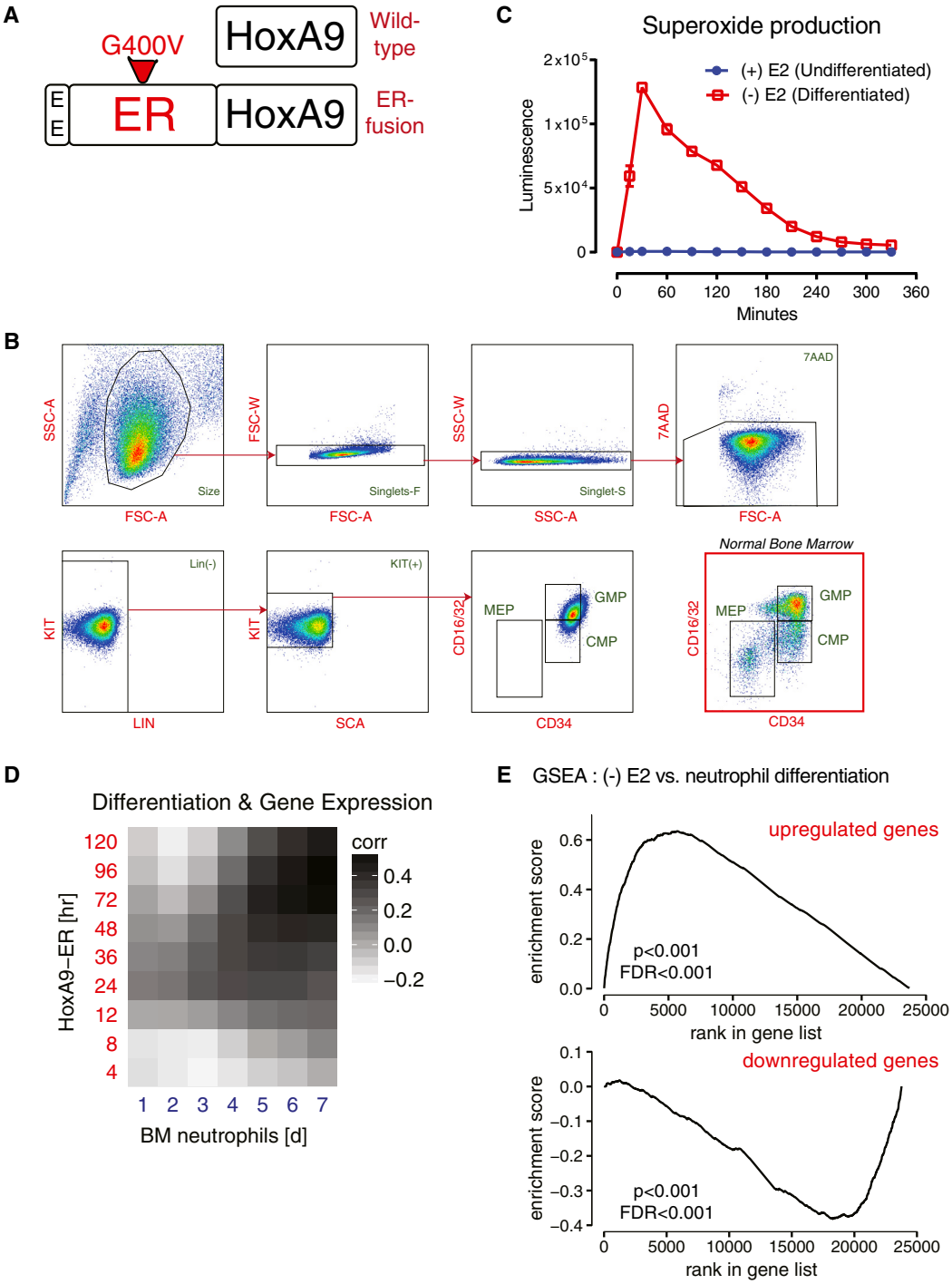


Figure S1. ER-HoxA9 Cells Are a Source of Unlimited GMPs, Related to Figure 1

(A) Fusion of the G400V variant of the human estrogen receptor to HoxA9 results in a protein that is constitutively expressed but whose function depends on the presence of beta-estradiol. A Glu-Glu (EE) epitope tag was added to the N terminus to allow for antibody detection. (B) Primary murine bone marrow cells transduced with MSCVneo-ER-HoxA9 grow as SCF-dependent GMP (KIT+, Linneg, Scaneg, CD34+, CD16/32+) cell lines in the presence of beta-estradiol (E2). The same staining and analysis of fresh