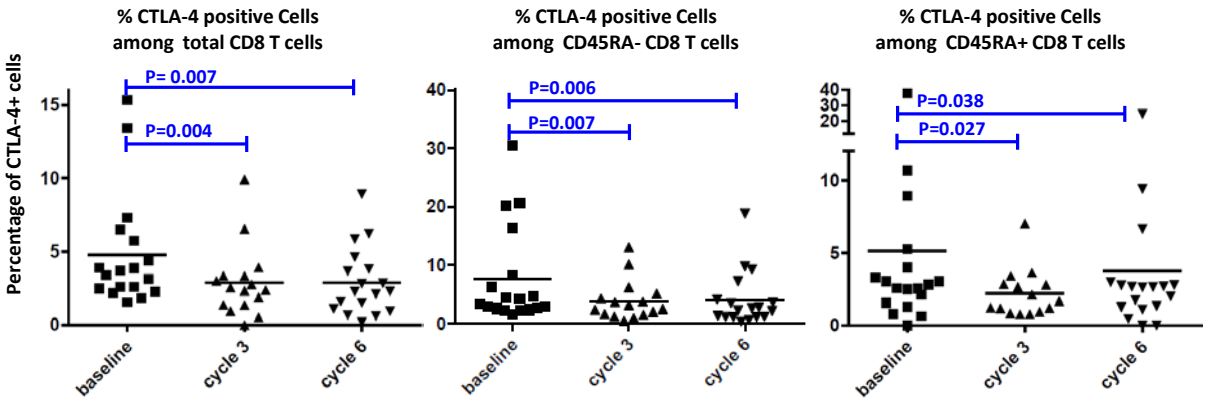


Figure S3: Treatment with ibrutinib, as well as with acalabrutinib, leads to a significant reduction in the frequency of PD-1 positive cells in CD4 T cell populations. **(A)**. Percentage of PD1 positive cells among different subsets of CD4 T cells from CLL patients before and after ibrutinib treatment. (n=17) **(B)**. Percentage of PD1 positive cells among different subsets of CD4 T cells from CLL patients before and after acalabrutinib treatment. (n=10)

Figure S4.

A. Patients treated with Ibrutinib



B. Patients treated with acalabrutinib

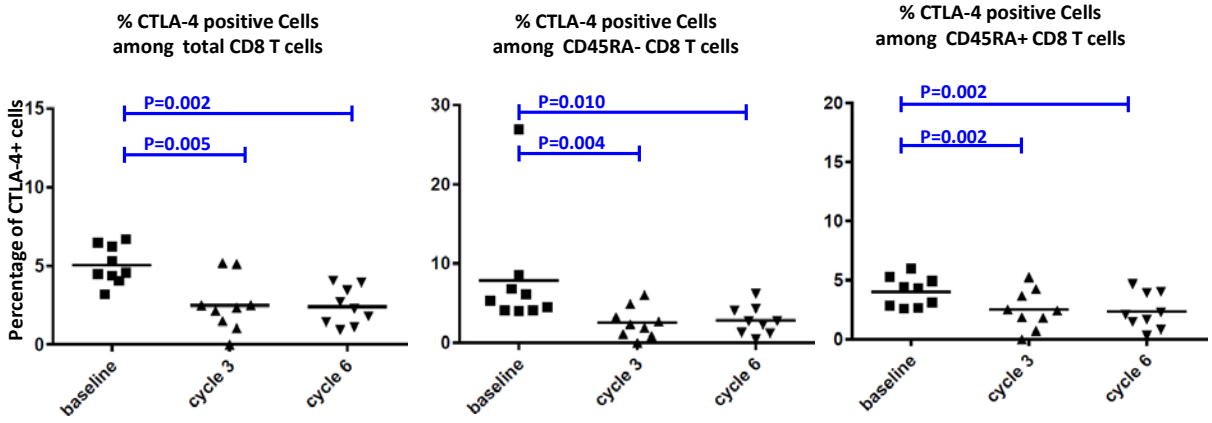


Figure S4: Treatment with ibrutinib, as well as with acalabrutinib, leads to a significant reduction in the frequency of intracellular CTLA4 positive cells in CD8 T cell populations. **(A)**. Percentage of CTLA4 (intracellular) positive cells among total CD8 T cells, CD45RA- CD8 T cells and CD45RA+ CD8 T cells from CLL patients before and after ibrutinib treatment.(n=18). **(B)**.Percentage of CTLA4 (intracellular) positive cells among total CD8 T cells, CD45RA- CD8 T cells and CD45RA+ CD8 T cells from CLL patients before and after acalabrutinib treatment.(n=9).

