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Supplementary Materials for

Epigenetic therapy overcomes treatment resistance in T cell prolymphocytic leukemia

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The PDF file includes:

Supplemental patient case information

Case 1 – Patient 1 is a 64 year old woman who presented with a recent diagnosis of T-cell prolymphocytic leukemia (T-PLL). She initially presented to her local hospital with symptoms of chronic back pain, fatigue, and abdominal swelling. She was found to have a white blood cell count of 237,000/μl with lymphocytic predominance. Further work up, including a bone marrow biopsy as well as left axillary lymph node biopsy, established a diagnosis of T-PLL. She was also noted to have bloody thoracentesis. At that point, she was transferred to our hematology service and was treated for disseminated intravascular coagulation (DIC) and received CVP (cyclophosphamide, vincristine and prednisone) therapy for her leukemia. She did not respond well and was switched to anti-CD52 therapy with alemtuzumab. She had a brisk but transient decline in her counts consistent with alemtuzumab-refractory disease. To circumvent this resistance, cladribine was added to her regimen. She did extremely well, and her white blood cell count decreased to 15,000/μl. She was previously anemic and thrombocytopenic, but after treatment, her hemoglobin and platelets completely normalized to 12.7/dl and 205,000/μl, respectively. Though her cell counts decreased, she continued to have a lymphocytic predominance (78%) in her differential white blood cell count. Her fibrinogen was 322, and her DIC appeared to have resolved at that point. After just 2 cycles of therapy, she achieved complete remission (CR). Her care was then transferred to her local hematologist. She remained in remission for a little over a year. At that time, her flow cytometry immunophenotyping findings were consistent with relapse, but her white blood cell counts were within the normal range, and she was asymptomatic. When her counts reached 20,000, she was retreated with 1 cycle of therapy. After this short course, she attained a partial remission (PR). Her PCR was still

positive for T-cell leukemic molecular rearrangements, but the patient requested a chemotherapy holiday.

She returned 8 months later with a white blood cell count of 108,000/μl. Treatment with cladribine and alemtuzumab had activated low-level expression of CD30, which was detected on immunophenotyping of a bone marrow specimen by flow cytometry and confirmed on peripheral blood by qRT-PCR. Due to slow response and alemtuzumab toxicity, this prompted us to start treatment immediately with the anti-CD30 antibody-drug conjugate brentuximab vedotin, with the plan of adding alemtuzumab and/or cladribine in case of suboptimal response. The patient achieved PR with white blood cell counts dropping to 35,000/μl. Alemtuzumab was added with good response, and follow up flow cytometry and qRT-PCR studies showed the disappearance of CD30 expression on her leukemic cells. She was maintained on alemtuzumab while she was being evaluated for reduced intensity allogeneic transplant. During this period her counts started rising, and she contracted varicella zoster (VZV) infection while on prophylactic acyclovir. This VZV strain was acyclovir and valacyclovir resistant but responded to treatment with foscarnet. Once it cleared, she was put back on alemtuzumab. She was started on brentuximab vedotin and pralatrexate, another therapeutic approved for T-cell malignancies, and showed a decrease in leukocytosis from 135,000 to 59,000. Nevertheless, her white blood cell count continued rising with a confirmed loss of CD52 and CD30 expression by flow cytometry (tested externally by Phenopath Laboratories). Loss of CD52 expression is rare and can be associated with prolonged alemtuzumab use.*(2,5,61)* She did not receive any therapy for a month as we waited for re-expression of the CD52 and/ or CD30 proteins. In that time, as a result of multiple therapies and

disease load, her performance status declined, and after much discussion she decided to discontinue therapy and enter hospice. She passed away one month later while in hospice care.

Case 2 – Patient 2 is a 74 year old man who presented to our service with relapsed T-PLL. His initial presentation before our evaluation was asymptomatic lymphocytosis thought to be B-cell CLL given his age and the gradual rise in white blood cell count. However, flow cytometry of peripheral blood revealed atypical lymphocytes of T-cell origin, expressing CD4, CD5, and CD7. He remained asymptomatic for 1.5 years, but his white blood cell count continued to rise from 12,000/μl to 73,000/μl (95% lymphocytes). At that time, CT scans showed diffuse scattered lymphadenopathy and mild to moderate splenomegaly. Bone marrow aspiration and biopsy demonstrated 25% involvement of the marrow with uniform CD52 expression, confirmed by flow cytometry and immunohistochemical staining. He had been previously treated with single agent alemtuzumab, after which he had a remarkably fast clinical response evidenced by rapid decrease in lymphocyte counts. Before initiation of therapy, his lymphocyte count was around 100,000/μl, which precipitously declined during three weeks of therapy. The total leukocyte count fell to 400/μl. Follow up peripheral smear, bone marrow studies, and scans confirmed CR. The possibility of a stem cell transplant to consolidate remission was discussed, but given his age, co-morbidities, and cardiac history, the patient decided not to undergo the procedure.

Several months later, his white blood cell count started to rise, indicating relapse. At this point, his care was transferred to our hematology service. He was started on single agent alemtuzumab, but his white blood cell count continued to rise, indicating resistance. At that point, cladribine was added to his regimen and led to a slight decline in white blood cell count. Vorinostat was

then added, and after just 2 cycles, he achieved CR. Treatment was discontinued. However, this second remission lasted only a few months. As his counts began to rise again, the above therapy was reinitiated, and he achieved PR. Unfortunately, due to the myelosuppressive nature of cladribine, he had to be taken off treatment and given intermittent blood and platelet transfusions and G-CSF support. His marrow recovered well over the next few months, but his counts rose again, prompting re-initiation of therapy. Again, after 2 weeks of therapy, his white blood cell count steadily decreased. This lasted a month, and he again relapsed and was started on alemtuzumab, cladribine, and vorinostat therapy for a fourth time. He developed severe pancytopenia after therapy. He was referred to hospice and died shortly thereafter.

Case 3 – Patient 3 is a 77 year old female who was referred to our clinic for T-PLL. She initially presented to her primary care physician with complaints of increasing fatigue over 3 months, weakness and swelling of the legs, dyspnea on exertion, lymphadenopathy in the neck and axilla, anorexia, and early satiety. Her spleen was measured to be 17 x 9.6 x 17 cm. Her primary care physician performed a CBC that revealed a white blood cell count of 256,000/μl, platelet count of 73,000/μl, and LDH of 987. Prolymphocytes were seen on her peripheral smear, and flow cytometry indicated T-PLL with 88% atypical lymphocytes expressing T-cell receptor gamma delta, CD4, CD5, and CD7. CT scans showed generalized lymphadenopathy with the largest node measuring 2.5 cm. We initiated therapy with cladribine and alemtuzumab. White blood cell count at the commencement of therapy was 301,000/μl but rapidly fell to 1700/μl after one cycle. LDH and platelets also improved from 987 to 701 and 68,000 to 138,000/μl, respectively. Flow cytometry and PCR after one cycle showed the absence of tumor cells but her lymphadenopathy and splenomegaly only partially resolved. She was continued on alemtuzumab therapy but

ultimately went off treatment after 3 months due to anemia and neutropenia. She achieved a PR. Her blood remained disease-free until her death, but her lymph nodes remained involved. Her cause of death was unknown, but she was referred to hospice after development of intractable lower back pain. No autopsy was performed, but T-PLL CNS involvement has been described and may have been related to this patient's case.*(62)*

Case 4 – Patient 4 is a 63 year old man with a previous history of sickle cell thalassemia and splenectomy. He had a mild leukocytosis for several years which was followed closely until an accelerated rise in lymphocytes. He had accompanying symptoms of fatigue, shortness of breath on exertion, and occasional night sweats. Bone marrow biopsy and flow cytometry showed CD2+/4+/5+/7+/3-/CD8- atypical T-lymphocytes consistent with T-PLL. We decided to start treatment with epigenetic combination therapy using cladribine and alemtuzumab. He had a dramatic response with decrease in white blood cell count from 248,000 to 2,700/μl and LDH from 2,769 to 600 within 4 weeks and achieved CR. Unfortunately, he developed cytomegalovirus (CMV) reactivation, and his alemtuzumab was stopped. He was followed thereafter, on a regular basis, with transfusion and G-CSF growth factor support. He continued to remain in remission for approximately 1 year, after which he presented with a white blood cell count of 54,000/μl suggestive of relapsed disease. At this point, he was severely anemic and supported with blood transfusions. After his anemia recovered, therapy with cladribine and alemtuzumab was re-initiated. He again had a rapid response. His white blood cell count fell from 54,000 to 3,300/μl after 1 cycle of cladribine and alemtuzumab. However, he had persistent anemia due to a combined effect of cladribine therapy and sickle cell disease, requiring regular blood transfusions and delay in chemotherapy administration. Meanwhile, his lymphocyte count

gradually rose, and after 4 weeks reached 9,900/μl, indicative of residual disease. Before he could receive further treatment, the patient succumbed to secondary CNS hemorrhage. This hemorrhage was likely due to a combination of his sickle cell, thrombocytopenia, and possibly alemtuzumab treatment *(33)*.

