SUPPLEMENTAL MATERIALS

Supplemental Methods

Next-Generation Sequencing (NGS) utilizing the MyAML panel. Confirmation of individual patient's *MLL*-r and identification of genetic co-variants was performed at Invivoscribe (San Diego, CA). Briefly, 5 µg of high quality DNA was isolated from mononuclear cells collected from either peripheral blood or bone marrow aspirate and sequenced using an NGS panel designed to capture both the coding and non-coding regions of 194 AML specific genes. Sequencing was performed with 300 bp paired end reads on the Illumina MiSeq platform with an average coverage of 1,000X. Utilizing a custom bioinformatics pipeline that aligned reads to the human genome assembly 19 (hg19) reference genome, genetic alterations including single nucleotide variants, indels, inversions, translocations, and copy number variants were identified.

Chromatin immunoprecipitation sequencing (ChIP-seq). Leukemic blasts from patients in the 90 mg/m^2/day 28-day CIV cohort were analyzed for H3K79me2 levels at specific MLL target loci and non-MLL target gene sites[1]. Briefly, hematopoietic cells were FACS-isolated from patient peripheral blood samples before treatment and various days during the pinometostat infusion cycles, as indicated. Crosslinking was performed for 5 minutes in phosphate buffered saline with 1% formaldehyde and stopped by addition of glycine to 0.125M and Tris to 0.1M. After cytoplasm lysis, the nuclei were re-suspended in buffer containing 1% SDS in the presence of protease inhibitors. Chromatin was sheared using an E220 Covaris sonicator. Immunoprecipitation of chromatin fragments was performed using a rabbit monoclonal antibody specific for H3K79me2 (#5427, Cell Signaling Technology; Danvers, MA) immobilized on protein-A magnetic Dynabeads (Thermo Fisher; Waltham, MA). Eluted DNA-protein complexes were reverse cross-linked in 0.1M NaHCO3, 0.2M NaCl, 1% SDS, pH8.0 and purified with the Agencourt AMPure XP magnetic beads (Beckman Coulter; Brea, CA). Isolated DNA fragments were barcoded with the NEBNext DNA Library Preparation Kit (New England Biolabs; Ipswich, MA) according to the manufacturer's protocol and sequenced on an Illumina HiSeq 2000 platform.

Real-Time quantitative reverse transcription PCR (qRT-PCR). Expression levels for the MLL-r target gene *HOXA9* in patient PBMCs were determined by qRT-PCR analysis performed

by EA Genomics of Q^2 Solutions (Morrisville, NC). *HOXA9* expression was normalized to *MLL* fusion transcript levels, which are unaltered over the course of pinometostat treatment (data not shown). The sequences flanking the specific *MLL* rearrangement in samples were determined by RNA-seq analysis and used to design custom *MLL* TaqMan Assays for each patient. When possible, primers spanning the *MLL* fusion boundary were identified, and used with 5'VIC-labeled probes that overlapped the *MLL* breakpoint to minimize detection of off-target product.

Total RNA was isolated from peripheral blood samples collected at the start of study and at multiple time points over the course of pinometostat treatment. Samples were reverse transcribed using 0.25 - 0.5µg RNA with the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher). Templates were amplified in triplicate in 20µL final reaction volumes containing 1x TaqMan Universal PCR Master Mix, 3µL of 1:4 cDNA, and 1x *HOXA9* (Hs00365956_m1) - FAM TaqMan Assay primer and probe set using the manufacturer's recommended conditions. *RTF1* (Hs00385532_m1 – FAM was used as an internal control assay. *MLL*-fusion assays with VIC-labeled probes were multiplexed with the *HOXA9* FAM-TaqMan Assays. Each processing batch included no template controls (NTC) and no reverse transcriptase enzyme controls (NEC). Reactions were run for 45 cycles on an Applied Biosystems Fast 7500 PCR machine using standard TaqMan cycling parameters. NECs and NTCs passed quality control metrics of CT>40 and the internal control assays were readily detected and had CTs within the QC range of 5-30.