Figure S5

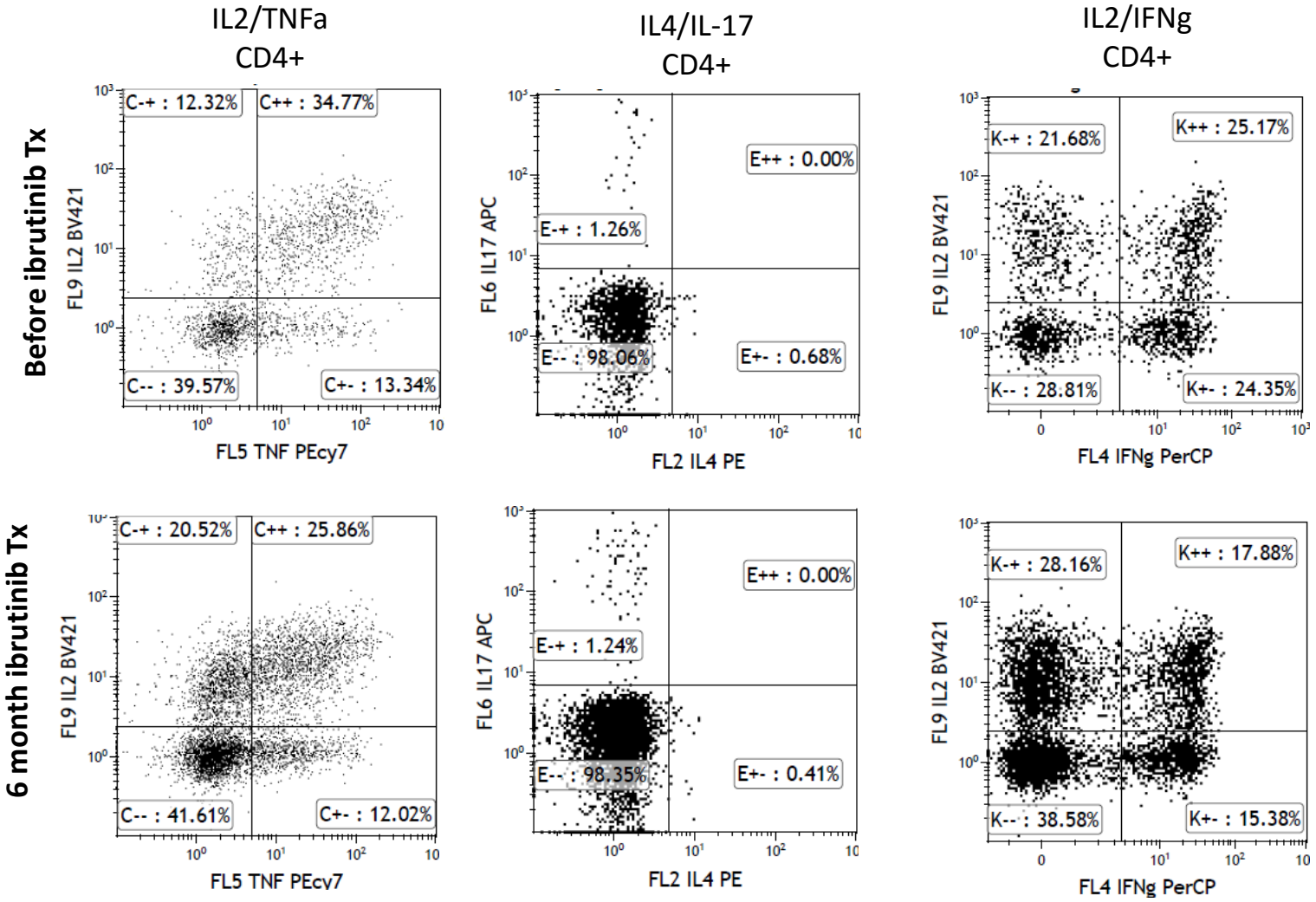


Figure S5: Representative FACS plot of intracellular cytokine staining(n=15). PBMC from CLL patient before and after ibrutinib treatment were stimulated with in vitro with PMA/Ionomycin in the presence of Monensin for 5 hours. Cytokine production (IFNγ, IL4, IL17, TNFα and IL2) were detected by intracellular cytokine staining.

Figure S6

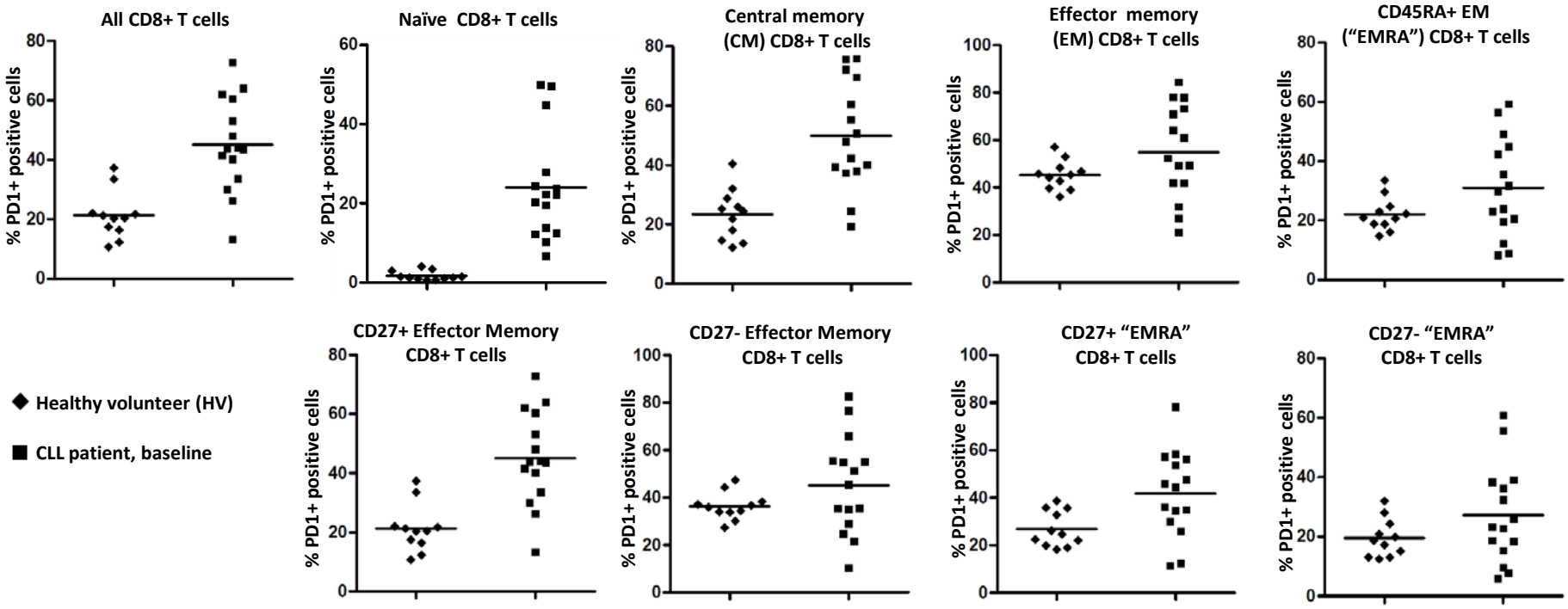


Figure S6: PD-1 expression is increased in all of the T cell subsets in CLL patients comparing to healthy donors. The increase is most prominent in naïve and central memory T cell compartment. Frequencies of PD-1 positive cells among different CD8 T cell subsets were shown. n=11 for healthy donor, n=15 for CLL patients.

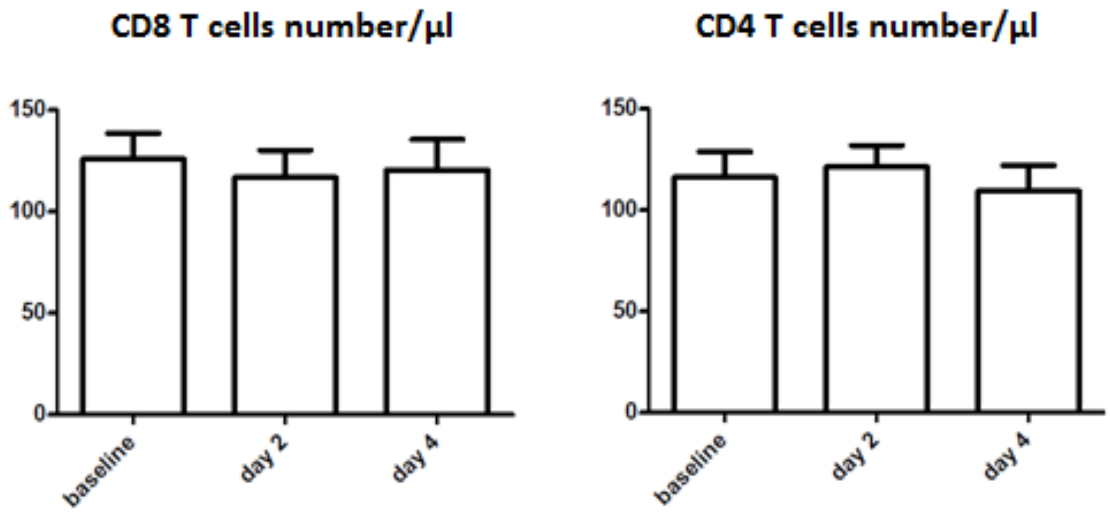


Figure S7: Short term Ibrutinib treatment (2 or 4 days) does not increase circulating T cell numbers. Wild type B6 mice were engrafted with CLL cells (splenocytes from leukemic Eμ-TCL1 transgenic mice). seven weeks post leukemia engraftment, the mice were treated with ibrutinib and the circulating T cell numbers in peripheral blood were monitored before starting ibrutinib, 2 days and 4 days post starting ibrutinib(corresponding to the time when CLL cells numbers were transiently increased in peripheral blood in this model(1)). N=14.