Case 5 – Patient 5 is a 70 year old man with a prior history of low grade B-cell lymphoma. He was followed closely for his indolent lymphoma but never required treatment. Suddenly, he presented with rapidly increasing white blood cell count and a skin rash on his chest. Flow cytometry was consistent with T-PLL in addition to a small population of B cells. We started therapy with cladribine, alemtuzumab, and vorinostat. His white blood cell count improved substantially from 211,000/μl to 1,100/μl after 3 weeks. Cladribine was stopped because of its myelosuppressive effects. Alemtuzumab was continued, and a CR was confirmed. His remission lasted 7 months, at which time he presented with a worsening skin rash and elevated white blood cell count (14,500/μl). A biopsy of the aforementioned lesions revealed T-PLL involvement of the skin. He restarted treatment with cladribine, alemtuzumab, and romidepsin which resulted in improvement of his white blood cell count (14,500 to 5,010 after 1 cycle) but had no effect on his skin. We chose to try romidepsin, because it is also approved for cutaneous T-cell lymphoma, a skin manifesting disease. In the interim, he complained of increasing sweats, chills, anorexia, fatigue, and weight gain secondary to edema.

Though his blood counts remained under control for several months, we observed that his skin rash had worsened noticeably in that time, turning purple in some places (Figure 4). His CT scans showed progressive increases in lymphadenopathy. A repeat skin biopsy revealed CD30

positivity by IHC (Figure 4) and RT-PCR of peripheral blood (Figure 3), which led to the initiation of brentuximab vedotin. Although alemtuzumab is an excellent biologic agent to target the circulating leukemic cells, it has limited efficacy in lymph nodes and skin.*(44)* Treatment with brentuximab and alemtuzumab was successful and led to resolution of skin lesions (Figure 4) with negative biopsy and qRT-PCR. However, after 9 months of brentuximab, treatment had to be stopped due to intracranial chronic small vessel disease and a complex anterior communicating artery trilobed aneurysm. The patient began to relapse after the halt in therapy and showed interval increases in lymph node and spleen size. Skin and white blood cells remained unaffected. At this point, we started him on alemtuzumab and proceeded with reduced intensity unrelated donor allogeneic stem cell infusion and low intensity matched unrelated donor allogeneic stem cell transplant. He seemed to recover well after the transplant and started gaining weight and having better energy levels, but his post-transplant course was soon complicated with hemolytic uremic syndrome and graft vs. host disease. He was admitted, and a skin biopsy and CT scan showed an increase in mediastinal, abdominal, and pelvic lymphadenopathy though his blood was cancer-free. Alemtuzumab was initiated along with broad spectrum antibiotics, but the patient succumbed while in the hospital. No autopsy was performed.

Case 6 – Patient 6 is a 57 year old man who was diagnosed with T-PLL a year before he presented to us. He was followed for mild leukocytosis for over 3 years until bone marrow biopsy showed 70% cellularity with 20-30% infiltration by CD4+ cells. He felt well except for mild anemia, thrombocytopenia, and left upper quadrant (LUQ) discomfort from a moderately enlarged spleen. Due to the nature of his disease, he sought a second opinion with us to explore

the options of any experimental treatments in combination with alemtuzumab. At presentation, he had increasing pain in the LUQ, fatigue, anorexia, and early satiety. He also developed small erythematous raised skin lesions a few days before presentation. We initiated therapy with cladribine and alemtuzumab. After one cycle of cladribine and alemtuzumab, his care was transferred to his local oncologist. He then completed a second cycle of cladribine and alemtuzumab and attained a CR. He underwent high dose chemotherapy with busulfan and fludarabine followed by a matched sibling allogeneic peripheral blood stem cell transplant. Apart from a viral infection, he did not suffer from any complications after transplantation. He has been in remission for over 4 years.

Case 7 – Patient 7 is a 63 year old man with T-PLL diagnosed by flow cytometry and T-cell gene rearrangement studies for elevated white blood cell count of 34,500/μl (50% lymphocytes). His white blood cell count continued to rise during this time to above 200,000/μl. He started treatment with subcutaneous alemtuzumab, but because of skin toxicity at the site of injection and documented lower efficacy of the subcutaneous route, he was changed to IV alemtuzumab. His blood did not respond to treatment. After lack of response of several IV doses of alemtuzumab, cladribine was added. His white blood cell count declined initially but then started to level off at around 100,000/μl before rising again. Vorinostat was denied by insurance, so valproic acid orally at 250 mg three times a day was added. Subsequently, his white blood cell count declined, and he achieved CR. He had minor infusion toxicity with alemtuzumab and no interim infections. He remained in CR until elevated white blood cell count with lymphocytosis was noted one year later. Flow cytometry was positive for relapsed T-PLL, and he is being re-treated with epigenetic therapy and referred for consideration of stem cell transplantation.

Case 8 – Patient 8 is a 67 year old man who presented with a white blood cell count of 110,000/μl, pneumonia, and splenomegaly. Flow cytometry revealed two separate T-cell populations. The major population was CD52/3/5/7/8 and TCR γ positive and negative for α/β TCR and CD30. The smaller subpopulation was CD2/3 and α/β TCR positive but CD8 negative. He received one cycle of alemtuzumab and achieved a CR. This CR lasted 16 months. At that time, he presented with lymphocytosis, constitutional symptoms, lymphadenopathy, and splenomegaly. He was restarted on alemtuzumab for 2 cycles. His T-PLL count decreased from 36 to 12% of total lymphocytes but remained high. Based on our success with other T-PLL patients, cladribine was added. He achieved CR, but his white blood cell counts are again rising. He is being treated with epigenetic drugs and alemtuzumab and being considered for allogeneic stem cell transplant.

SUPPLEMENTARY FIGURES

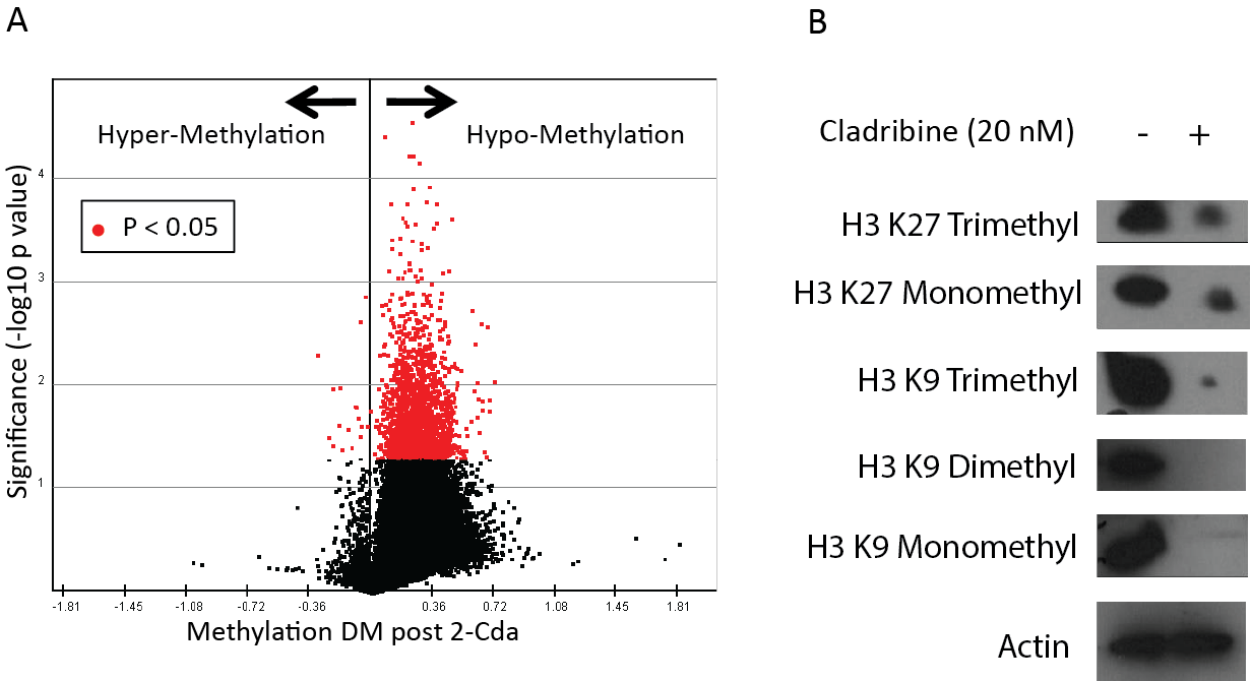


Figure S1: Cladribine inhibits DNA methylation in vivo and histone methylation in vitro. **(A)** Volcano plot showing the difference of mean DNA methylation in 2 CLL patient samples after cladribine treatment (X axis) vs. significance (Y axis) using HpaII tiny fragment Enrichment by Ligation-mediated PCR (HELP) 3 days after treatment. Red dots indicate cutoff of significance, $p<0.05$ by t-test. Of 25,626 probes, 1,992 were significant ($p<0.05$, red). Of those 1,992, 1,974 were confirmed by paired t-test to be hypomethylated after cladribine treatment. **(B)** Western blot showing the effect of cladribine on histone methylation in the Granta 519 cell line (MCL) 24 hours after 20 nM cladribine treatment. Control is DMSO-treated.

