Inhibitors of poly ADP-ribose polymerase (PARP) induce apoptosis of myeloid leukemic cells: potential for therapy of myeloid leukemia and myelodysplastic syndromes

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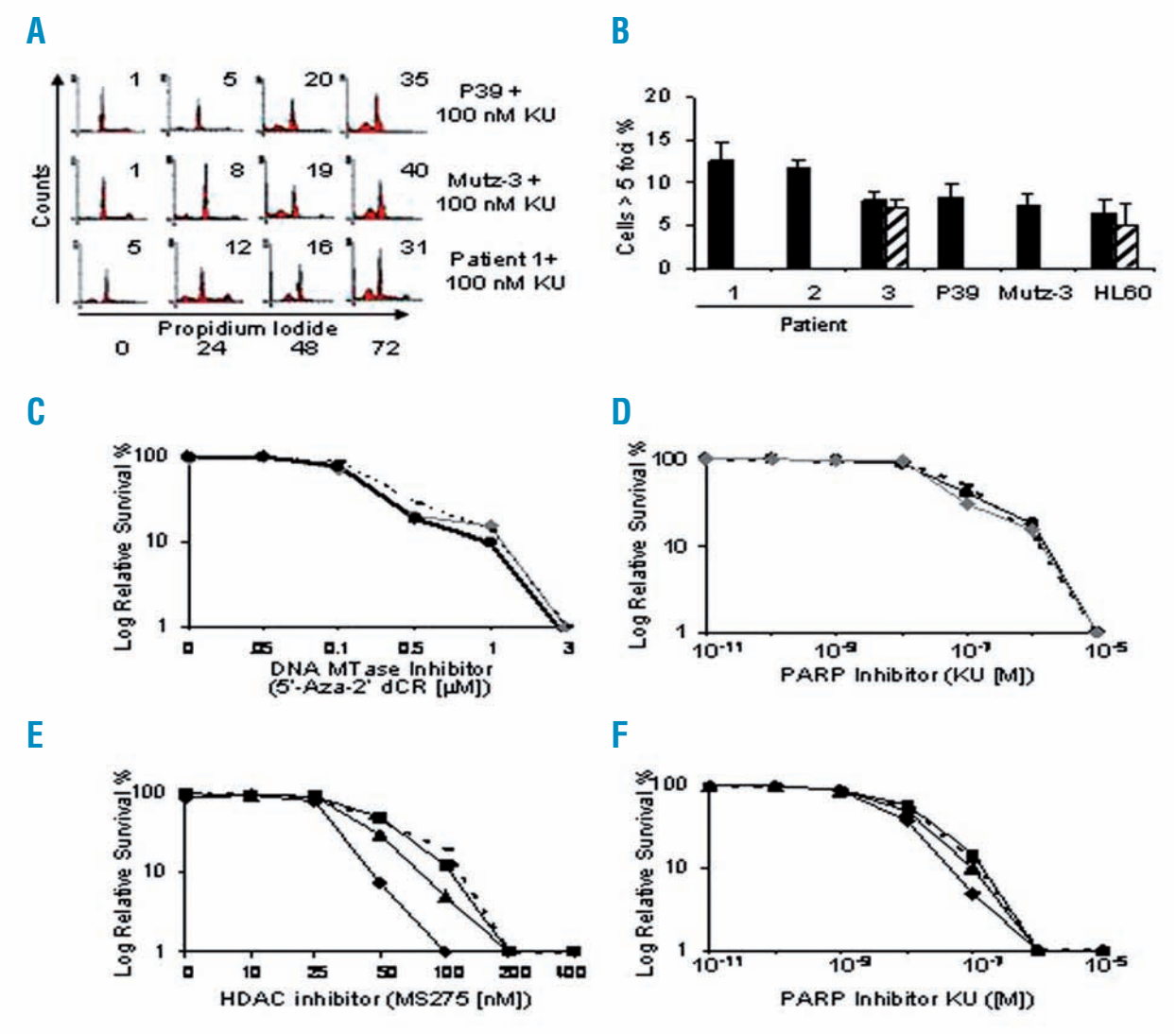