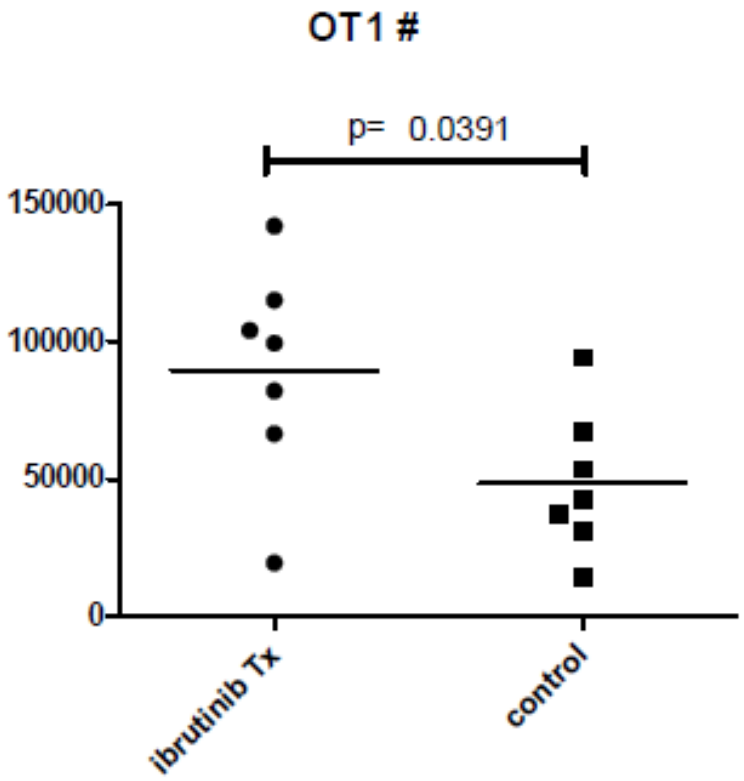


Figure S8: Ibrutinib treatment increases the number of activated leukemia specific T cells. Mice were engrafted with AML cell line (C1498) expressing OVA (a model antigen). OT-1 transgenic T cells (recognize OVA) were then adoptively transferred into AML engrafted mice. The mice were treated with Ibrutinib versus vehicle. Mice were sacrificed at day 6, spleens were harvested, the frequency and number of leukemia specific OT-1 T cells were counted and plotted. N=7 for each group.

Figure S9

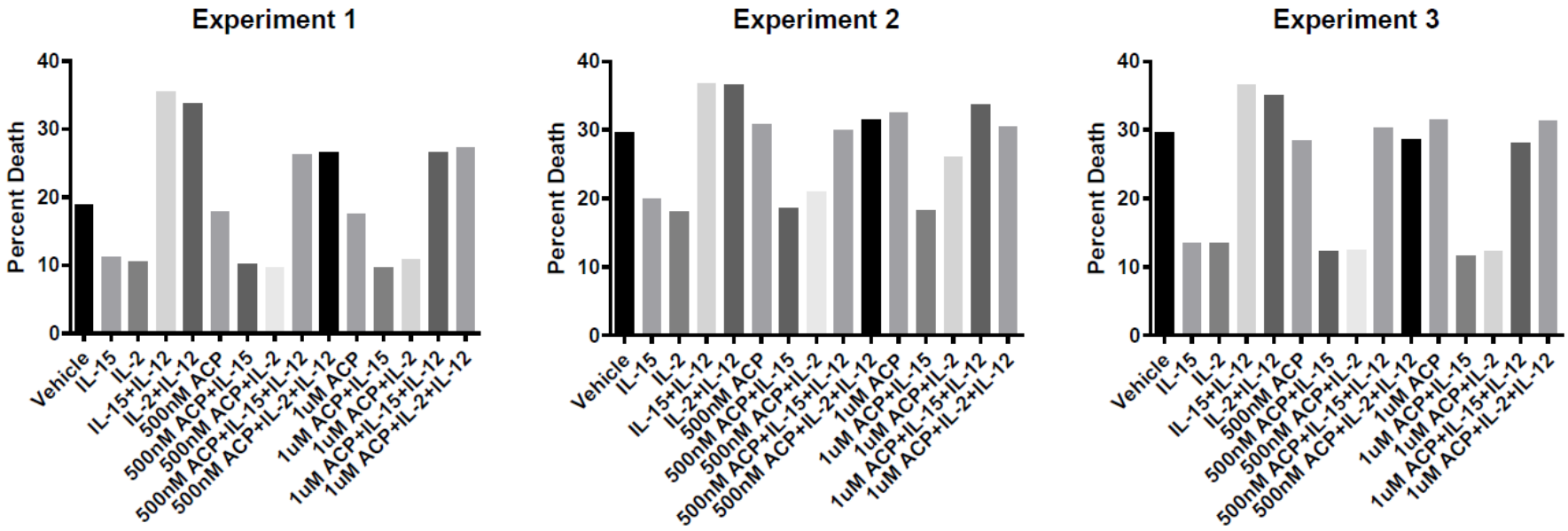


Figure S9: Human CD56+/CD3-/14-/20- NK cells were isolated from peripheral blood from normal donors by negative enrichment, and were then sorted to greater than 99% purity by FACSAria II sorter. Purified NK cells were plated at 5x104 cells/well and were cultured for three days. IL-15 and IL-2 were added as indicated for a final concentration of 10ng/mL. IL-12 (Miltenyi Biotec) was added where indicated at a concentration of 10ng/mL to induce activation induced cell death. Vehicle control versus acalabrutinib 500nM , acalabrutinib 1000nM were added to rescue cytokine induced NK cell deah.. Bar graphs that show the percentage of non-viable (apoptotic + necrotic, as defined by Annexin V positive and TO-PRO-3 positive cells) cells after induction of AICD. ACP: acalabrutinib (ACP-196)

Table S1: IC50 values for inhibition of enzymatic activity by ibrutinib versus acalabrutinib.