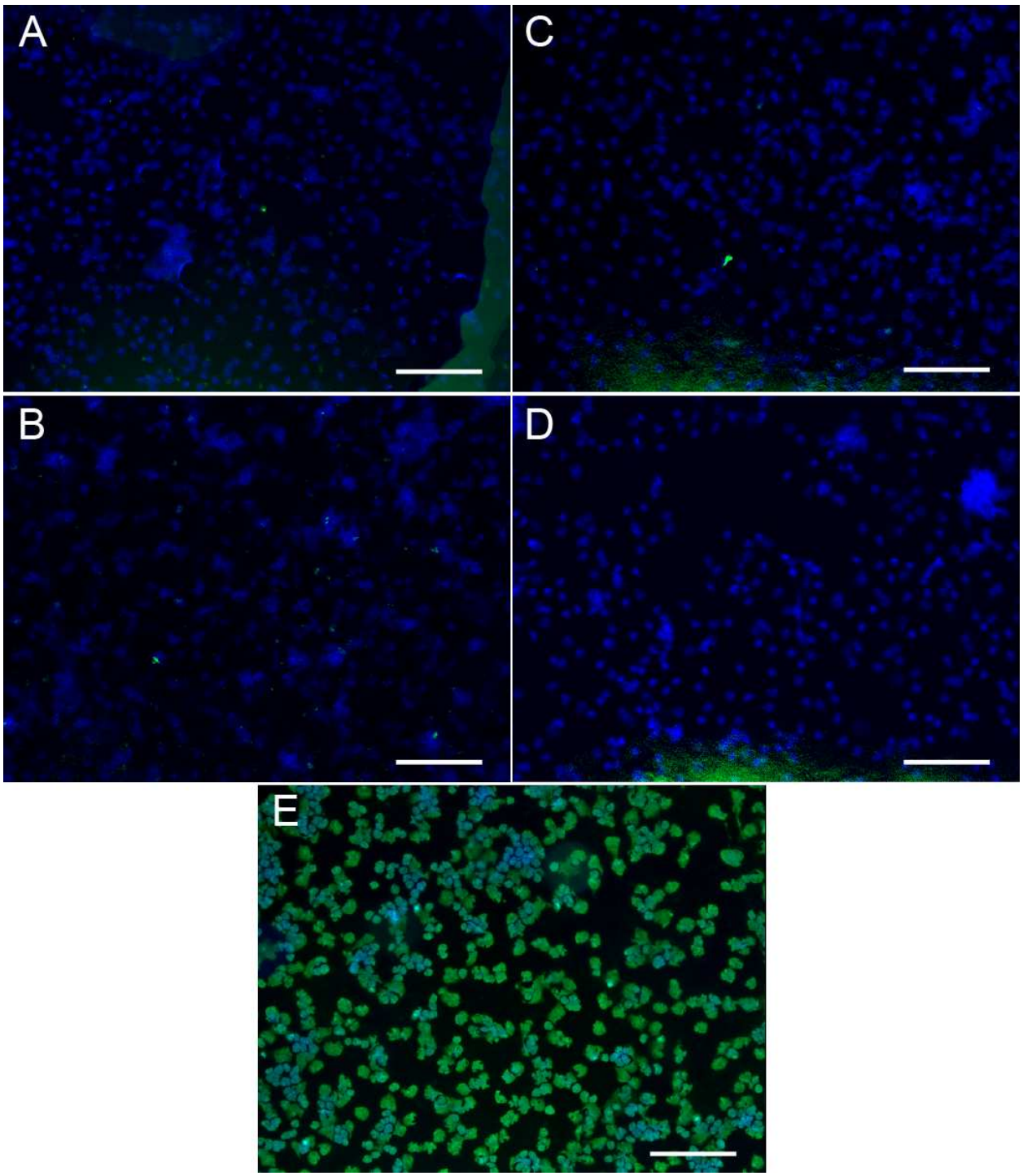
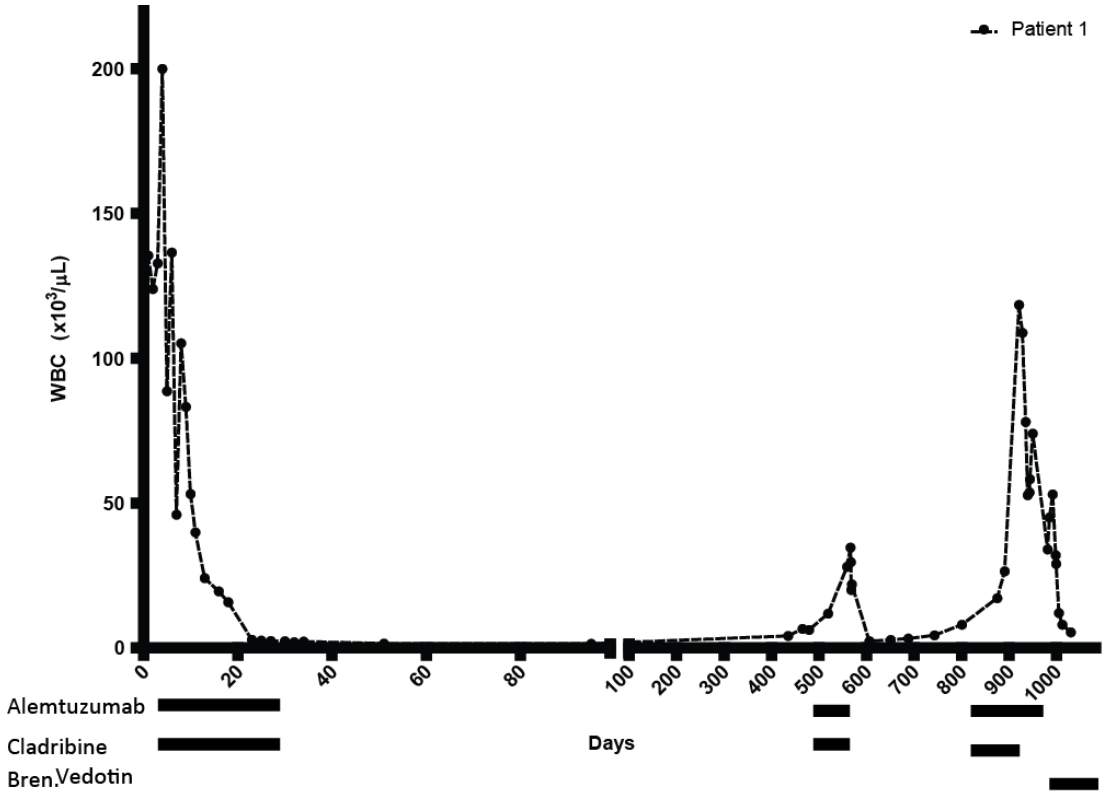
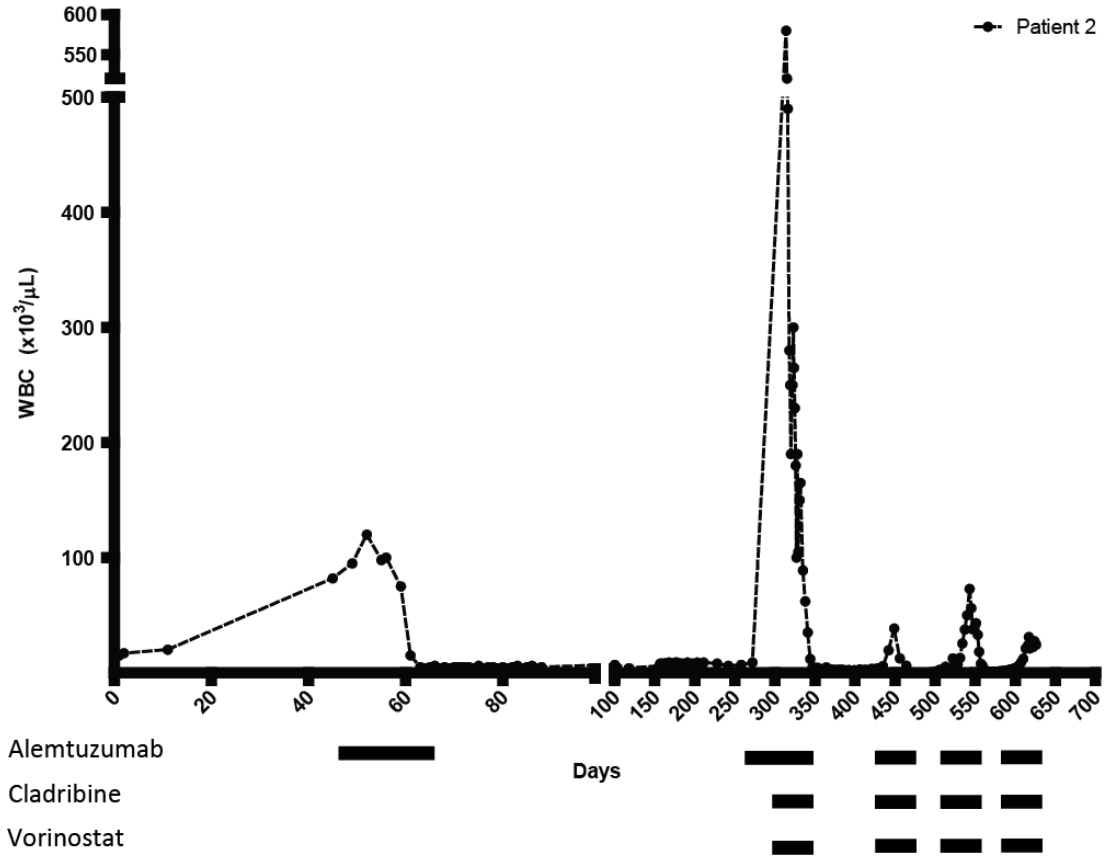