Supplemental Tables

Table S1. Listing of identified patient 11q23 rearrangements and genetic co-variants

Patient ID	Disease	Tissue	Days on Study	Local 11q23 rearrangement (cytogenetics)	Central 11q23 rearrangement and fusion partner (NGS)	co-Variants
A2	AML	BMA	8	(4;11)	(4;11) AFF1	IZKF1 (R83*); MTOR (T1881M); CSF1R (V32G); JAK2 (N1108); ASXL1 (R1316L)
A4	AML	PBMC	49	(6;11)	(6;11) MLLT4	NRAS (Q61K); PTPN11 (A72T); TET2 (H667Y, N1260S, G1606A); CSF1R (T507M); SRRM2 (R1689C)
A5	AML	BMA	65	(11;19)	(11;19) ELL	SRMM2 (K807T, T2289A)
A6	AML	PBMC	196	(11;19)	(11;19) MLLT1	TET2 (K244*, C1374W); SRSF2 (P95L); CBFB (N104S)
A7	AML	BMA	38	+11	MLL-PTD	DNMT3A (N879D); IDH1 (R132C, Q138K); KRAS (G12C); JAK1 (1 bp deletion); HDAC2 (1 bp insertion)
A8	AML	BMA	136	(11;19)	(11;19) ELL	NRAS (G12C); DIS3 (1 bp deletion)
A9	AML	BMA	31	non-MLLr	(6;11) MLLT4	SETD2 (R1543W); WT1 (17 bp deletion)
A10	AML	BMA	86	dup(11)(q22.1;q25)	non-MLLr	CEBPA (L331Q, N292S, Y181*); SRSF2 (P95H); ASXL1 (Y591*); STAG2 (W1103*); IDH1 (F32V); TET2 (A1153P); JAK2 (A800D)
A11	AML	PBMC	59	t(5;11)(q13;q21)	non-MLLr	KRAS (Q61L); PTPN11 (E76K); SF3B1 (H662Q)
A12	AML	PBMC	38	del(11)(q23q25)	(11;19) ELL	TP53 (Y126S); NSD1 (R1233Q)
A13	AML	PBMC	71	+11	MLL-PTD	DNMT3A (W89S); FLT3 D835V);IDH2 (R140Q)
A14	ALL	PBMC	80	(11;19)	(11;19) MLLT1	NRAS (G12D); ASXL1 (D799Y)
A15	AML	PBMC	13	(6;11)	(6;11) MLLT4	CBX5 (E61*)
A16	AML	PBMC	53	No Result	(9;11) MLLT3	FLT3 (D835H); WT1 (S381*)
A17	AML	PBMC	70	(9;11)	(9;11) MLLT3	GATA2 (D209Tfs*9)
A18	AML	PBMC	36	(10;11)	not sequenced	No sequencing result (Low DNA yield)
B1	AML	BMA	21	(3;11)	(3;11) TFG	U2AF1 (Q157P); PTPRT (R401W)
B3	AML	PBMC	21	(6;11)	(6;11) MLLT4	NOTCH1 (T1379P); BCR (T1018A)
B4	ALL	PBMC	82	(6;11)	(6;11) MLLT4	TET1 (K22fs*23); DIS3 (*959Q, stop loss); U2AF2 (1bp deletion)
B6	AML	PBMC	29	(9;11)	(9;11) MLLT3	FLT3 (Q580_E598dup); KIT (D816V);
B7	AML	PBMC	18	(10;11)	(10;11) MLLT10	TP53 (A138Pfs*7)
B8	ALL	PBMC	19	(4;11)	(4;11) AFF1	No variants detected
B9	AML	PBMC	21	(6;11)	(6;11) MLLT4	CBL (R718*); NRAS (Q61K); CEBPA (R323P); SUZ12 (M1?); WT1 (R471Pfs*30)
B10	AML	PBMC	17	(11;19)	(11;19) ELL	KRAS (G12D)
C3	AML	PBMC	33	(6;11)	(6;11) MLLT4	FLT3 (ITD 193 bp, ITD 39 bp, ITD 84 bp, ITD 36 bp)
C4	AML	PBMC	49	(1;11), (11;19)	(11;19) MLLT1	ASXL1 (S846N)
D2	AML	BMA	110	(11;19)	(11;19) ELL	GATA1 (L194P)
D3	AML	BMA	50	add 11q23	(10;11) MLLT10	SETD2 (R1625H); TET2 (P1655L); FANCC (1 bp deletion)
D4	AML	PBMC	44	(11;19)	(11;19) ELL	NRAS (Q61K)