Kinase	IC50 (nM) of acalabrutinib	IC50 (nM) of ibrutinib
BTK	5.1 ± 1.0	1.5 ± 0.2
BMX	46 ± 12	0.8 ± 0.1
ITK	>1000	4.9 ± 1.2
TEC	93 ± 35	7 ± 2.5
TXK	368 ± 141	2.0 ± 0.3
EGFR	>1000	5.3 ± 1.3
ERBB2	~ 1000	6.4 ± 1.8
ERBB4	16 ± 5	3.4 ± 1.3
JAK3	>1000	32 ± 15
BLK	>1000	0.1 ± 0.0
FGR	>1000	3.3 ± 1.1
FYN	>1000	29 ± 0
HCK	>1000	29 ± 0
LCK	>1000	6.3 ± 1.3
LYN	>1000	20 ± 1
SRC	>1000	19 ± 1
YES1	>1000	4.1 ± 0.2
CSK	86	2.25
BRK	79	3.34
FLT3	100	72.9

Table S2: detailed patient information

						Cygoegenetics/FISH results															
Exp	gender	time of treatment	year of diagnosis	Rai stage at the time of treatment	line of prior Tx	Del 13	Del 11q	Del 17q	Trisomy 12	6q21	2p12	14q32	8q24	complex karyotype ?	IgVH mutation status (%)	% bone marrow involvement	baseline ALC	cycle 3 ALC	Cycle 6 ALC	age at treatment	age at diagnosis
1	F	10/11/2013	2009	3	Kipps Regimen x1 cycle March 2013	positive		positive						no	7.6	>90	40.85	81.87	73.56	71	66
2	M	7/9/2013	2012	4	BR x 6, 7/2012 - 12/2012 5/16/13: Ofatumumab + Dinaciclib on protocol 11120. . Aa by 2015: pancytopenia, platelet transfusion dependent.	81	83.7							no	0.4	90	15.71	76.60	47.63	62	61
3	M	1/18/2014	2006	2	FCR X6 finished by 8/2009.	93.4	91.5			84.8		88.6		yes	0	70	90.21	153.45	15.25	51	44
4	F	4/1/2014	2007	4	FR X8 by 8/2012, Rituxan + steroid for AIHA 2014.	no	87	87	no	no	no		73.2	yes	0.3	80-90	22.52	24.53	10.43	79	72
5	F	10/29/2012	2001	3/4	1st: chlorambucil from May 2005 through June 2006 with rituximab given in spring 2006. She had a partial response, lasted 2 years (progressive lymphocytosis and abdominal pain and GI symptoms with bulky adenopathy in the abdomen) 2nd: chlorambucil in October 2008 given with prednisone in February 2009. No response 3rd: PCR (pentostatin, cyclophosphamide, rituximab) for two cycles in March and April 2009- this was complicated by pneumonia. Treatment free for one year. 4th: rituximab weekly x 4 (March – April 2010), progressive lymphocytosis, bulky abdominal nodes an splenomegaly, six months 5th: CVP for one cycle in October 2010, then R-CVP on 11/17/2010. complicated by jaw pain and constipation – she had decreased adenopathy though. Treatment free for 3 months,, 6th bendamustine + rituximab x 2 cycles February & March 2011 7th: High dose methylprednisone + rituximab June - July 2012 - pretreatment - WBC 208.3, Hgb 8.4, Platelets 70,000, following therapy on 7/23/12, she had WBC 113.7 , hemoglobin 10.5, and platelets 68. She had increasing adenopathy. 8th: Ofatumumab: 8 weekly doses from July to Sep 2012. Last dose was 09/24/12 2012.		85.8							no	0.3	96	29.88	202.70	47.56	60	49
6	F	6/11/2012	2010	4	Ofatumumab 1-3/2012. per 10023	94.3								no	0.3	>90	18.40	50.46	41.96	78	76
7	M	7/24/2012	2001	4	ofatumumab starting 10/2011 and received 8 weeks followed by 1 maintenance dose in February 2012.	53.7								no	3.68	>80	51.78	112.02	126.80	51	40
8	F	7/18/2012	2006	4	March 2011 weekly x 4 rituximab ending 3/30/2012.		95	88.5						yes	0	95	61.85	169.59	86.69	56	50
9	M	10/21/2012	2003	4	2008 - FCR + Campath X6 CR 2/2012 - Rit + Revlamid 3 cycles - oneumonia	26.9	93							no	1.1		9.38	93.79	55.10	66	56
10	M	10/17/2012	1980	4	#1: 1995. chlorambucil, prednisone, and fludarabine . #2: 9 cycles of CHOP which led to reduction in the number of leukemic cells in his bone marrow. #3: allo SCT in 03/1997. remission for about 9 years till 2006, #4: single agent Rituxan for about 2 years, and subsequently with rituxan-bendamustine combination. #5: Revlimid , at first at 10mg every other day. #6: January 2011, briefly treated on study with ofatumumab , ,pancytopenia and sepsis after 1st dose he had a good hematologic response	84.4								no	2.5	90	81.48	99.10	31.09	71	39
11	M	9/26/2012	2009	4	1. 6 cycles of R-CVP, completing in April 2010 in Tampa Florida. He returned to Dayton, OH and had a bone marrow biopsy because of thrombocytopenia in July 2010 which showed persistent CLL involvement of somewhere between 30-50%. He was feeling well with normal counts and continued to be monitored. His WBC count 5/2011 had risen to the 30,000 range and his physician repeated his BM biopsy which showed 80% cellularity with 70% involvement by CLL. 2. 6 cycles of BR from Aug 2011 to Jan 2012 at local facility 3. 4 weekly Rituxan and prednisone in June 2012 due to AIHA	75.4		93.8		80.1				yes	0	70	19.13	59.19	22.62	77	74
12	M	11/5/2012	2002	4	* Radiation: to cervical LAD in 2004 * Rituxan: 4 weekly treatments in 2004 * Rituxan: 4 weekly treatments in 2005 * Chlorambucil: 2006-2008 * Rituxan: on/off 2007-2008 along with Chlorambucil * FCR: 2008-2009 x 6 cycles in CR * Rituxan/solumedrol: 08/2012 * Revlimid: on study in Jacksonville, FL at Mayo clinic 09/07/12-10/01/12	93.5						67.5		no	0	90	29.52	194.49	58.56	67	56
13	M	11/7/2012	2001	4	1. FCR x 6 cycles: 6/2003 through 11/2003; best response = CR 2. R-lenalinomide: 6/2008 through 12/2008; discontinued secondary to severe thrombocytopenia 3. FCR x 4 cycles 2009/2010 4. Kipps regimen (solu-medrol, rituximab) x 2 cycle starting 8/20/2012		96.4			88.2				yes	0	70	17.34	92.82	41.86	66	55
14	M	12/4/2012	1989	4	- fludarabine x 6 in April 1993 - fludarabine x 2 in October 2001 - rituximab weekly x 4 in early 2002 - rituximab weekly in May 2005 - rituximab weekly in January 2007 - rituximab + fludarabine on an abbreviated schedule x 3 in 7/2008 - fludarabine + rituximab +Neulasta x 4 cycles in 2010, - rituximab weekly in October 2012	93.5								yes	6	90	19.62	101.19	65.33	66	43
15	F	2/20/2013	2007	4	FCR done in 2008, maintenance R X 6 months. BR starting 8/2011, only 1 cycle. ofatumumab locally from Jan to July 2012..	95.6	96.6							yes	0.3	99	154.84	280.57	248.83	60	54
16	F	1/28/2013	2004	4	1. FC X 1, FCR x 1 (cytopenias, rituximab infusion reaction) 2. CVP x 6 --> rituximab weekly x 4 3. FCR x 4 (completed 3/2006) 4. BR + CAL101 (5/2011), discontinued secondary to rash 5. Ofatumumab (7-12/2011)									no	0	90	126.52	178.32	47.26	61	53
17	M	3/25/2013	2001	4	• 7/16/2001-12/2001 Fludarabine x 6. Nearly achieved CR. • 5/2004: FCR x 4 for progressive lymphocytosis to prepare for alloSCT - dose reduction of cyclophosphamide and fludarabine secondary to cytopenias. Achieved significant cytoreduction. • 9/2004: RIC flu/bu/tbi alloSCT from matched sibling (brother), Some rash, but unclear if GVHD. 10/05: Bone marrow showed CR	89.6	92					55.6		yes	0.3	85	38.56	55.10	29.10	50	39
18	F	7/2/2013	2002	4	> FCR x 6 cycles in 8/2004	45.9								no	6.1	>90	35.40	96.52	70.29	64	53
19	M	6/25/2013	2001	4	• 1/2005: eight weekly doses of rituximab→ PR. • 10/2006 to 4/2007 with eight treatments two times a month. • fenretinide from July of 2007 to January of 2008 on a clinical trial with four doses of rituximab from 7-8/2007 and then continued on oral fenretinide. • 6 cycles of FCR from March to August 2008									no	0.3	90	20.66	206.78	37.47	62	51

Table S3, list of antibodies used.