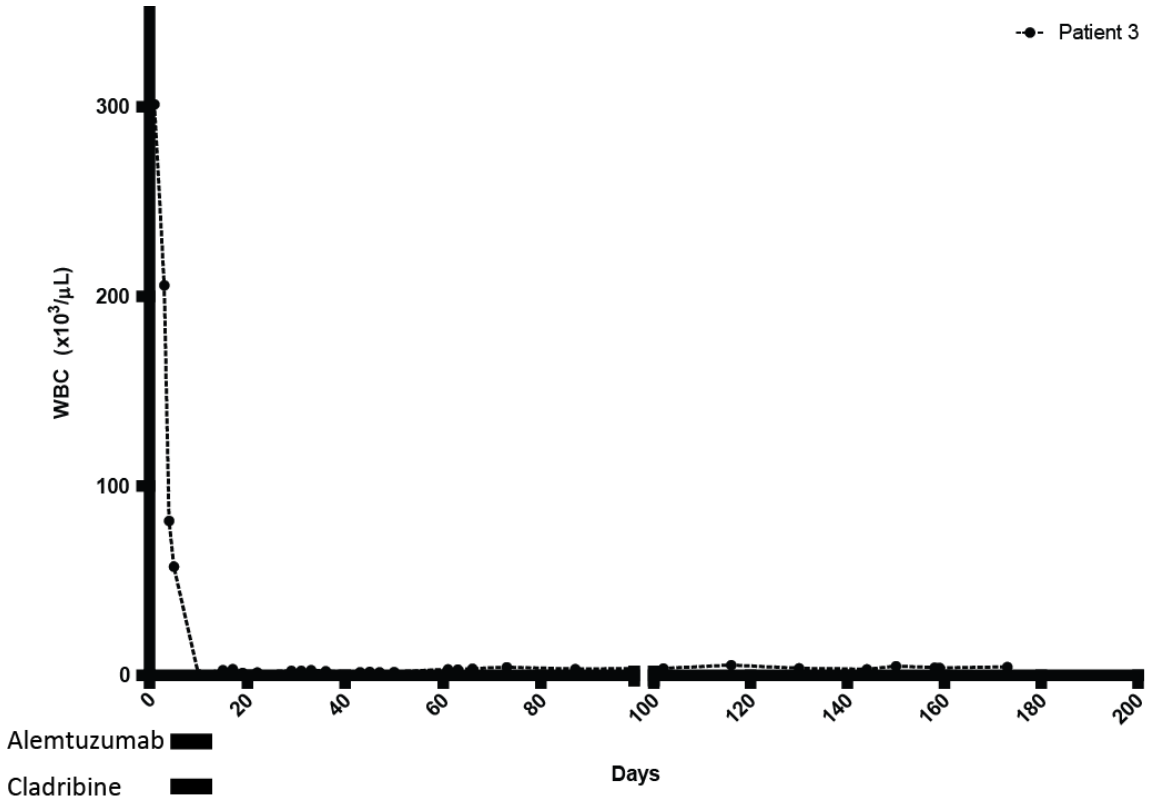
Figure S2: Epigenetic therapy does not induce apoptosis in T-PLL.

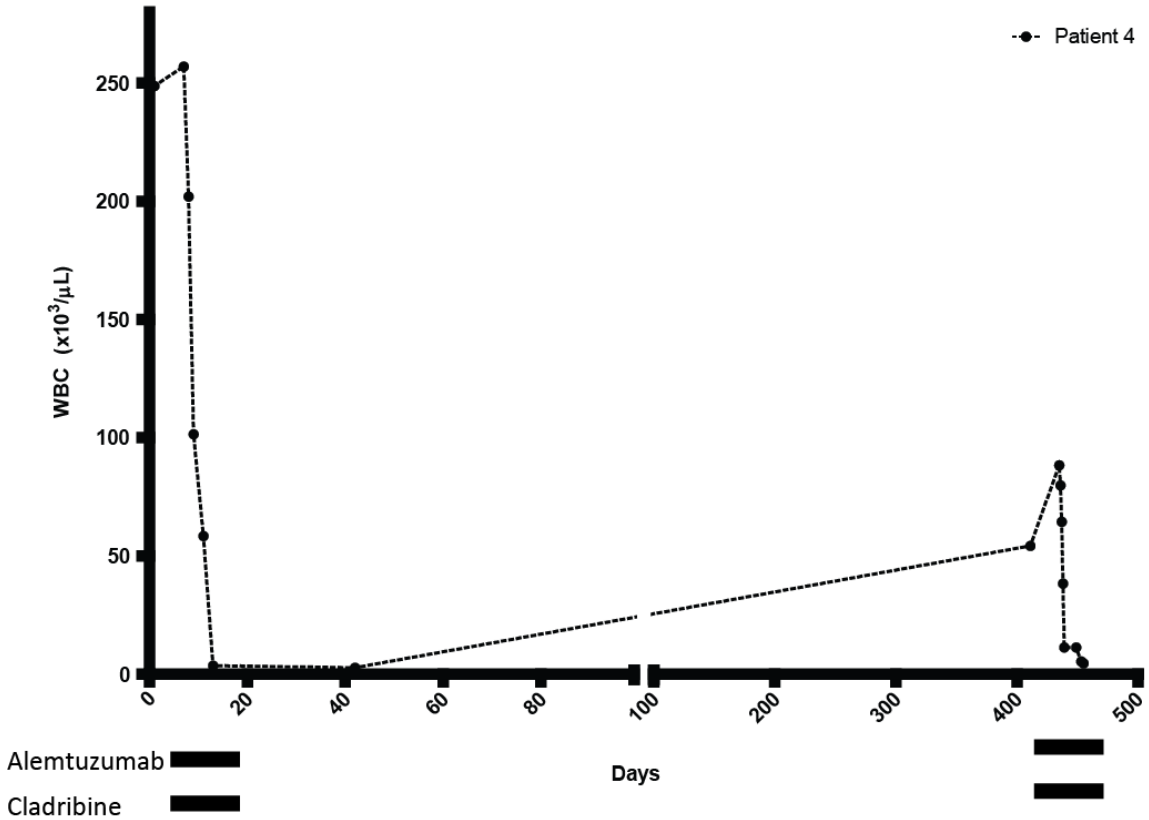
T-PLL cells from patient 2 before **(A)** and 5 days after **(B)** treatment with alemtuzumab, cladribine, and vorinostat. T-PLL cells taken from patient 3 before **(C)** and 5 days after **(D)**

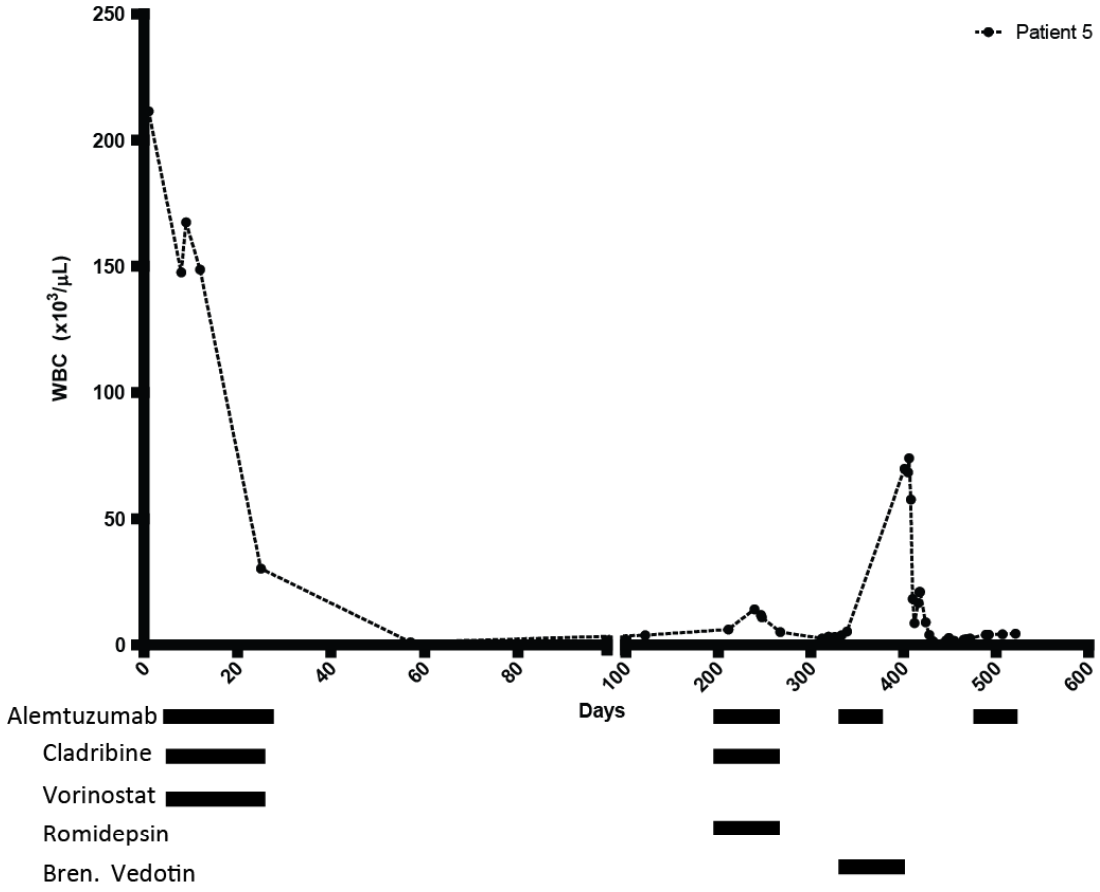
treatment with alemtuzumab and cladribine. Primary B-cells treated with DNAse were used as positive control **(E)**. Fragmented apoptotic DNA, green, Nuclei DAPI, blue. Scale bar is 100 μM.