Patient ID	Disease	Tissue	Days on Study	Local 11q23 rearrangement (cytogenetics)	Central 11q23 rearrangement and fusion partner (NGS)	co-Variants
D5	AML	PBMC	80	(11;1;10)	(10;11) MLLT10	U2AF1 (S34F)
D6	AML	PBMC	13	(11;19)	(11;19) ELL	JAK2 (N1108S); KRAS (G12D); NRAS (G13D); PRDM16 (Y68*); RPS6KA6 (G157*)
D7	AML	PBMC	3	(9;11;16)(p22;q23;q22)	non-MLLr	WT1 (S381*); SF1 (P609_P610del); ZRSR2 (R433_S434insSRGRGSR)
D8	AML	PBMC	109	(11;19)	(11;19) ELL	WT1 (A382Gfs*69); KIT (V399I); PRDM16 (S497Afs*42); PTEN (C304*)
F2	AML	PBMC	41	non-MLLr	MLL-PTD	FLT3 (D835V, 33 bp ITD); DNMT3A (R882H); KRAS (Q61R); U2AF1 (S34F); IDH1 (H40N); MECOM (G614fs*30); NOTCH1 (T2511P); WAPAL (splice donor variant); BCOR (1 bp deletion)
F3	ALL	BMA	15	(4;11)	(4;11) AFF1	FLT3 (N626S); TP53 (V197M); ASXL2 (S114N); TET2 (K1911I); CBL (in frame 3 bp deletion YD455-456Y); CTCF (2 bp deletion)
F4	AML	PBMC	28	No Result	MLL-PTD	FLT3 (57 bp ITD); DNMT3A (V636M); U2AF1 (S34F); ASXL3 (Q2111E)
F5	AML	PBMC	10	(10;11)	not sequenced	No sequencing result (Low DNA yield)
F6	AML	PBMC	50	non-MLLr	MLL-PTD	BCOR (V379Afs*62); DNMT3A (R882H); NF1 (R1276*); NRAS (Q61H); NRAS (G12D); PTPN11 (E76G); U2AF1 n(S34F); KMT2C (M305Dfs*28)
F7	MLL	PBMC	43	non-MLLr	non-MLLr	IDH2 (R140Q); RUNX1 (N448Rfs*147); SRSF2 (p95H)
G1	AML	NA	20	MLL-r	not sequenced	Sample unavailable
G2	ALL	NA	12	MLL-r	not sequenced	Sample unavailable

NA = not available

Table S2. Treatment-emergent AEs that occurred in 5 or more patients

	Dose Escalation						Expansion		
Primary System Organ Class Preferred Term, n (%)	12 mg/m²/day 21-day CIV (N=1)	24 mg/m²/day 21-day CIV (N=5)	36 mg/m²/day 21-day CIV (N=4)	54 mg/m²/day 21-day CIV (N=6)	80 mg/m²/day 21-day CIV (N=3)	90 mg/m²/day 28-day CIV (N=7)	54 mg/m²/day 28-day CIV (N=8)	90 mg/m²/day 28-day CIV (N=17)	Total (N=51)
Patients with at least 1 TEAE, n (%)	**1 (100)**	**5 (100)**	**4 (100)**	**6 (100)**	**3 (100)**	**7 (100)**	**8 (100)**	**17 (100)**	**51 (100)**
Metabolism and nutrition disorders	**0**	**4 (80)**	**3 (75)**	**4 (67)**	**3 (100)**	**6 (86)**	**4 (50)**	**14 (82)**	**38 (75)**
Hypokalaemia	0	0	1 (25)	1 (17)	1 (33)	4 (57)	4 (50)	5 (29)	16 (31)
Hypocalcaemia	0	0	1 (25)	2 (33)	0	3 (43)	0	9 (53)	15 (29)
Hypomagnesaemia	0	1 (20)	1 (25)	1 (17)	2 (67)	2 (29)	2 (25)	3 (18)	12 (24)
Hyperuricaemia	0	2 (40)	1 (25)	0	0	0	0	3 (18)	6 (12)
Hypophosphataemia	0	0	0	1 (17)	0	0	2 (25)	3 (18)	6 (12)
Decreased appetite	0	1 (20)	1 (25)	1 (17)	0	1 (14)	0	1 (6)	5 (10)
Gastrointestinal disorders	**0**	**2 (40)**	**2 (50)**	**3 (50)**	**1 (33)**	**5 (71)**	**8 (100)**	**16 (94)**	**37 (73)**
Nausea	0	1 (20)	2 (50)	2 (33)	1 (33)	2 (29)	4 (50)	8 (47)	20 (39)
Constipation	0	0	0	2 (33)	0	3 (43)	4 (50)	9 (53)	18 (35)
Vomiting	0	0	0	0	0	2 (29)	2 (25)	9 (53)	13 (25)
Diarrhoea	0	0	2 (50)	1 (17)	1 (33)	1 (14)	4 (50)	2 (12)	11 (22)
Abdominal pain	0	0	0	0	0	2 (29)	3 (38)	5 (29)	10 (20)
General disorders and administration site conditions	**0**	**2 (40)**	**3 (75)**	**6 (100)**	**1 (33)**	**7 (100)**	**5 (63)**	**12 (71)**	**36 (71)**
Fatigue	0	1 (20)	1 (25)	2 (33)	1 (33)	3 (43)	5 (63)	7 (41)	20 (39)
Oedema peripheral	0	1 (20)	0	1 (17)	0	5 (71)	1 (13)	5 (29)	13 (25)
Pyrexia	0	1 (20)	0	2 (33)	1 (33)	4 (57)	0	2 (12)	10 (20)
Mucosal inflammation	0	1 (20)	1 (25)	1 (17)	1 (33)	3 (43)	0	2 (12)	9 (18)
Chills	0	0	0	0	0	1 (14)	0	5 (29)	6 (12)
Malaise	0	0	0	0	0	1 (14)	1 (13)	4 (24)	6 (12)
Blood and lymphatic system disorders	**1 (100)**	**0**	**3 (75)**	**4 (67)**	**1 (33)**	**4 (57)**	**7 (88)**	**11 (65)**	**31 (61)**
Febrile neutropenia	0	0	1 (25)	1 (17)	0	3 (43)	5 (63)	8 (47)	18 (35)
Leukocytosis	0	0	1 (25)	1 (17)	1 (33)	0	3 (38)	5 (29)	11 (22)
Anaemia	1 (100)	0	0	3 (50)	0	1 (14)	3 (38)	2 (12)	10 (20)