Antigen	fluorochrome	vendor	Catalog #	clone
BTLA	PE	Biolegend	344505	MIH26
CCR7	PE Cy7	Biolegend	353225	G043H7
CD11b	PerCP-Eflour710	eBiosciences	46-0118-41	ICRF44
CD11c	PE Efluor 610	eBiosciences	61-0116	3.9
CD122	BV421	BD Biosciences	562887	Mik-β3
CD123	PE Efluor 610	eBiosciences	61-1239-41	6H6
CD14	PE-CF594	BD Biosciences	562334	MφP9
CD16	FITC	eBiosciences	11-0168-41	eBioCB16 (CB16)
CD160	APC	Biolegend	341203	BY55
CD160	PE Cy7	Biolegend	341211	BY55
CD180 MD1	PE	eBiosciences	12-1809-41	MHR73-11
CD19	PerCP-Eflour710	eBiosciences	46-0198-41	SJ25C1
CD19	APC-R700	BD Biosciences	564977	HIB19,
CD19	BB515	BD Biosciences	564456	HIB19
CD2	fitxc	BD Biosciences	347593	S5.2
CD200	APC	eBiosciences	17-9200-41	OX104
CD24	PE-CF594	BD Biosciences	562405	ML5
CD244	PE		329507	C1.7
CD244	PerCP-Cy5.5	biolegend	329515	C1.7
CD25	PE Cy7	eBiosciences	25-0259-41	BC96
CD25	PE cy7	eBiosciences	25-0259-41	BC96
CD26	PE-CF594	BD Biosciences	565158	M-A261
CD26	PerCp cy5.5	biolgend	302715	BA5b
CD27	PE	BD Biosciences	560985	M-T271
CD27	eFluor® 450	eBiosciences	48-0279-41	O323
CD27	BB515	BD Biosciences	564643	M-T271
CD28	PE	eBiosciences	12-0289-41	CD28.2
CD290 TLR10	PE	eBiosciences	12-2909-41	3C10C5
CD3	APC-R700	BD Biosciences	659110	SK7 (Leu-4)
CD31	APC	eBiosciences	17-0319-41	WM59
CD33	APC	eBiosciences	17-0338-41	WM-53
CD38	PE Cy7	eBiosciences	25-0388-41	HB7
CD4	Apc R700	BD Biosciences	564976	RPA-T4
CD4	BB515	BD Biosciences	564420	RPA-T4
CD45RA	PerCP-Cy5.5	BD Biosciences	563429	HI100
CD5	BV510	BD Biosciences	563380	UCHT2

CD57	PE-CF594	BD Biosciences	562488	NK-1
CD62L	BV510	BD Biosciences	563203	DREG-56
CD7	BB515	BD Biosciences	565211	M-T701
CD8	BV510	Biolegend	344731	SK1
CD8	PECF594	BD Biosciences	562311	RPA-T8
CD8	PE-Cy7	eBiosciences	25-0087-41	SK1
CD9	PerCp cy5.5	BD Biosciences	561329	M-L13
CD95	BB515	BD Biosciences	564597	DX2
CTLA4	BV421	BD Biosciences	562743	BNI3
CTLA4	PE-CF594	BD Biosciences	562742	BNI3
EOMES	PE	eBiosciences	12-4877-41	WD1928
FCRL3	BB515	BD Biosciences	565026	H5
Foxp3	PE	BD Biosciences	560082	259D/C7
HLA-A2	PECy7	eBiosciences	25-9876-41	BB7.2
HLADR	BV421	BD Biosciences	562805	G46-6
IDO	eflour 660	eBiosciences	50-9477-41	eyedio
IFNg	PerCP-Cy5.5	eBiosciences	45-7319-41	4S.B3
IgD	BV421	BD Biosciences	562518	IA6-2
IgG	Alexa700	BD Biosciences	561296	G18-145
IgM	APC	BD Biosciences	561010	G20-127
IL-10	PE	eBiosciences	12-7108-41	JES3-9D7
IL-17A	APC	eBiosciences	17-7179-41	eBio64DEC17
IL2	BV421	BD Biosciences	562914	5344.111
IL-4	PE	BD Biosciences	554516	8D4-8
KLRG1	BV421	biolgend	138413	2F1
LAG3	PE Cy7	eBiosciences	25-2239-41	3DS223H
LAG3	APC	eBiosciences	17-2239-41	3DS223H
PD1	BB515	BD Biosciences	564494	EH12.1
PD1	BV421	BD Biosciences	563842	MIH18
PTK7	PE	miltenyibiotec	130-099-109	188B
Tim3	APC	eBiosciences	17-3109-41	F38-2E2
Tim3	BV421	Biolgend	345007	F38-2E2
TNFa	PE Cy7	eBiosciences	25-7349-41	MAb11
TNFa	eFluor® 450	eBiosciences	48-7349-41	MAb11

Table S4. P-values from initial and updated cohorts

An initial analysis was performed using data from 17 patients treated with ibrutinib and 9 patients treated with acalabrutinib. Later, an additional 2 patients with ibrutinib and 4 patients with acalabrutinib were added to the original cohorts and the analysis was redone; this second analysis was not planned at the time of the first analysis. P-values from the initial and new analyses are shown below for comparison purposes. Note that in the original analysis, p-values within each figure were adjusted for multiple comparisons using Hochberg's procedure, while unadjusted p-values are presented in the new analysis. Not all experiments were performed on each patient's serial sample, therefore the actual "N" for each experiment was less than 19 and 13 for ibrutinib and acalabrutinib treated patients, respectively.