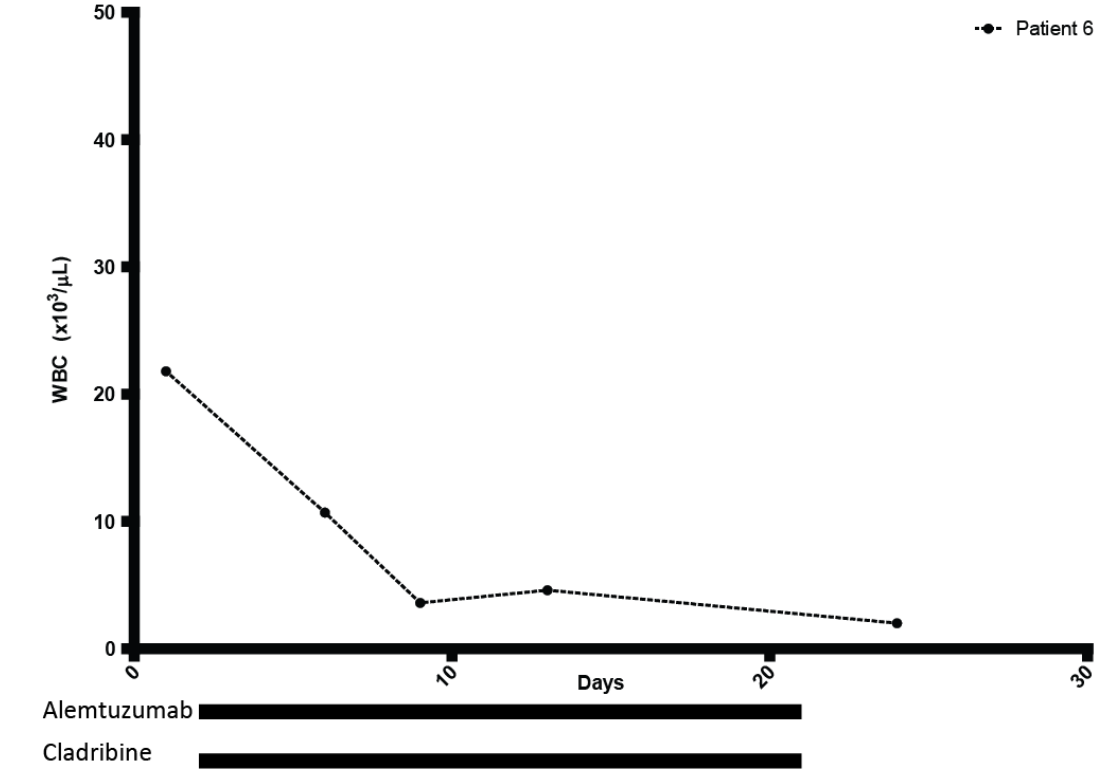

Patient 1
WBC (x10³/μL)
200
150
100
50
0
0
20
40
60
80
100
200
300
400
500
600
700
800
900
1000
Days
Alemtuzumab
Cladribine
Bren.Vedotin

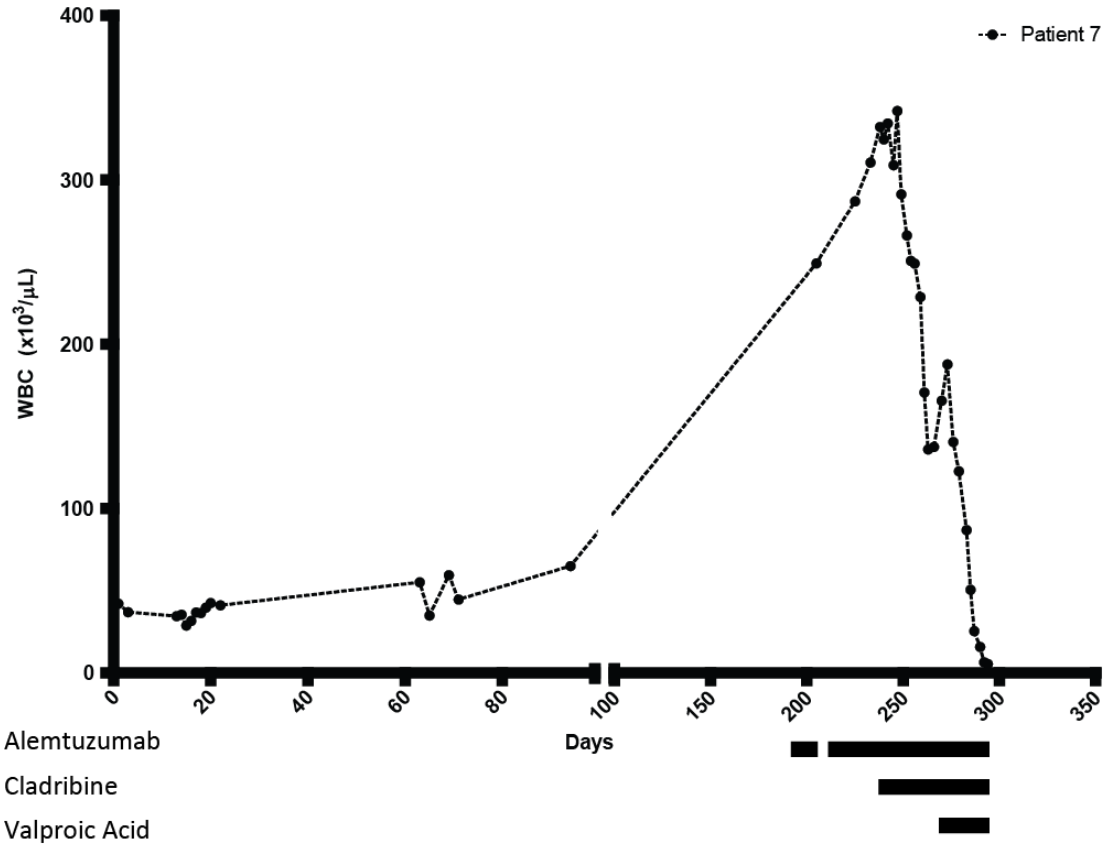

Patient 2
WBC (x10³/μL)
600
550
500
400
300
200
100
0
20
40
60
80
100
150
200
250
300
350
400
450
500
550
600
650
700
Days
Alemtuzumab
Cladribine
Vorinostat


Patient 3
300
200
100
0
WBC (x10³/μL)
0
20
40
60
80
100
120
140
160
180
200
Days
Alemtuzumab
Cladribine


Patient 4
250
200
150
100
50
0
WBC (x10³/μL)
0
20
40
60
80
100
200
300
400
500
Days
Alemtuzumab
Cladribine


Patient 5
WBC (x10³/μL)
250
200
150
100
50
0
0
20
40
60
80
100
200
300
400
500
600
Days
Alemtuzumab
Cladribine
Vorinostat
Romidepsin
Bren. Vedotin


Patient 6
WBC (x10³/μL)
50
40
30
20
10
0
0
10
20
30
Days
Alemtuzumab
Cladribine


Patient 7
WBC (x10³/μL)
400
300
200
100
0
0
20
40
60
80
100
150
200
250
300
350
Days
Alemtuzumab
Cladribine
Valproic Acid

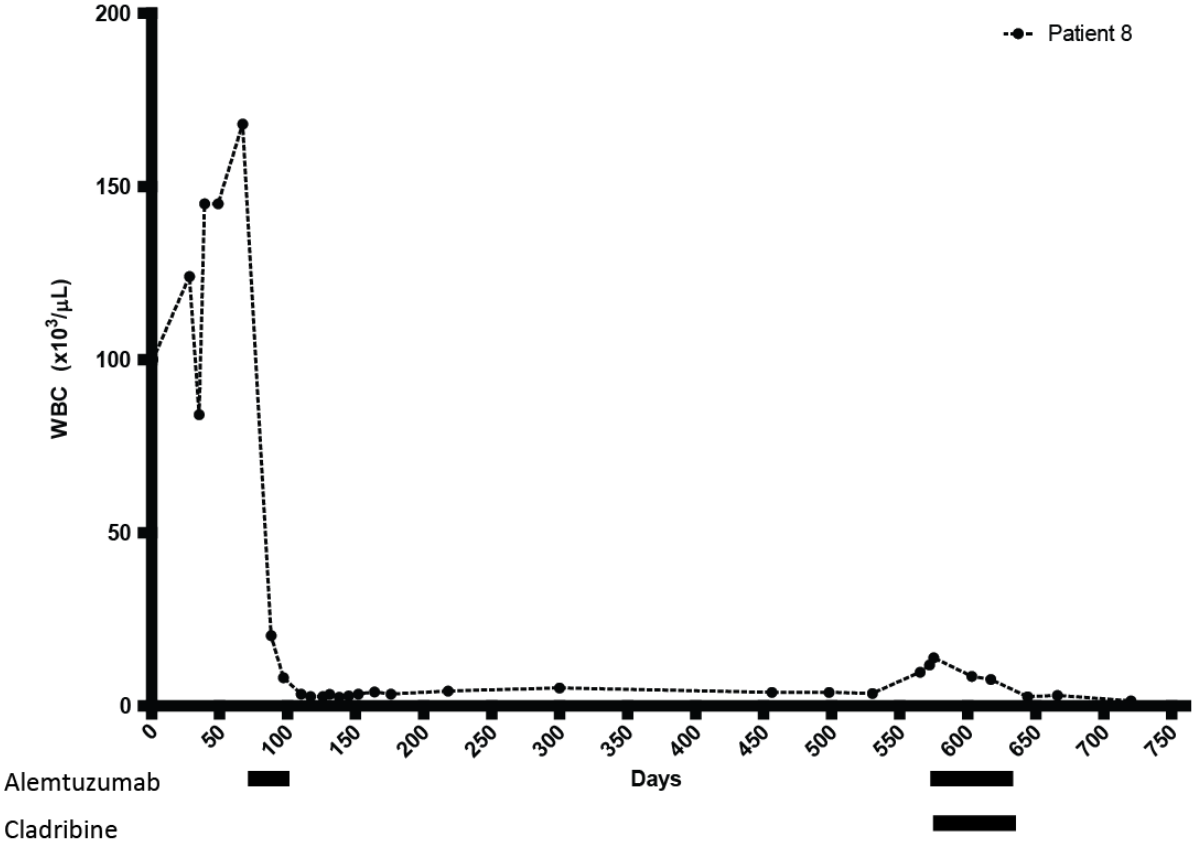

Patient 8
WBC (x10³/μL)
200
150
100
50
0
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
Days
Alemtuzumab
Cladribine