continued

Table S2. Treatment emergent AEs that occurred in 5 or more patients (continued)

	Dose Escalation						Expansion		
Primary System Organ Class Preferred Term, n (%)	**12 mg/m²/day 21-day CIV (N=1)**	**24 mg/m²/day 21-day CIV (N=5)**	**36 mg/m²/day 21-day CIV (N=4)**	**54 mg/m²/day 21-day CIV (N=6)**	**80 mg/m²/day 21-day CIV (N=3)**	**90 mg/m²/day 28-day CIV (N=7)**	**54 mg/m²/day 28-day CIV (N=8)**	**90 mg/m²/day 28-day CIV (N=17)**	**Total (N=51)**
Thrombocytopenia	1 (100)	0	1 (25)	0	0	2 (29)	0	2 (12)	6 (12)
Respiratory, thoracic and mediastinal disorders	**1 (100)**	**2 (40)**	**1 (25)**	**5 (83)**	**1 (33)**	**4 (57)**	**4 (50)**	**13 (76)**	**31 (61)**
Cough	1 (100)	0	0	2 (33)	0	3 (43)	2 (25)	3 (18)	11 (22)
Dyspnoea	0	0	0	1 (17)	1 (33)	1 (14)	1 (13)	7 (41)	11 (22)
Respiratory failure	0	0	0	0	0	2 (29)	0	5 (29)	7 (14)
Oropharyngeal pain	0	1 (20)	0	2 (33)	0	1 (14)	1 (13)	1 (6)	6 (12)
Pleural effusion	0	1 (20)	0	0	0	2 (29)	1 (13)	1 (6)	5 (10)
Investigations	**1 (100)**	**4 (80)**	**2 (50)**	**2 (33)**	**0**	**4 (57)**	**3 (38)**	**12 (71)**	**28 (55)**
Blood alkaline phosphatase increased	0	2 (40)	0	1 (17)	0	0	1 (13)	2 (12)	6 (12)
Platelet count decreased	0	0	0	0	0	0	2 (25)	4 (24)	6 (12)
Blood bilirubin increased	0	2 (40)	0	1 (17)	0	0	0	2 (12)	5 (10)
Blood creatinine increased	0	2 (40)	0	0	0	1 (14)	0	2 (12)	5 (10)
ECG QT prolonged	0	0	1 (25)	0	0	0	1 (13)	3 (18)	5 (10)
International normalised ratio increased	0	0	0	0	0	1 (14)	0	4 (24)	5 (10)
WBC count increased	0	0	2 (50)	0	0	0	0	3 (18)	5 (10)
Nervous system disorders	**0**	**2 (40)**	**3 (75)**	**5 (83)**	**2 (67)**	**3 (43)**	**1 (13)**	**9 (53)**	**25 (49)**
Dizziness	0	0	0	1 (17)	1 (33)	1 (14)	0	2 (12)	5 (10)
Headache	0	1 (20)	0	1 (17)	0	0	0	3 (18)	5 (10)
Infections and infestations	**0**	**1 (20)**	**2 (50)**	**4 (67)**	**3 (100)**	**3 (43)**	**3 (38)**	**7 (41)**	**23 (45)**
Pneumonia	0	0	0	2 (33)	3 (100)	2 (29)	0	2 (12)	9 (18)
Musculoskeletal and connective tissue disorders	**0**	**3 (60)**	**3 (75)**	**2 (33)**	**1 (33)**	**2 (29)**	**3 (38)**	**5 (29)**	**19 (37)**
Arthralgia	0	1 (20)	2 (50)	1 (17)	1 (33)	0	0	1 (6)	6 (12)
Pain in extremity	0	1 (20)	1 (25)	0	1 (33)	0	2 (25)	0	5 (10)
Vascular disorders	**0**	**1 (20)**	**1 (25)**	**0**	**1 (33)**	**2 (29)**	**1 (13)**	**5 (29)**	**11 (22)**
Hypotension	0	0	1 (25)	0	1 (33)	1 (14)	0	3 (18)	6 (12)