normal bone marrow cells (red frame) is shown for comparison. (C) Upon differentiation, the ER-HoxA9 cells demonstrate terminal effector function such as the production of superoxide. (D and E) Heat map and gene set enrichment analysis demonstrates a high correlation of the gene-expression changes that accompany differentiation of the ER-HoxA9 cells over the five-day time course and unmanipulated primary bone marrow myeloid cells over a seven-day time course.

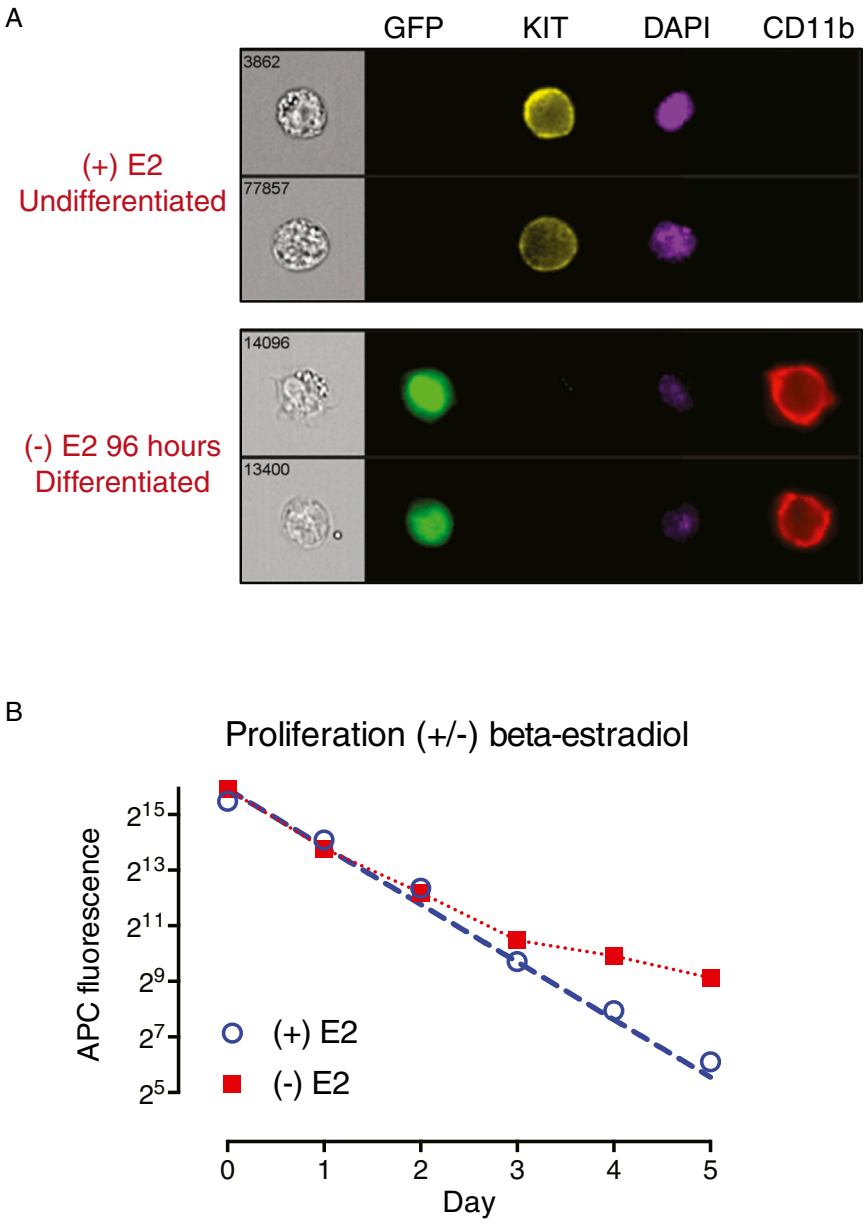


Figure S2. Lys-GFP-ER-HoxA9 Cells Differentiate and Stop Proliferating upon Removal of Beta-Estradiol, Related to Figure 2