Figure S3: Epigenetic therapy lowered leukemic white blood cell counts despite multiple relapses.

Patient white blood cell counts were monitored during treatment. Day 0 was the initial white blood cell count at presentation**.** The bars beneath the graph represent treatment received by the patient over the corresponding time periods. Each patient's white blood cell counts are represented individually.

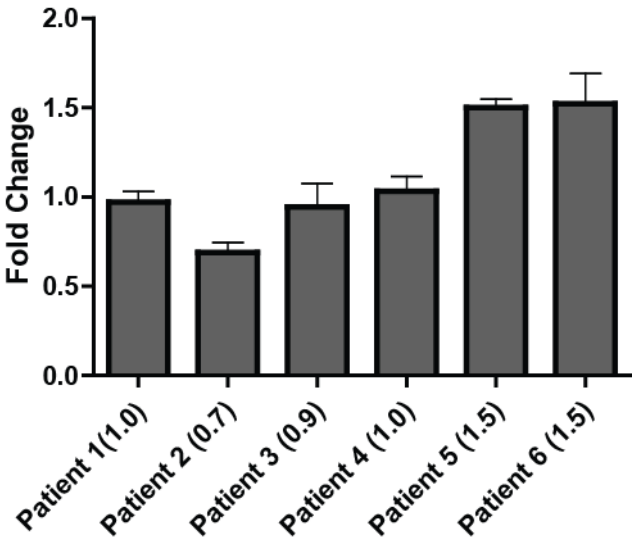


Figure S4: *CD52* expression was unchanged by epigenetic therapy.

T-PLL patient mRNA samples were assayed for *CD52* expression by qRT-PCR before and 5 days after treatment with alemtuzumab, cladribine, and/or vorinostat. Bars represent mean fold change +/- SEM. N=3.

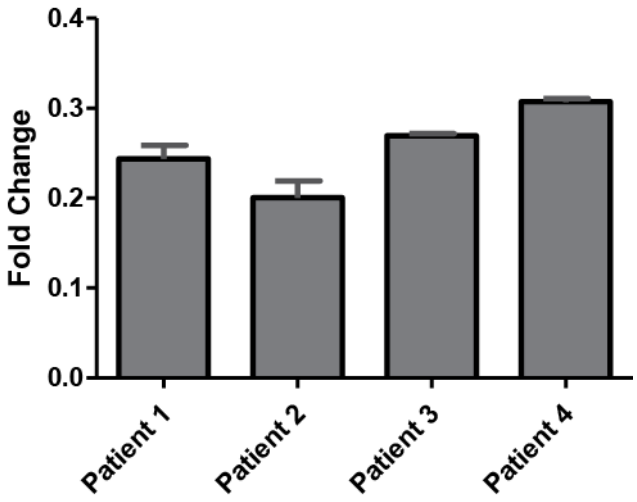


Figure S5: *CD30* induction was not observed in circulating MCL cells.

We used qRT-PCR to assay mRNA samples from patients with mantle cell lymphoma for *CD30* expression before and 5 days after treatment with cladribine. Bars indicate mean fold change +/- SEM. N=3.

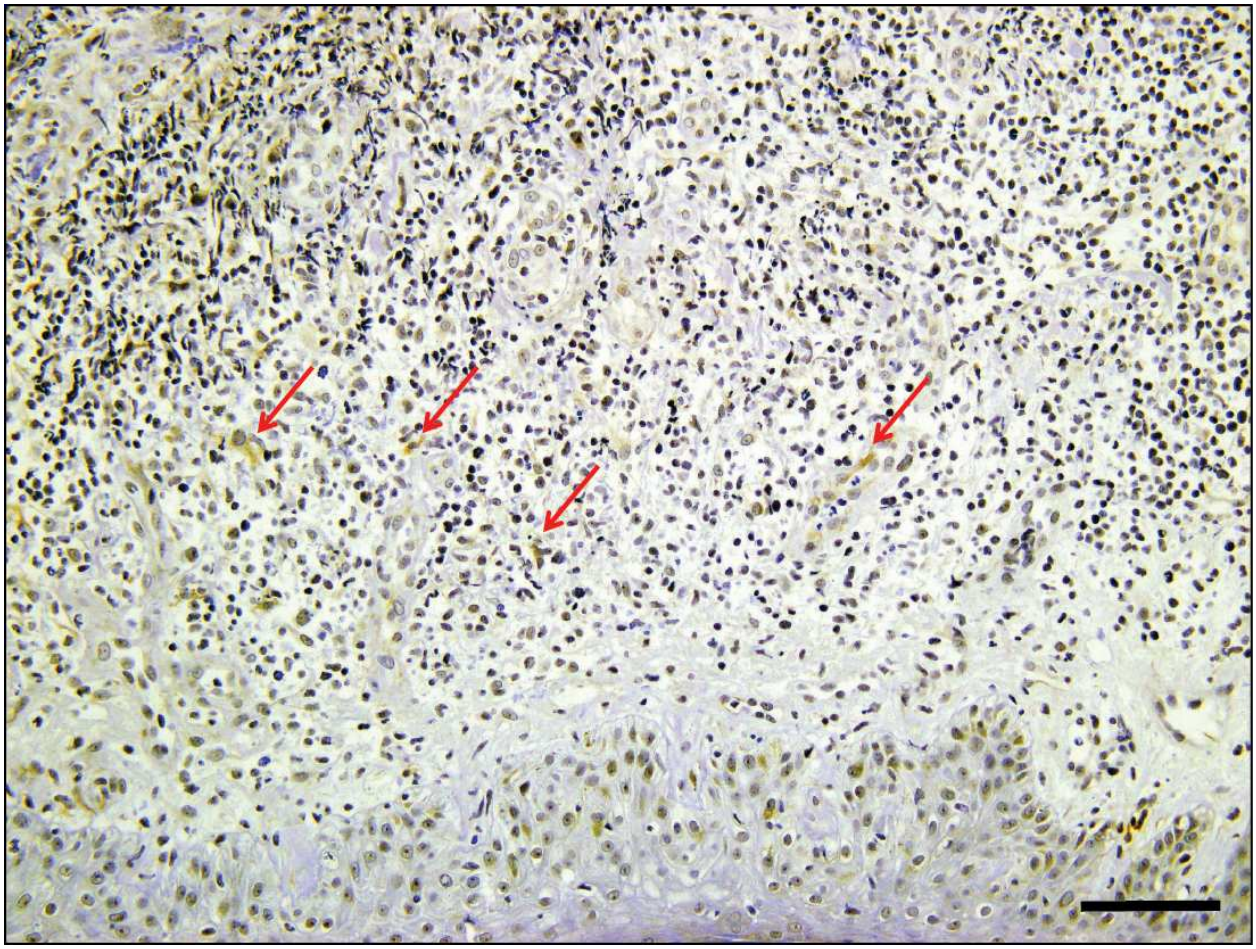


Figure S6: Skin biopsy from patient 5 shows CD52-positive dermis-infiltrating lymphocytes.

Tissue sections were paraffin-embedded and stained by immunohistochemistry for CD52 expression (brown). The bottom of the image is the epidermis. Red arrows indicate CD52-positive lymphocytes in the dermis. Scale bar is 100 μM.