Supplemental Figures

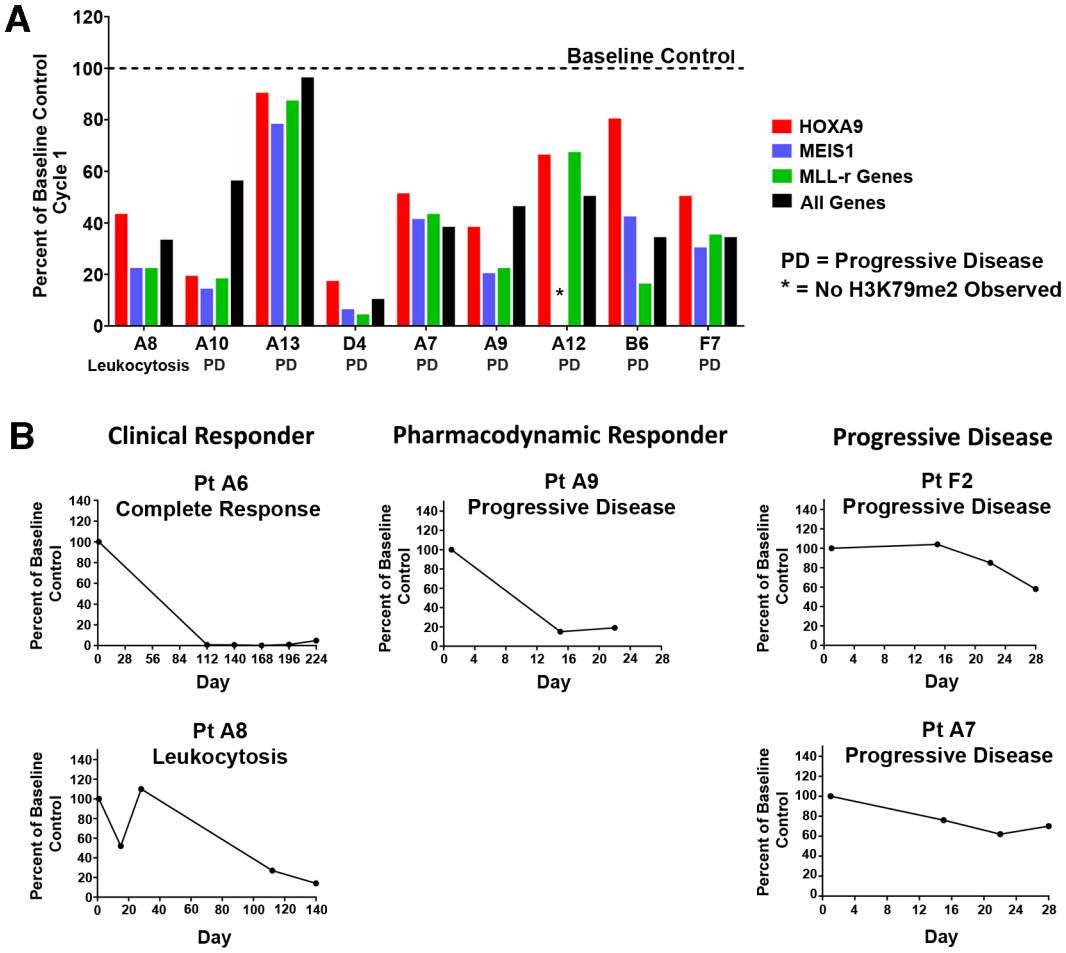

Figure S1. Decreased levels of H3K79me2 and transcription at MLL-r target gene loci. (A) H3K79me2 ChIP-seq was performed on leukemic blasts isolated from individual patients in the 90 mg/m^2/day DEsc cohort with enough material to obtain 200,000 viable cells. Results from ChIP-seq analysis demonstrate that pinometostat inhibits H3K79 methylation both globally (black bars) and at MLL-r target genes (green bars). Further analysis of MLL-r target genes *HOXA9* (red bars) and *MEIS1* (blue bars) demonstrate that pinometostat also inhibits H3K79me2 specifically at loci implicated in MLL-r driven leukemogenesis. PD, progressive disease. (B) Real-time quantitative reverse transcriptase PCR analysis of *HOXA9* mRNA expression in frozen PBMC pellets collected from patients with different response