Figure	Comparison	Initial Cohort		Updated Cohort	
		N	P-value	N	P-value
1A (ibrutinib): CD8+ T cells	Total CD8+ T cells: cycle 3 vs baseline	16	0.001	18	<.001
	Total CD8+ T cells: cycle 6 vs baseline	16	0.007	18	0.006
	Naïve CD8+ T cells: cycle 3 vs baseline	16	0.001	18	<.001
	Naïve CD8+ T cells: cycle 6 vs baseline	16	0.053	18	0.035
	CM CD8+ T cells: cycle 3 vs baseline	16	0.001	18	0.001
	CM CD8+ T cells: cycle 6 vs baseline	16	0.418	18	0.370
	EM CD8+ T cells: cycle 3 vs baseline	16	0.001	18	<.001
	EM CD8+ T cells: cycle 6 vs baseline	16	0.005	18	0.009
	EMRA CD8+ T cells: cycle 3 vs baseline	16	<.001	18	<.001
	EMRA CD8+ T cells: cycle 6 vs baseline	16	<.001	18	0.001
1A (ibrutinib): CD4+ T cells	Total CD8+ T cells: cycle 3 vs baseline	16	0.002	18	<.001
	Total CD8+ T cells: cycle 6 vs baseline	16	0.019	18	0.009
	Naïve CD8+ T cells: cycle 3 vs baseline	16	0.010	18	0.002
	Naïve CD8+ T cells: cycle 6 vs baseline	16	0.494	18	0.185
	CM CD8+ T cells: cycle 3 vs baseline	16	0.052	18	0.026
	CM CD8+ T cells: cycle 6 vs baseline	16	0.745	18	0.549
	EM CD8+ T cells: cycle 3 vs baseline	16	0.001	18	0.001
	EM CD8+ T cells: cycle 6 vs baseline	16	0.007	18	0.006
	EMRA CD8+ T cells: cycle 3 vs baseline	16	0.001	18	<.001
	EMRA CD8+ T cells: cycle 6 vs baseline	16	<.001	18	<.001
1B (acalabrutinib): CD8+ T cells	Total CD8+ T cells: cycle 3 vs baseline	8	0.960	12	0.999
	Total CD8+ T cells: cycle 6 vs baseline	8	0.960	12	0.329
	Naïve CD8+ T cells: cycle 3 vs baseline	8	0.813	12	0.772
	Naïve CD8+ T cells: cycle 6 vs baseline	8	0.813	12	0.857
	CM CD8+ T cells: cycle 3 vs baseline	8	0.960	12	0.556
	CM CD8+ T cells: cycle 6 vs baseline	8	0.813	12	0.127
	EM CD8+ T cells: cycle 3 vs baseline	8	0.960	12	0.848
	EM CD8+ T cells: cycle 6 vs baseline	8	0.960	12	0.353
	EMRA CD8+ T cells: cycle 3 vs baseline	8	0.813	12	0.694
	EMRA CD8+ T cells: cycle 6 vs baseline	8	0.813	12	0.319
1B (acalabrutinib): CD4+ T cells	Total CD8+ T cells: cycle 3 vs baseline	9	0.960	12	0.984
	Total CD8+ T cells: cycle 6 vs baseline	9	0.960	12	0.893
	Naïve CD8+ T cells: cycle 3 vs baseline	8	0.612	12	0.264
	Naïve CD8+ T cells: cycle 6 vs baseline	8	0.813	12	0.653
	CM CD8+ T cells: cycle 3 vs baseline	8	0.960	12	0.939

Figure	Comparison	Initial Cohort		Updated Cohort	
		N	P-value	N	P-value
	CM CD8+ T cells: cycle 6 vs baseline	8	0.960	12	0.940
	EM CD8+ T cells: cycle 3 vs baseline	8	0.960	12	0.841
	EM CD8+ T cells: cycle 6 vs baseline	8	0.960	12	0.942
	EMRA CD8+ T cells: cycle 3 vs baseline	8	0.070	12	0.527
	EMRA CD8+ T cells: cycle 6 vs baseline	8	0.135	12	0.691
2C (ibrutinib): Absolute cell #	Cycle 3 vs. baseline	13	0.049	15	0.002
	Cycle 6 vs. baseline	13	0.074	15	0.845
2C (ibrutinib): Percentage	Cycle 3 vs. baseline	13	0.298	15	0.564
	Cycle 6 vs. baseline	13	0.016	15	0.094
3A (ibrutinib): CD8 T cell subsets	Total CD8 T cells: cycle 3 vs baseline	15	0.041	17	0.001
	Total CD8 T cells: cycle 6 vs baseline	15	0.004	17	<.001
	Naïve CD8+ T cells: cycle 3 vs baseline	15	0.317	17	0.075
	Naïve CD8+ T cells: cycle 6 vs baseline	15	0.125	17	0.015
	T-CM CD8+ cells: cycle 3 vs baseline	15	0.116	17	0.002
	T-CM CD8+ cells: cycle 6 vs baseline	15	0.001	17	<.001
	T-EM CD8+ cells: cycle 3 vs baseline	15	0.115	17	0.007
	T-EM CD8+ cells: cycle 6 vs baseline	15	0.017	17	<.001
	T-EMRA CD8+ cells: cycle 3 vs baseline	15	0.622	17	0.351
	T-EMRA CD8+ cells: cycle 6 vs baseline	15	0.117	17	0.005
	T-EM CD8+ cells (CD27+): cycle 3 vs baseline	15	0.399	17	0.118
	T-EM CD8+ cells (CD27+): cycle 6 vs baseline	15	0.117	17	0.010
	T-EM CD8+ cells (CD27-): cycle 3 vs baseline	15	0.117	17	0.008
	T-EM CD8+ cells (CD27-): cycle 6 vs baseline	15	0.020	17	<.001
	T-EMRA CD8+ cells (CD27+): cycle 3 vs baseline	15	0.117	17	0.012
	T-EMRA CD8+ cells (CD27+): cycle 6 vs baseline	15	0.020	17	0.001
	T-EMRA CD8+ cells (CD27-): cycle 3 vs baseline	15	0.622	17	0.257
	T-EMRA CD8+ cells (CD27-): cycle 6 vs baseline	15	0.053	17	0.002
3B (acalabrutinib): CD8 T cell subsets	Total CD8 T cells: cycle 3 vs baseline	8	0.053	10	0.001
	Total CD8 T cells: cycle 6 vs baseline	8	0.025	10	<.001
	Naïve CD8+ T cells: cycle 3 vs baseline	8	0.053	10	0.006
	Naïve CD8+ T cells: cycle 6 vs baseline	8	0.028	10	0.001
	T-CM CD8+ cells: cycle 3 vs baseline	8	0.229	10	0.006
	T-CM CD8+ cells: cycle 6 vs baseline	8	0.034	10	<.001
	T-EM CD8+ cells: cycle 3 vs baseline	8	0.094	10	0.006
	T-EM CD8+ cells: cycle 6 vs baseline	8	0.148	10	0.006
	T-EMRA CD8+ cells: cycle 3 vs baseline	8	0.651	10	0.114
	T-EMRA CD8+ cells: cycle 6 vs baseline	8	0.106	10	0.002
	T-EM CD8+ cells (CD27+): cycle 3 vs baseline	8	0.245	10	0.019
	T-EM CD8+ cells (CD27+): cycle 6 vs baseline	8	0.229	10	0.005
	T-EM CD8+ cells (CD27-): cycle 3 vs baseline	8	0.028	10	0.957
	T-EM CD8+ cells (CD27-): cycle 6 vs baseline	8	0.113	10	0.245
	T-EMRA CD8+ cells (CD27+): cycle 3 vs baseline	8	0.181	10	0.005
	T-EMRA CD8+ cells (CD27+): cycle 6 vs baseline	8	0.004	10	<.001
	T-EMRA CD8+ cells (CD27-): cycle 3 vs baseline	8	0.910	10	0.672
	T-EMRA CD8+ cells (CD27-): cycle 6 vs baseline	8	0.910	10	0.769
4A (ibrutinib): CTLA4	Total CD4 T cells: cycle 3 vs baseline	16	0.001	18	<.001
	Total CD4 T cells: cycle 6 vs baseline	16	<.001	18	<.001
	CD45RA- CD4 T cells: cycle 3 vs baseline	16	0.001	18	<.001
	CD45RA- CD4 T cells: cycle 6 vs baseline	16	<.001	18	<.001

