Figure 5. Effect of DAC on the level of DNMT1 and its chromatin trapping. Cells were exposed to drug(s) for 48 hrs (A and B) or 24 hrs (C) and analyzed by Western blotting for changes in the level of DNA methyltransferases DNMT1 and DNMT3A/3B (A and B) and chromatin-bound DNMT1 (C) as described under Materials and Methods. Results are representatives of two independent experiments. Bu, busulfan; 4HC, 4-hydroperoxycyclophosphamide; DAC, decitabine.

Figure 6. Effect of FLT3-ITD status on the response of AML cell lines to cytotoxic drug treatments. Genomic DNA was isolated from FLT3 wild type (KBM3/Bu250[6], OCI-AML3 and THP1) and FLT3-ITD-positive AML cell lines (MV4-11 and MOLM13) and used as template for PCR analysis. PCR products were resolved on 2% agarose gel with ethidium bromide staining (A). The sensitivity of the five AML cell lines to Sor was compared by analyzing cell proliferation using MTT assay (B). The cytotoxicity of [Bu+4HC+DAC] in the absence (C) or presence (D) of Sor was determined in FLT3-ITD cells. Drug synergism (E) was analyzed as in Figure 1. Western blot analysis (F) shows increased cleavage of PARP1 and caspase 3 and phosphorylation of H2AX, and decreased levels of FLT3 downstream targets, when Sor was combined with [Bu+4HC+DAC]. The levels of phosphorylated proteins were first normalized to their non-phosphorylated forms, and the ratio of the normalized phosphoprotein level relative to the untreated control was calculated. All cells were exposed to drug(s) for 48 hrs (B-F). Results are representatives of two (A, E, F) or average±SD of three (B-D) independent experiments . Bu, busulfan; 4HC, 4-hydroperoxycyclophosphamide; DAC, decitabine; Sor, sorafenib.

Supplemental Materials

Table 1. List of primary antibodies, their sources and dilutions

Antigen	Source/Cat. #	Clone type*	Dilution**
β-ACTIN	Sigma/A5316	mAb	6000
ATM	Santa Cruz Biotech/25921	mAb	750
P-ATM (S1981)	Rockland/200-301-400	mAb	2000
CHK2	Cell Signaling/2662	pAb	2500
P-CHK2 (S19)	Cell Signaling/2666	pAb	2500
c-MYC	Cell Signaling/9402	pAb	2000
Cleaved CASPASE 3	Cell Signaling/9661	pAb	2500
COX IV	Cell Signaling/4850	mAb	4500
Cytochrome c	BD Pharm/556433	mAb	2000
DNMT1	Santa Cruz Biotech/10222	pAb	700
DNMT3A	Cell Signaling/3598	mAb	3000
DNMT3B	Santa Cruz Biotech/10236	pAb	700
γ-Histone 2AX	EMD Millipore/05-636	mAb	3000
Histone 3	Abcam//1791	pAb	3000
KAP1	Bethyl Lab/A300-275	pAb	3000
P-KAP1 (S824)	Cell Signaling/4127	pAb	2000
Nucleoporin	Santa Cruz Biotech/48373	mAb	700
PARP1	Santa Cruz Biotech/8007	mAb	1000
P16	Cell Signaling/4824	pAb	1200
P21	Upstate Biotech/05-345	mAb	1000
P-P53 (S15)	Cell Signaling/9284	pAb	2000
P53	Santa Cruz Biotech/126	mAb	1000
SMAC/DIABLO	Cell Signaling/2954	mAb	2500
STAT3	Cell Signaling/9132	pAb	2000
STAT5	Santa Cruz Biotech/835	pAb	700
P-STAT5 (T694)	Cell Signaling/9359	mAb	2000

* pAb: polyclonal antibody; used anti-rabbit IgG for secondary antibody from Bio-Rad Lab

mAb: monoclonal antibody; used anti-mouse IgG for secondary antibody from Bio-Rad Lab

mAb-R: rabbit monoclonal antibody

**Fold dilution in PBS with 0.05% Tween 20