profiles. Patients were selected based on the availability of material and sequence evidence describing the *MLL* breakpoint junction. *HOXA9* expression was normalized for changes in blast counts by utilizing each patient's unique *MLL* fusion breakpoint. Patients showing evidence of clinical responses tended to show reduced *HOXA9* levels relative to those with progressive disease.

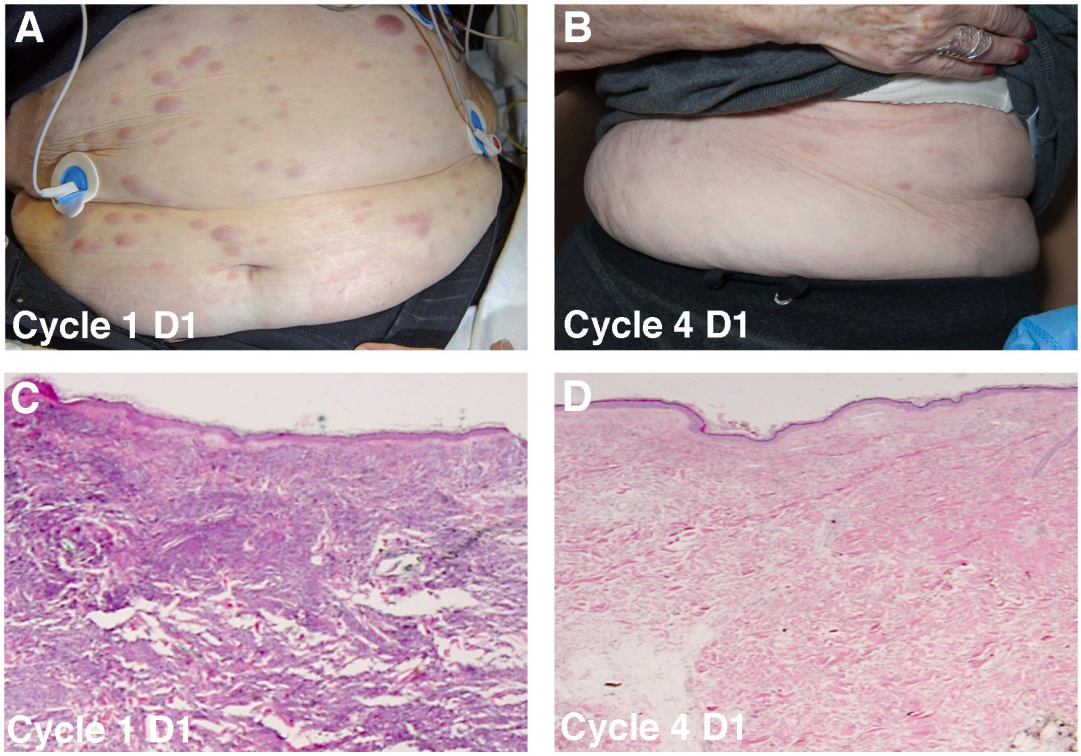

Figure S2. Histological resolution of leukemia cutis. (A) Abdominal lesions in CMML patient that resolved over the course of pinometostat treatment cycles (B). Histological characterization of abdominal lesion from the same patient at the start of treatment using hematoxylin and eosin staining, showing dense cutaneous infiltration by monocytic cells (C). By the start of the fourth treatment cycle, the infiltrate had largely cleared (D).