(A) Imaging flow cytometry demonstrates upregulation of GFP and CD11b expression as well as the downregulation of KIT expression in Lys–GFP–ER–HoxA9 cells during differentiation in the absence of estradiol (-E2). (B) Cells stained with CFSE were analyzed by flow cytometry daily for APC fluorescence, demonstrating that Lys–GFP–ER–HoxA9 cells divide every 14 hr in the presence of estradiol, and undergo proliferation-arrest following 5 divisions in the absence of estradiol.

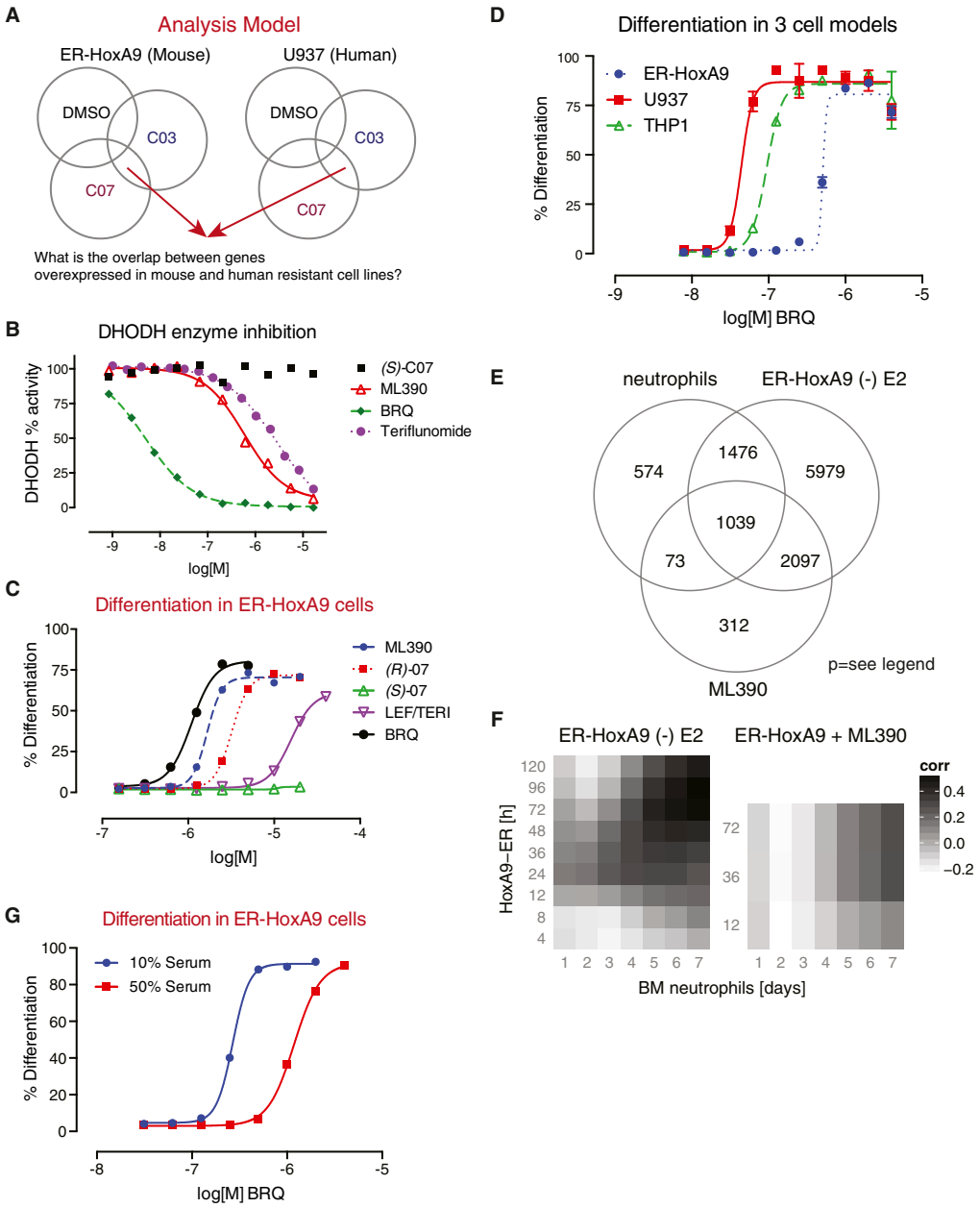
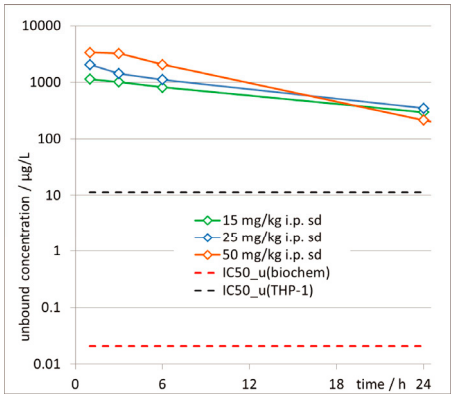