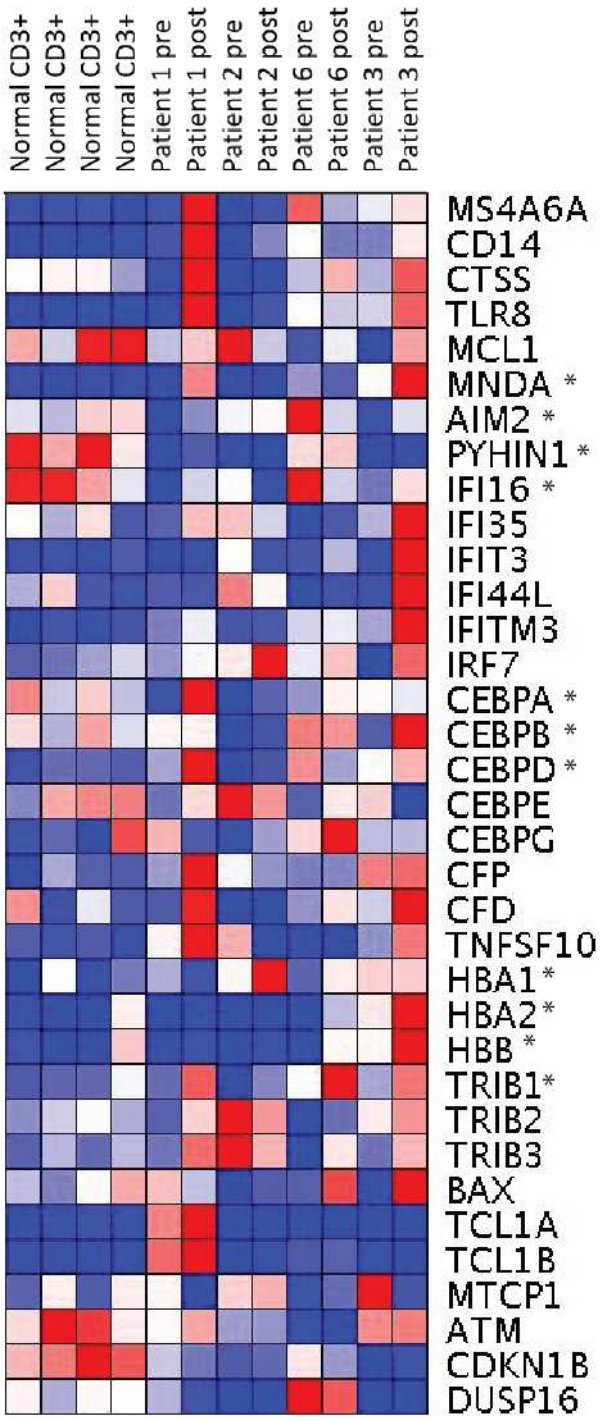

Normal CD3+
Normal CD3+
Normal CD3+
Normal CD3+
Patient 1 pre
Patient 1 post
Patient 2 pre
Patient 2 post
Patient 6 pre
Patient 6 post
Patient 3 pre
Patient 3 post
MS4A6A
CD14
CTSS
TLR8
MCL1
MNDA *
AIM2 *
PYHIN1 *
IFI16 *
IFI35
IFIT3
IFI44L
IFITM3
IRF7
CEBPA *
CEBPB *
CEBPD *
CEBPE
CEBPG
CFP
CFD
TNFSF10
HBA1*
HBA2*
HBB *
TRIB1*
TRIB2
TRIB3
BAX
TCL1A
TCL1B
MTCP1
ATM
CDKN1B
DUSP16

Figure S7: Epigenetic treatment of T-PLL causes mRNA expression changes in the HIN-200, CEBP, and tribbles families.

Samples are listed across the top and genes of interest are listed along the right column. Normal samples were mRNA from magnetic bead-separated CD3+ cells from normal donors. Only four patients had samples of sufficient quality and quantity for microarray experimentation. Starred genes were found to be upregulated after treatment and confirmed further by q-RT-PCR. Blue = low expression, white = medium expression, red = high expression

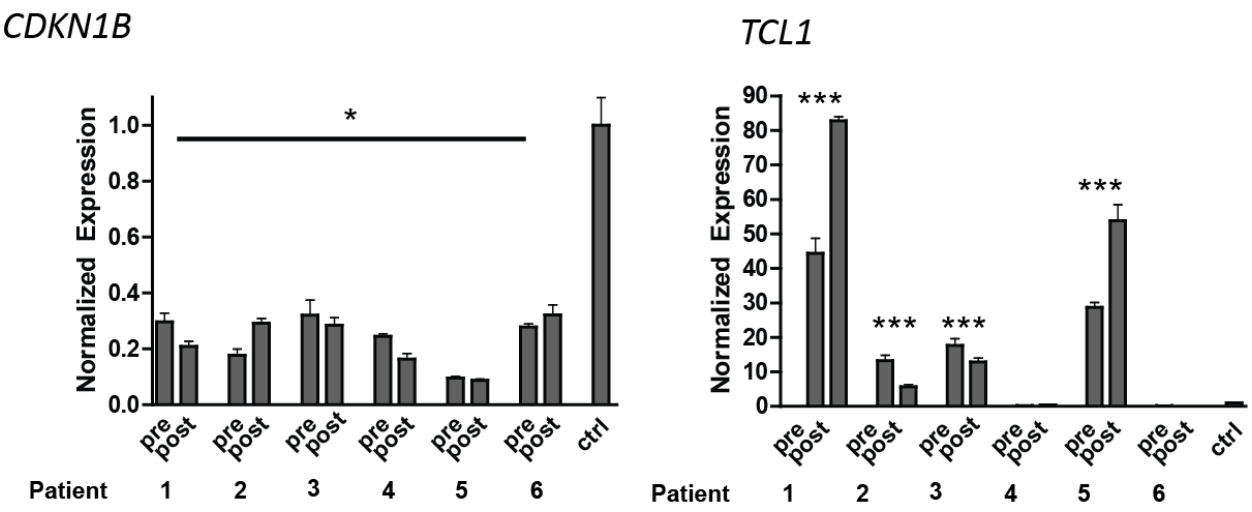


Figure S8: All tested patients showed *CDKN1B* haploinsufficiency, and four of six were *TCL1*-positive.

Bar graphs indicate *CDKN1B* and *TCL1* mRNA expression before and 5 days after treatment with epigenetic therapy, quantified by qRT-PCR. Bars represent mean expression +/- SEM. N=3. Values are normalized to an *HPRT* control. Significance is calculated from pre or post treatment values compared to control, not pre vs. post. * $p<0.05$, *** $p<0.0005$

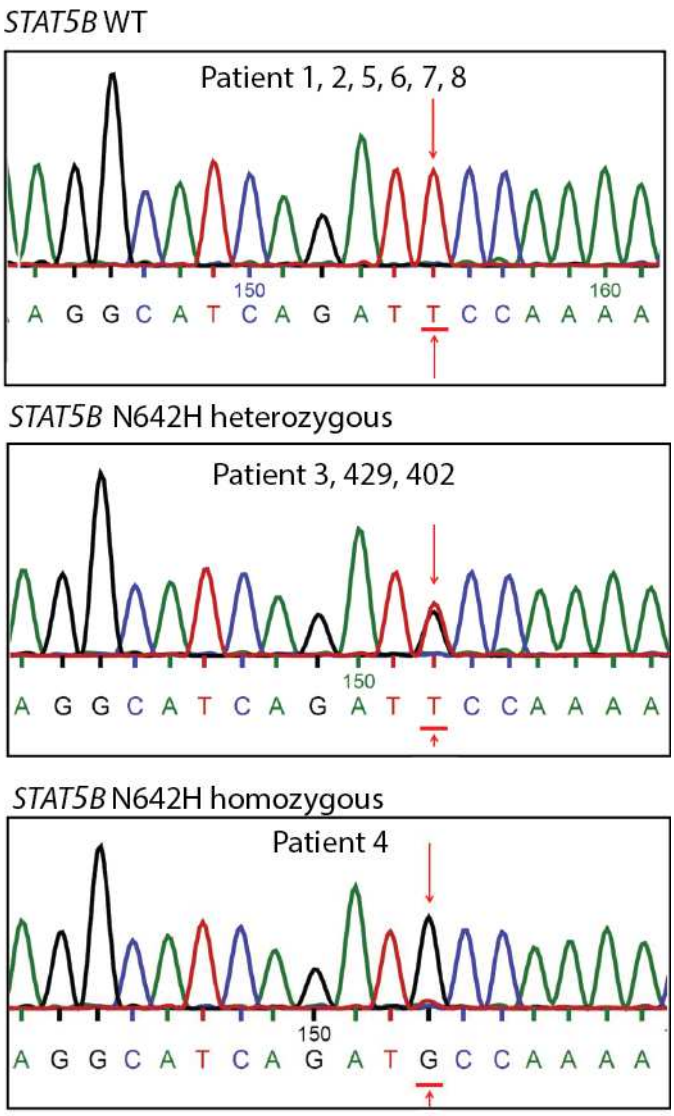


Figure S9: Forty percent of patients were positive for the N642H *STAT5B* mutation.

Mutational status of all patients was found to be *STAT5B* wild type (WT, top), N642H/WT heterozygous (middle), or N642H homozygous (bottom). Patients whose cells had each genotype are indicated in the corresponding images. The mutation site (T to G) is shown between the two red arrows and is underlined.

Patient	WBC k/μL	PMN k/μL	Lymph k/μL	Hb g/dL	Plts k/μL	Organ Involvement					Immunophenotyping Blood
						Blood	Marrow	Spleen	Lymph nodes	Other	
1	200	16	183.9	8.3	105	Yes	Yes	No	Yes	Pleura	CD2, CD4, CD5, CD7, CD45
2	121	15.7	95.7	13	203	Yes	Yes	Yes	Yes	—	CD2, CD3, CD4, CD5, CD7, CD8, CD45
3	256	17.9	215.2	13	73	Yes	Yes	Yes	Yes	—	CD2, CD4, CD5, CD7, CD45
4	248	12.4	231	6	181	Yes	Yes	No	No	—	CD2, CD4, CD5, CD7, CD8, CD45
5	211	8.5	198.7	13	185	Yes	Yes	No	Yes	Skin	CD2, CD4, CD5, CD7, CD8, CD45
6	21.8	1.5	19.9	13	123	Yes	Yes	Yes	No	Skin	CD2, CD3, CD4, CD5, CD7, CD45
7	34.5	9.3	23.3	10.9	543	Yes	Yes	Yes	No	—	CD2, CD3, CD5, CD7, CD8, CD45
8	145	12.5	126	12	119	Yes	Yes	Yes	Yes	—	CD3, CD5, CD7, CD8

Table S1: Patient presentation at diagnosis.