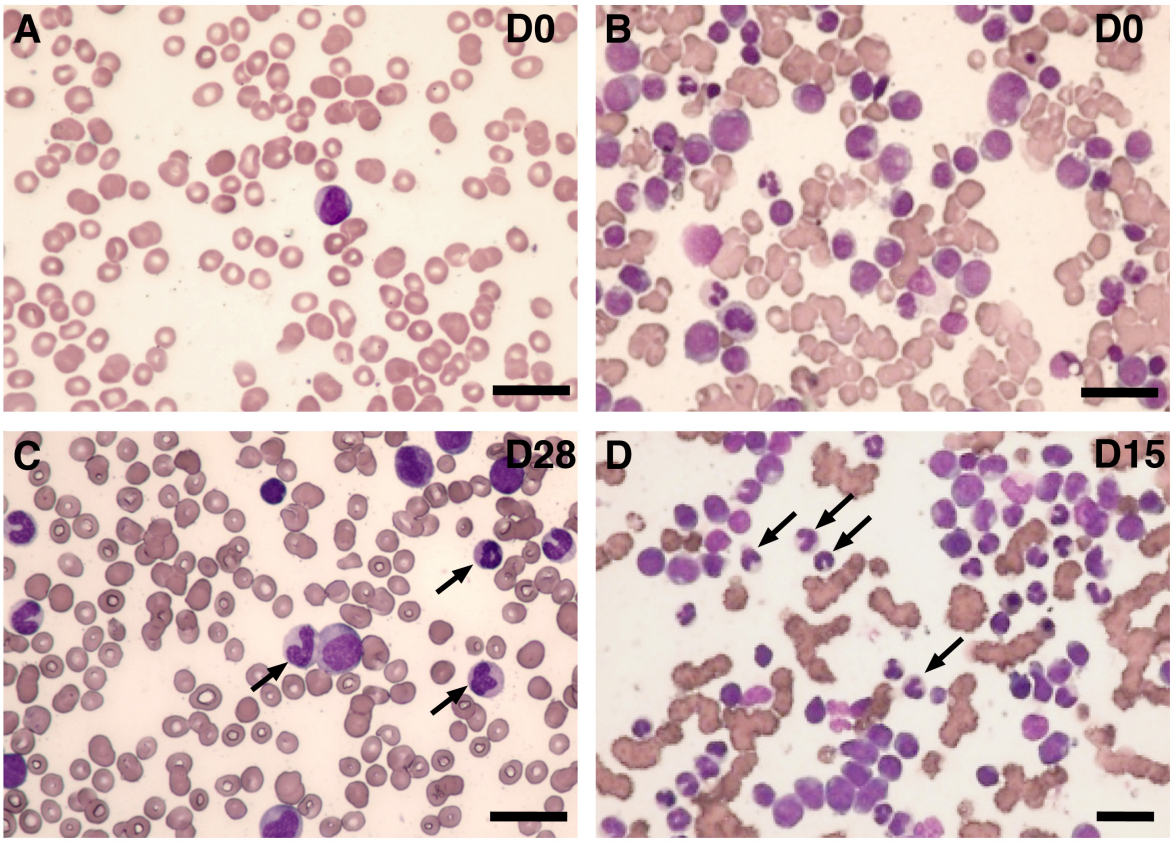

Figure S3. Pinometostat-induced differentiation of leukemic blasts in *MLL-r* patients. Pleomorphic leukocytosis in PBMC samples before treatment (A) and at the end of cycle 1 following 28-day (D28) pinometostat CIV infusion (C). Leukemic blasts in a pretreatment bone marrow sample showing increased numbers of immature cells (B). After 15 days of pinometostat treatment, bone marrow samples show evidence of increased myeloid differentiation (D). Arrowheads in C and D show differentiated leukocytes following pinometostat treatment. All panels, hematoxylin and eosin staining. Scale Bar = 50 μm for all.

REFERENCES

1. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukemia stem-cell development. *Nat Rev Cancer.* 2007;7(11):823-833.