Figure S3. ML390 and Other Inhibitors of DHODH Trigger Myeloid Differentiation, Related to Figure 3

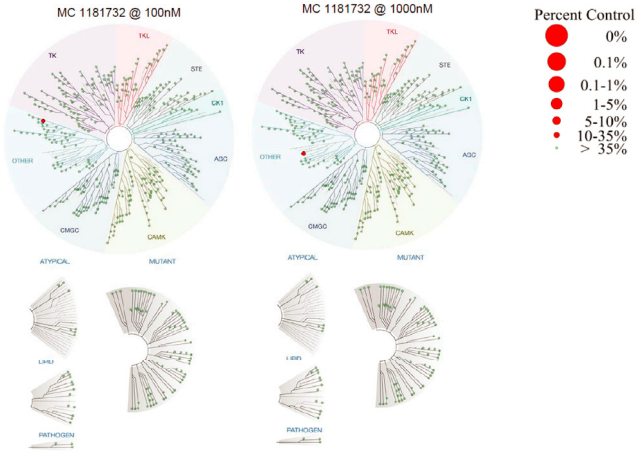
(A) Schematic depicting our approach to analyzing the gene-expression data by comparing those genes upregulated in the C03 and C07-resistant cell lines to the DMSO control in ER–HoxA9 cells, and then overlapping this group of genes with the same analysis in the U937 cells. Genes that exhibited a greater than 2-fold change, with a p-value of less than 0.01 were considered differentially expressed genes. This resulted in a list of only eight syntenic genes common to the human (chromosome 16) and mouse (chromosome 8) cell lines, as depicted in Figure 3B and Figure 3C. (B) Known DHODH inhibitors brequinar (BRQ) and teriflunomide are compared to ML390 in the in vitro recombinant DHODH enzyme inhibition assay. (C) Brequinar, leflunomide, teriflunomide, *R*-07, *S*-07, and ML390 are compared in the ER-HoxA9 cellular differentiation assay. (D) Brequinar triggers differentiation in ER-HoxA9, U937, and THP1 cells at an EC_{50} of < 1 μM. (E) A Venn diagram depicts the overlap in gene-expression changes that accompanies the differentiation of the ER-HoxA9 cells out of estrogen, the differentiation of primary murine bone marrow myeloid cells, and the differentiation of the ER-HoxA9 cells treated with ML390 at 10 μM for 72 hr. For each sample, genes with a corrected p-value (Benjamini-Hochberg) below 0.01 were considered differentially expressed. Pairwise comparisons show that the overlap between samples is highly significant (neutrophils versus ER-HoxA9: $p = 2.9e^{-81}$; neutrophils versus ML390: $p = 2.3e^{-54}$; ER-HoxA9 versus ML390: $p = 4.3e^{-214}$; Fisher's exact test). (F) Heatmap showing the correlation of the gene expression changes that accompany differentiation of the ER-HoxA9 cells over the five-day time course (left panel), unmanipulated primary bone marrow myeloid cells over a seven-day time course (horizontal-axis), and ML390 treatment of the ER-HoxA9 cells (right panel).