Figure	Comparison	Initial Cohort		Updated Cohort	
		N	P-value	N	P-value
	CD45RA+ CD4 T cells: cycle 3 vs baseline	16	0.005	18	0.002
	CD45RA+ CD4 T cells: cycle 6 vs baseline	16	0.001	18	<.001
4B (acalabrutinib): CTLA4	Total CD4 T cells: cycle 3 vs baseline	8	0.182	9	0.122
	Total CD4 T cells: cycle 6 vs baseline	8	0.182	9	0.103
	CD45RA- CD4 T cells: cycle 3 vs baseline	8	0.182	9	0.089
	CD45RA- CD4 T cells: cycle 6 vs baseline	8	0.182	9	0.062
	CD45RA+ CD4 T cells: cycle 3 vs baseline	8	0.050	9	0.007
	CD45RA+ CD4 T cells: cycle 6 vs baseline	8	0.104	9	0.062
5A (ibrutinib): cytokines	IFNγ: cycle 3 vs. baseline	14	0.803	15	0.999
	IFNγ: cycle 6 vs. baseline	14	0.803	15	0.756
	TNFα: cycle 3 vs. baseline	14	0.803	15	0.571
	TNFα: cycle 6 vs. baseline	14	0.803	15	0.213
	IL-2: cycle 3 vs. baseline	14	0.803	15	0.876
	IL-2: cycle 6 vs. baseline	14	0.803	15	0.524
	IL-4: cycle 3 vs. baseline	14	0.803	15	0.245
	IL-4: cycle 6 vs. baseline	14	0.803	15	0.650
	IL-17: cycle 3 vs. baseline	14	0.803	15	0.257
	IL-17: cycle 6 vs. baseline	14	0.059	15	0.008
5B (acalabrutinib): cytokines	IFNγ: cycle 3 vs. baseline	7	0.075	11	0.007
	IFNγ: cycle 6 vs. baseline	7	0.349	11	0.003
	TNFα: cycle 3 vs. baseline	7	0.357	11	0.033
	TNFα: cycle 6 vs. baseline	7	0.667	11	0.022
	IL-2: cycle 3 vs. baseline	7	0.987	11	0.767
	IL-2: cycle 6 vs. baseline	7	0.987	11	0.523
	IL-4: cycle 3 vs. baseline	7	0.987	11	0.210
	IL-4: cycle 6 vs. baseline	7	0.832	11	0.042
	IL-17: cycle 3 vs. baseline	7	0.987	11	0.427
	IL-17: cycle 6 vs. baseline	7	0.987	11	0.880
6B (ibrutinib): Foxp3+ cells	Percentage: cycle 3 vs. baseline	16	<.001	18	<.001
	Percentage: cycle 6 vs. baseline	16	<.001	18	<.001
	Absolute number: cycle 3 vs. baseline	16	0.411	18	0.282
	Absolute number: cycle 6 vs. baseline	16	0.700	18	0.694
6C (acalabrutinib): Foxp3+ cells	Percentage: cycle 3 vs. baseline	8	0.386	11	0.553
	Percentage: cycle 6 vs. baseline	8	0.239	11	0.346
	Absolute number: cycle 3 vs. baseline	8	0.547	11	0.747
	Absolute number: cycle 6 vs. baseline	8	0.448	11	0.331
7A (ibrutinib): CD200, BTLA	CD200: Cycle 3 vs. baseline	16	<.001	18	<.001
	CD200: Cycle 6 vs. baseline	16	<.001	18	<.001
	BTLA: Cycle 3 vs. baseline	14	<.001	16	<.001
	BTLA: Cycle 6 vs. baseline	14	<.001	16	<.001
7B (acalabrutinib): CD200, BTLA	CD200: Cycle 3 vs. baseline	8	0.002	12	0.001
	CD200: Cycle 6 vs. baseline	8	0.002	12	<.001
	BTLA: Cycle 3 vs. baseline	8	<.001	12	<.001
	BTLA: Cycle 6 vs. baseline	8	<.001	12	<.001
8C (ibrutinib): IL10+ cells	5 hours (B10): Cycle 3 vs. baseline	6	0.313	13	0.064
	5 hours (B10): Cycle 6 vs. baseline	6	0.313	13	0.155
	48 hours (B10 Pro): Cycle 3 vs. baseline	16	<.001	18	<.001
	48 hours (B10 Pro): Cycle 6 vs. baseline	16	<.001	18	<.001