All patients were positive for TCR γ/δ rearrangements by PCR. WBC, white blood cell count; PMN, neutrophils; Lymph, lymphocytes; Hb, hemoglobin; Plts, platelets.

Patient	Gender	Age at diagnosis	Comorbidities	Major Adverse Events
1	F	64	none	lung infection (pneumonia): grade 2 neutrophil count decreased: grade 4 skin infection (VZV): grade 3 oral mucositis (pralatrexate): grade 3
2	M	73	hypertension, type 2 diabetes, gout, peripheral vascular disease, hyperlipidemia, coronary artery disease, GERD, BPH	platelet count decreased: grade 4 neutrophil count decreased: grade 4 anemia: grade 3
3	M	77	hypertension, arthritis, hyperlipidemia, IBS, spinal stenosis	anemia: grade 2 neutrophil count decreased: grade 3
4	F	63	sickle cell/beta thalassemia, iron overload, elevated liver enzymes, history of DVT, history of low grade non-Hodgkin's lymphoma	neutrophil count decreased: grade 4 anemia: grade 3 lung infection (CMV): grade 2 intracranial hemorrhage: grade 5
5	M	70	low grade B-cell lymphoma, hypercholesterolemia, asthma	febrile neutropenia: grade 3 neutrophil count decreased: grade 4 platelet count decreased: grade 4 CMV reactivation: grade 1
6	M	57	depression	neutrophil count decreased: grade 3 febrile neutropenia: grade 3
7	M	63	Prostate cancer post x-ray therapy	neutrophil count decreased: grade 3
8	M	66	type 2 diabetes, hypertension, depression, BPH	neutrophil count decreased: grade 4 platelet count decreased: grade 3

Table S2: Patient characteristics and adverse events.

The NCI CTCAE v4.0 was used to grade adverse events. VZV, varicella zoster virus; CMV, cytomegalovirus; CNS, central nervous system; GERD, gastroesophageal reflux disease; BPH, benign prostatic hypertrophy; DVT, deep vein thrombosis; IBS, irritable bowel syndrome.

Assay	Primer Name	Gene Represented	Sequence
ChIP	CD30 Prox Forward	*TNFRSF8* - promoter	ACC ACT AGT TCC GTC AAA TCC G
ChIP	CD30 Prox Reverse	*TNFRSF8* - promoter	CAC TTA GCT ACA AGC AGC GAA G
ChIP	CD30 +350 Forward	*TNFRSF8* - promoter	TTC GCT GCT TGT AGC TAA GTG
ChIP	CD30 +350 Reverse	*TNFRSF8* - promoter	AGT GGT TGT TCC TCC GAG
ChIP	CD30 -400 Forward	*TNFRSF8* - promoter	GGA TGG GTG GGT GAT GGT TG
ChIP	CD30 -400 Reverse	*TNFRSF8* - promoter	TGC CCT TGA GAT TCT TTG GAA G
qRT-PCR	CD30 Primer and Probe	*TNFRSF8*	Hs00174277_m1 (taqman assay)
qRT-PCR	18S Primer and Probe	*RNA 18 S5*	Hs03928992_g1 (taqman assay)
qRT-PCR	HBA Forward	*HBA1/2*	CGG AGG CCC TGG AGA GGA TGT T
qRT-PCR	HBA Reverse	*HBA1/2*	TGG CTC AGG TCG AAG TGC GG
qRT-PCR	HBB Forward	*HBB*	AGA ACT TCA GGC TCC TGG GCA AC
qRT-PCR	HBB Reverse	*HBB*	GGA CAG CAA GAA AGC GAG CTT AGT G
qRT-PCR	AIM2 Forward	*AIM2*	TGG GCA TGC TCT CCT GAG TCC T
qRT-PCR	AIM2 Reverse	*AIM2*	TGA CAA CTT TGG GAT CAG CCT CCT
qRT-PCR	IFI16 Forward	*IFI16*	ACT CCT GGA GCT CAG AAC CCG AAA
qRT-PCR	IFI16 Reverse	*IFI16*	ATC ACT GGG CGT TTT TGG AGA ACA T
qRT-PCR	MNDA Forward	*MNDA*	TAC TCC GAA TCA GGA AAC CCA GGC
qRT-PCR	MNDA Reverse	*MNDA*	CCA CCA CTG TCA CTG GGT CGT
qRT-PCR	PYHIN Forward	*PYHIN1*	CCC AAC ACT TCC TCA ACT GAG AGC C
qRT-PCR	PYHIN Reverse	*PYHIN1*	TCG CGA TTA TTG GGT CTT CTC GGA
qRT-PCR	Trib 1 Forward	*TRIB1*	CTG CGC TGC AAG GTG TTT CCC A
qRT-PCR	Trib 1 Reverse	*TRIB1*	GCG ATG GCA GCT GGA TGT AAG G
qRT-PCR	CEBPa Forward	*CEBPA*	CGC CAT GCC GGG AGA ACT CTA ACT
qRT-PCR	CEBPa Reverse	*CEBPA*	CTC TGC AGG TGG CTG CTC ATC GG
qRT-PCR	CEBPb Forward	*CEBPB*	CGC CGC CGC CTG CCT TTA AAT
qRT-PCR	CEBPb Reverse	*CEBPB*	TAC GCA GCA GCC AAG CAG TCC G
qRT-PCR	CEBPd Forward	*CEBPD*	CAG CCT CGC TTG GAC GCA GA
qRT-PCR	CEBPd Reverse	*CEBPD*	GTA GAA GGG CGC AGG CTC CG
qRT-PCR	HPRT Forward	*HPRT1*	CGT CTT GCT CGA GAT GTG ATG
qRT-PCR	HPRT Reverse	*HPRT1*	GCA CAC AGA GGG CTA CAA TGT G
qRT-PCR	p27 Forward	*CDKN2B*	TCG GAC AGC CAG ACG GGG TT
qRT-PCR	p27 Reverse	*CDKN2B*	GAA GAA TCG TCG GTT GCA GGT CGC
qRT-PCR	TCL1 Forward	*TCL1A*	CGC CTG GCT GCC CTT AAC CA
qRT-PCR	TCL1 Reverse	*TCL1A*	GAC GAC GTC TTC CCG ACG CA
mutant analysis	STAT5B N642H Forward	*STAT5B*	TGT TGG GGT TTT AAG ATT TCC
mutant analysis/sequencing	STAT5B N642H Reverse	*STAT5B*	CAA ATC AGA ATG CGA ACA TTG

Table S3: Primer sequences.

Figure 2A P values

Patient	*TNFRSF8*
1	0.0001
2	n.s.
3	n.s.
4	0.0012
5	n.s.
6	0.0048

Figure 2B P values

Patient 1	PolII	5meC	H3Ly9Me3	H3Ly27Me3
-400	n.s.	n.s.	n.s.	0.005
prox	0.0178	n.s.	n.s.	n.s.
+300	0.0375	0.0015	0.0025	0.0059

Figure 2C P values

Patient 5	PolII	5meC	H3Ly9Me3	H3Ly27Me3
-400	n.s.	n.s.	n.s.	n.s.
prox	0.02	0.002	0.006	0.0003
+300	n.s.	0.0006	0.007	0.04

Figure 3 P values

Day	*TNFRSF8*
383	0.0001
390	n.s.
411	n.s.

Figure 5 P values

Patient	*AIM2*	*MNDA*	*IFI16*	*PYHIN*	*CEBPA*	*CEBPB*	*CEBPD*	*HBA*	*HBB*
1	0.00040	0.0001	0.01790	0.00080	0.00010	n.s.	0.00040	n.s.	0.0024
2	0.00030	0.0013	0.00630	0.00040	0.00170	0.00380	0.00090	0.0015	0.0322
3	0.04730	0.0190	0.00220	0.03450	n.s.	0.01370	0.00540	n.s.	n.s.
4	0.00010	0.0059	0.02990	n.s.	n.s.	n.s.	0.00980	n.s.	0.0063
5	0.00110	0.0001	n.s.	0.02200	0.02390	0.00230	n.s.	0.0007	0.0026
6	n.s.	0.0237	n.s.	n.s.	0.00950	0.01050	0.03610	0.0005	0.0006

Figure 6A P values

Patient	*TRIB1*
1	0.0015
2	0.0002
3	0.0026
4	0.0011
5	n.s.
6	n.s.

Figure 6B P values

Patient	PolII	5meC	H3Ly9Me3	H3Ly27Me3	Acetyl
1	0.0229	n.s.	0.0316	n.s.	0.0051
5	0.0066	0.0001	0.0003	0.0037	0.0001

Figure S8 P values

Patient	*CDKN1B*	*TCL1*
1	0.0014	0.0001
2	0.0021	0.0023
3	0.0022	0.0004
4	0.0011	n.s
5	0.0008	0.0004
6	0.0029	n.s

Table S4: Significant *P* values for all statistically significant comparisons in figures.

P values are shown for $p<0.05$.