A Brequinar plasma concentration after single IP dose



Dose	mg/kg	15	25	50
AUC(0-t_{last})	µg·h/L	1052599	1442857	1638106
AUC(0-t_{last})$_{norm}$	kg·h/L	70.2	57.7	32.8
t_{last}	h	24	24	48
C_{max}	µg/L	81275	147158	168786
$C_{max,norm}$	kg/L	5.42	5.89	3.38
T_{max}	h	1	1	1
C(24 h)/C_{max}		26%	17%	6.4%
$t_{1/2}$	h	12.0	10.5	5.82
%AUC(t_{last}-oo)		35%	26%	0.34%
AUC	µg·h/L	1418663	1819382	1643706
AUC_{norm}	kg·h/L	94.6	72.8	32.9
AUC	µM·h	3570	4579	4137
AUC_u	µM·h	50	64	58

B Brequinar kinase-inhibitory activity in the DiscoverX Kinase assay



C

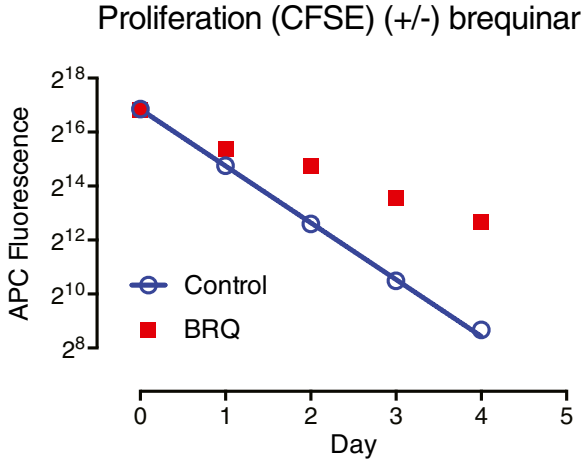


Figure S4. BRQ Has Desirable Pharmacokinetic Properties and Lacks Kinase-Inhibitory Activity, Related to Figure 4

(A) The concentration of brequinar in plasma was monitored in healthy mice after a single IP dose of 15 mg/kg, 25 mg/kg, or 50 mg/kg and compared to the expected in vitro EC_{50} for differentiation in THP1 cells cultured in RPMI with 10% FBS (dotted black line). (B) The kinase-inhibitory activity of brequinar (at concentrations of 100 nM and 1 µM) was assayed across a panel of known kinases (DiscoverX). Brequinar has negligible kinase inhibition against this panel of known kinases (note the general absence of red marker dots). (C) Cells stained with CFSE were analyzed daily by flow cytometry for APC fluorescence, demonstrating a growth-inhibitory effect of brequinar in Lys–GFP–ER–HoxA9 cells (cf. Figure S2B).