Figure	Comparison	Initial Cohort		Updated Cohort	
		N	P-value	N	P-value
8C (acalabrutinib): IL10+ cells	5 hours (B10): Cycle 3 vs. baseline	5	0.118	10	0.028
	5 hours (B10): Cycle 6 vs. baseline	5	0.313	10	0.211
	48 hours (B10 Pro): Cycle 3 vs. baseline	7	0.007	12	<.001
	48 hours (B10 Pro): Cycle 6 vs. baseline	7	0.031	12	<.001
S1A (ibrutinib): CD8+ T cells	Total CD8+ T cells: cycle 3 vs baseline	16	0.848	18	0.904
	Total CD8+ T cells: cycle 6 vs baseline	16	0.101	18	0.066
	Naïve CD8+ T cells: cycle 3 vs baseline	16	0.310	18	0.232
	Naïve CD8+ T cells: cycle 6 vs baseline	16	0.034	18	0.046
	CM CD8+ T cells: cycle 3 vs baseline	16	0.453	18	0.255
	CM CD8+ T cells: cycle 6 vs baseline	16	0.026	18	0.006
	EM CD8+ T cells: cycle 3 vs baseline	16	0.310	18	0.868
	EM CD8+ T cells: cycle 6 vs baseline	16	0.056	18	0.569
	EMRA CD8+ T cells: cycle 3 vs baseline	16	0.994	18	0.206
	EMRA CD8+ T cells: cycle 6 vs baseline	16	0.185	18	0.017
S1A (ibrutinib): CD4+ T cells	Total CD8+ T cells: cycle 3 vs baseline	16	0.848	18	0.712
	Total CD8+ T cells: cycle 6 vs baseline	16	0.092	18	0.051
	Naïve CD8+ T cells: cycle 3 vs baseline	16	0.360	18	0.915
	Naïve CD8+ T cells: cycle 6 vs baseline	16	0.048	18	0.553
	CM CD8+ T cells: cycle 3 vs baseline	16	0.026	18	0.015
	CM CD8+ T cells: cycle 6 vs baseline	16	0.001	18	0.001
	EM CD8+ T cells: cycle 3 vs baseline	16	0.067	18	0.806
	EM CD8+ T cells: cycle 6 vs baseline	16	0.001	18	0.102
	EMRA CD8+ T cells: cycle 3 vs baseline	16	0.056	18	0.010
	EMRA CD8+ T cells: cycle 6 vs baseline	16	0.101	18	0.106
S1B (acalabrutinib): CD8+ T cells	Total CD8+ T cells: cycle 3 vs baseline	9	0.734	12	0.574
	Total CD8+ T cells: cycle 6 vs baseline	9	0.382	12	0.032
	Naïve CD8+ T cells: cycle 3 vs baseline	8	0.786	12	0.708
	Naïve CD8+ T cells: cycle 6 vs baseline	8	0.734	12	0.960
	CM CD8+ T cells: cycle 3 vs baseline	8	0.851	12	0.321
	CM CD8+ T cells: cycle 6 vs baseline	8	0.602	12	0.405
	EM CD8+ T cells: cycle 3 vs baseline	8	0.715	12	0.559
	EM CD8+ T cells: cycle 6 vs baseline	8	0.786	12	0.846
	EMRA CD8+ T cells: cycle 3 vs baseline	8	0.731	12	0.151
	EMRA CD8+ T cells: cycle 6 vs baseline	8	0.734	12	0.778
S1B (acalabrutinib): CD4+ T cells	Total CD8+ T cells: cycle 3 vs baseline	9	0.734	12	0.569
	Total CD8+ T cells: cycle 6 vs baseline	9	0.382	12	0.024
	Naïve CD8+ T cells: cycle 3 vs baseline	8	0.397	12	0.165
	Naïve CD8+ T cells: cycle 6 vs baseline	8	0.715	12	0.605
	CM CD8+ T cells: cycle 3 vs baseline	8	0.715	12	0.778
	CM CD8+ T cells: cycle 6 vs baseline	8	0.602	12	0.698
	EM CD8+ T cells: cycle 3 vs baseline	8	0.648	12	0.460
	EM CD8+ T cells: cycle 6 vs baseline	8	0.930	12	0.942
	EMRA CD8+ T cells: cycle 3 vs baseline	8	0.648	12	0.243
	EMRA CD8+ T cells: cycle 6 vs baseline	8	0.760	12	0.132
S3A (ibrutinib): CD4 T cell subsets	Total CD4 T cells: cycle 3 vs baseline	14	0.977	17	0.593
	Total CD4 T cells: cycle 6 vs baseline	14	0.040	17	0.001
	Naïve CD4+ T cells: cycle 3 vs baseline	14	0.977	17	0.968
	Naïve CD4+ T cells: cycle 6 vs baseline	14	0.720	17	0.009
	T-CM CD4+ cells: cycle 3 vs baseline	14	0.977	17	0.242

Figure	Comparison	Initial Cohort		Updated Cohort	
		N	P-value	N	P-value
	T-CM CD4+ cells: cycle 6 vs baseline	14	0.019	17	<.001
	T-EM CD4+ cells: cycle 3 vs baseline	14	0.977	17	0.726
	T-EM CD4+ cells: cycle 6 vs baseline	14	0.025	17	0.001
	T-EMRA CD4+ cells: cycle 3 vs baseline	14	0.975	17	0.042
	T-EMRA CD4+ cells: cycle 6 vs baseline	14	0.001	17	<.001
	T-EM CD4+ cells (CD27+): cycle 3 vs baseline	14	0.977	17	0.782
	T-EM CD4+ cells (CD27+): cycle 6 vs baseline	14	0.007	17	<.001
	T-EM CD4+ cells (CD27-): cycle 3 vs baseline	14	0.977	17	0.471
	T-EM CD4+ cells (CD27-): cycle 6 vs baseline	14	0.720	17	0.014
	T-EMRA CD4+ cells (CD27+): cycle 3 vs baseline	14	0.977	17	0.194
	T-EMRA CD4+ cells (CD27+): cycle 6 vs baseline	14	0.001	17	<.001
	T-EMRA CD4+ cells (CD27-): cycle 3 vs baseline	14	0.977	17	0.743
	T-EMRA CD4+ cells (CD27-): cycle 6 vs baseline	14	0.977	17	0.793
S3B (acalabrutinib): CD4 T cell subsets	Total CD4 T cells: cycle 3 vs baseline	8	0.339	10	0.018
	Total CD4 T cells: cycle 6 vs baseline	8	0.562	10	0.099
	Naïve CD4+ T cells: cycle 3 vs baseline	8	0.132	10	0.007
	Naïve CD4+ T cells: cycle 6 vs baseline	8	0.079	10	0.002
	T-CM CD4+ cells: cycle 3 vs baseline	8	0.213	10	0.002
	T-CM CD4+ cells: cycle 6 vs baseline	8	0.040	10	<.001
	T-EM CD4+ cells: cycle 3 vs baseline	8	0.766	10	0.217
	T-EM CD4+ cells: cycle 6 vs baseline	8	0.766	10	0.023
	T-EMRA CD4+ cells: cycle 3 vs baseline	8	0.766	10	0.097
	T-EMRA CD4+ cells: cycle 6 vs baseline	8	0.024	10	<.001
	T-EM CD4+ cells (CD27+): cycle 3 vs baseline	8	0.766	10	0.163
	T-EM CD4+ cells (CD27+): cycle 6 vs baseline	8	0.416	10	0.005
	T-EM CD4+ cells (CD27-): cycle 3 vs baseline	8	0.766	10	0.026
	T-EM CD4+ cells (CD27-): cycle 6 vs baseline	8	0.911	10	0.158
	T-EMRA CD4+ cells (CD27+): cycle 3 vs baseline	8	0.766	10	0.078
	T-EMRA CD4+ cells (CD27+): cycle 6 vs baseline	8	0.017	10	<.001
	T-EMRA CD4+ cells (CD27-): cycle 3 vs baseline	8	0.769	10	0.399
	T-EMRA CD4+ cells (CD27-): cycle 6 vs baseline	8	0.769	10	0.159
S4A (ibrutinib): CTLA4	Total CD4 T cells: cycle 3 vs baseline	16	0.029	18	0.004
	Total CD4 T cells: cycle 6 vs baseline	16	0.012	18	0.007
	CD45RA- CD4 T cells: cycle 3 vs baseline	16	0.052	18	0.007
	CD45RA- CD4 T cells: cycle 6 vs baseline	16	0.012	18	0.006
	CD45RA+ CD4 T cells: cycle 3 vs baseline	16	0.059	18	0.027
	CD45RA+ CD4 T cells: cycle 6 vs baseline	16	0.059	18	0.038
S4B (acalabrutinib): CTLA4	Total CD4 T cells: cycle 3 vs baseline	8	0.013	9	0.002
	Total CD4 T cells: cycle 6 vs baseline	8	0.016	9	0.005
	CD45RA- CD4 T cells: cycle 3 vs baseline	8	0.021	9	0.004
	CD45RA- CD4 T cells: cycle 6 vs baseline	8	0.039	9	0.010
	CD45RA+ CD4 T cells: cycle 3 vs baseline	8	0.018	9	0.002
	CD45RA+ CD4 T cells: cycle 6 vs baseline	8	0.015	9	0.002