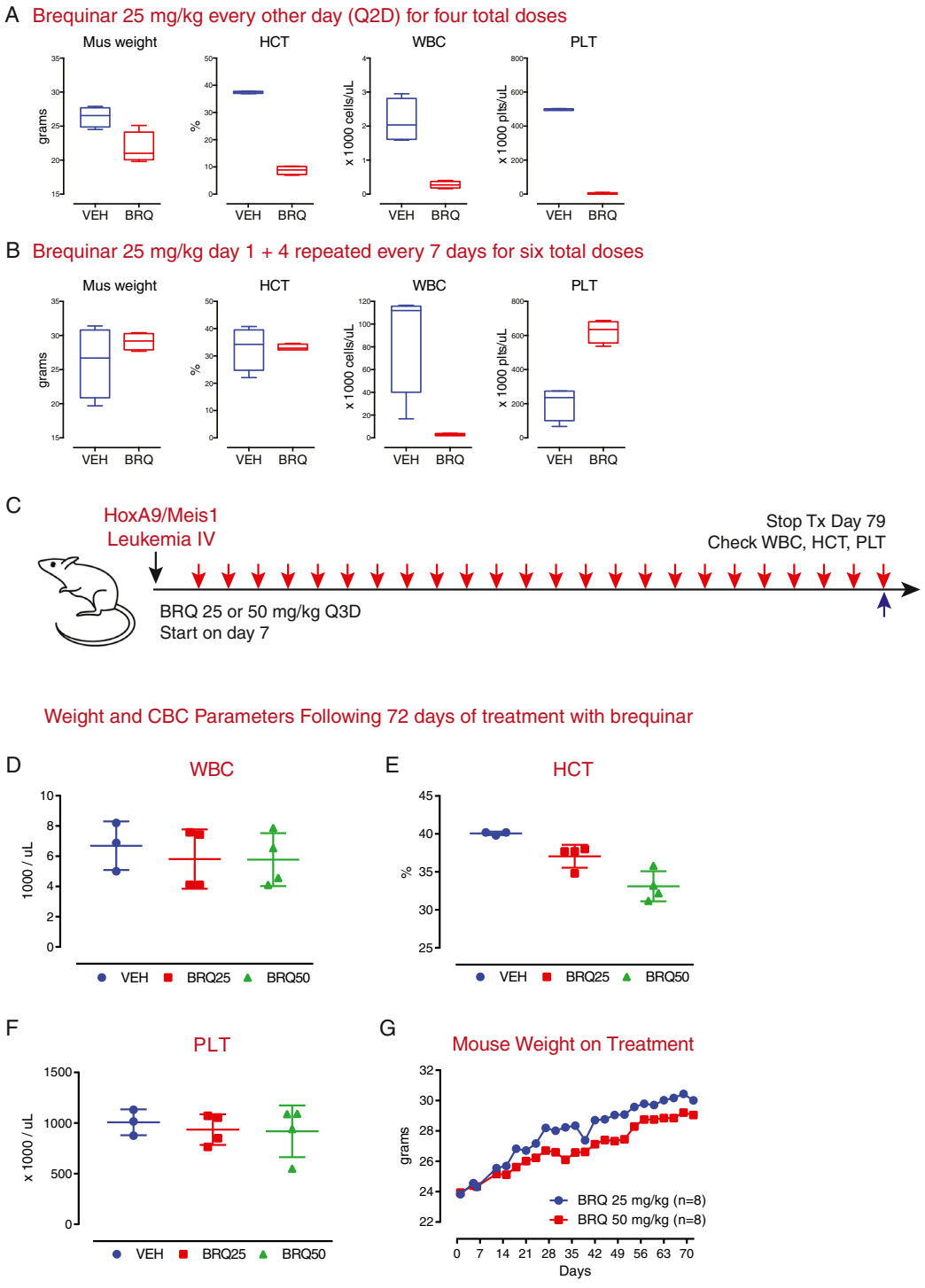


Figure S5. The Adverse Effects of DHODH Inhibition Are Abrogated by Changes in Drug Schedule, Related to Figure 6

The weight, hematocrit (HCT), peripheral white blood cell (WBC) count, and platelet (PLT) count were monitored in (A) mice that were treated with 25 mg/kg brequinar given every other day for a total of 4 doses or (B) 25 mg/kg given on day 1 and 4 of a 7-day schedule for a total of 6 doses. The weight loss, anemia, and thrombocytopenia seen with the every-other-day dosing were not observed with the day 1 + 4 dosing highlighting an important relationship of dose schedule and normal tissue toxicities. (C) Leukemia-bearing mice were treated with brequinar 25 mg/kg or 50 mg/kg every three days for a total of 24 doses (72 days). The mice exhibited a mild anemia (E), but normal WBC (D), normal PLT (F), and normal weight gain (G). Data in (A) and (B) are box and whisker plots where the mean, the minimum, and the maximum are indicated. Data in (D), (E) and (F) are represented as the mean ± SD.

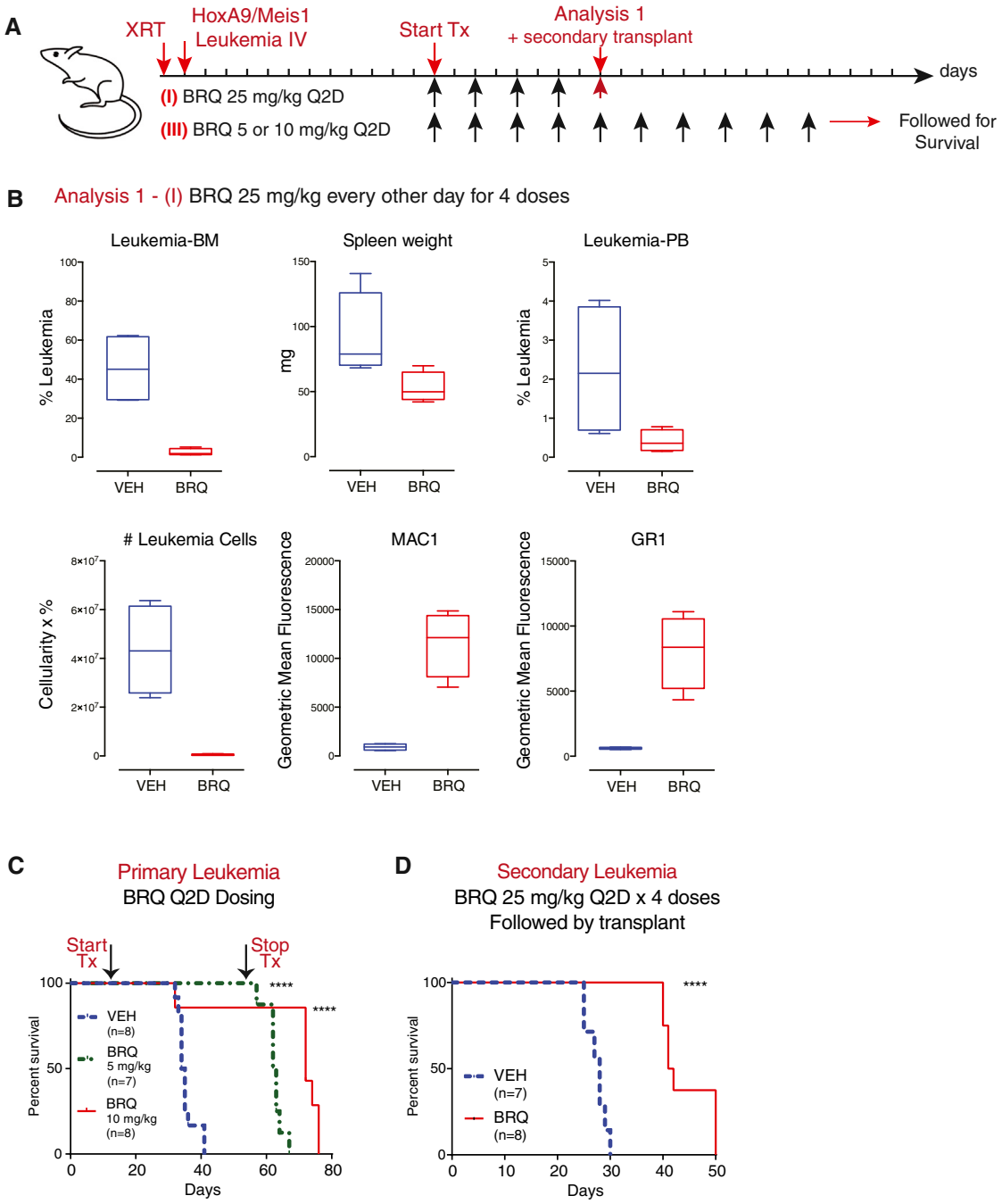


Figure S6. BRQ, Given on an Every 2 Day Schedule, Is Effective in a Model of HoxA9+Meis1 Syngeneic Leukemia, Related to Figure 6

(A) Mice were treated with brequinar IP given at 5, 10, or 25 mg/kg brequinar every other day as indicated. (B) Brequinar 25 mg/kg given for a total of 4 doses results in decreased leukemic burden in the bone marrow, normalization of spleen weight, decreased leukemia in the peripheral blood, and increased differentiation markers compared to vehicle-treated mice. (C) Treatment with brequinar results in prolonged survival. (D) Treatment with brequinar results in a decrease in the number of leukemia initiating cells. Data in (B) are box and whisker plots where the mean, the minimum, and the maximum are indicated. Data in (D), (E) and (F) are represented as the mean ± SD.

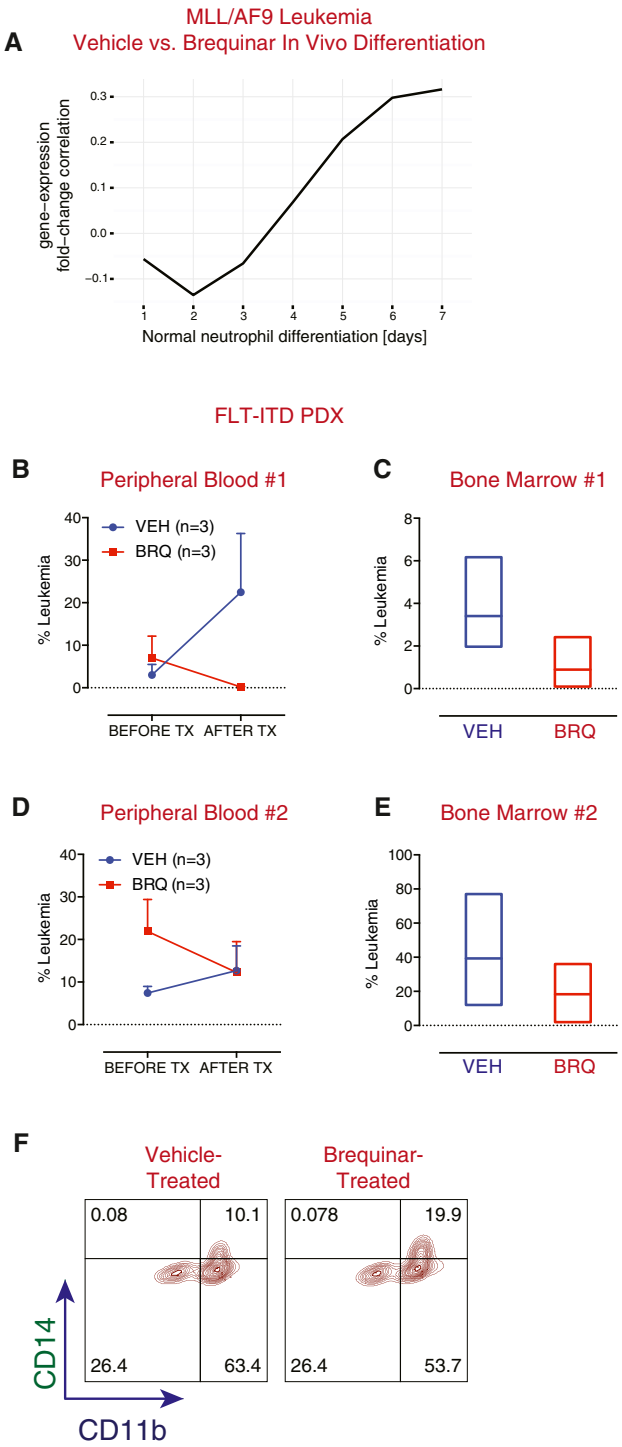


Figure S7. BRQ Causes Differentiation in Models of MLL/AF9 Syngeneic AML as well as PDX AML, Related to Figure 7

(A) MLL/AF9 leukemia cells were FACS-sorted from mice treated with vehicle or brequinar. RNA-sequencing demonstrated in vivo gene expression changes consistent with myeloid differentiation. (B) NSG mice were engrafted with a FLT3-ITD PDX AML. Treatment of one cohort with four doses of brequinar (25 mg/kg) led to decreased leukemia burden in the peripheral blood (B) and bone marrow (C). Treatment of a second cohort with two doses of brequinar (25 mg/kg) lead to decreased leukemia burden in the peripheral blood (D) and bone marrow (E) as well as evidence of in vivo differentiation as indicated by an increase in CD14 expression (F). Data in (C) and (E) are box and whisker plots where the mean, the minimum, and the maximum are indicated. Data in (B) and (D) are represented as the mean ± SD.