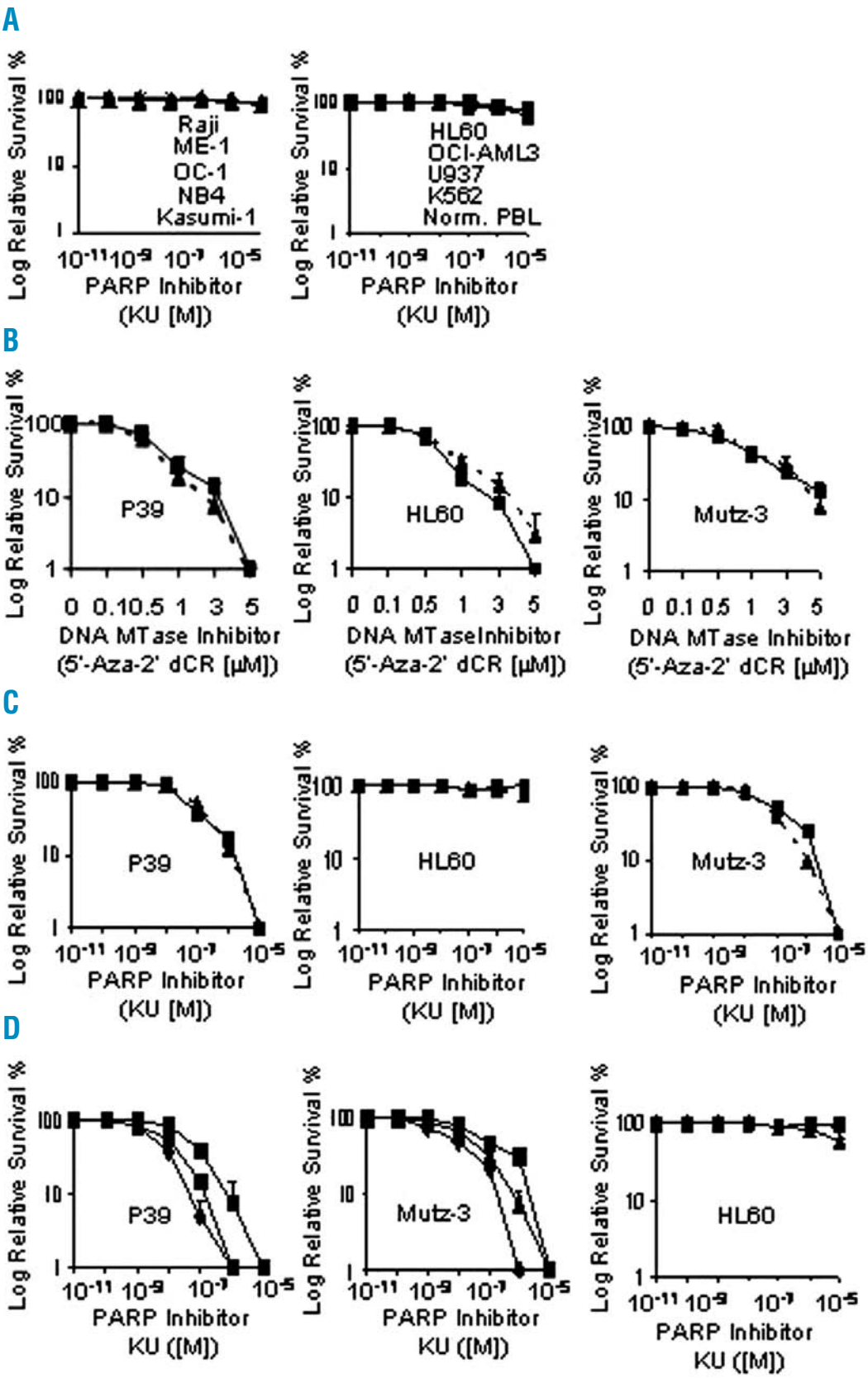
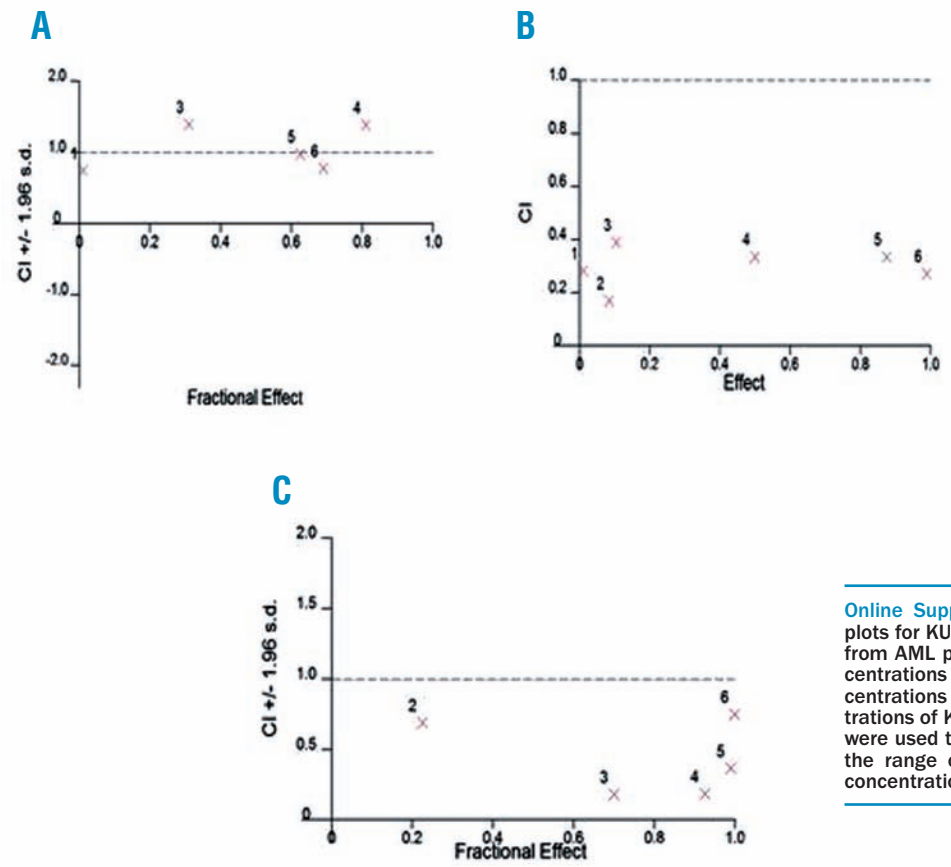


Online Supplementary Figure S1 The effect of PARP inhibitors on leukemic cell lines and primary AML cells that are defective in DNA repair. (A) 100 nM KU was added to P39, Mutz-3 or cells from AML patient n. 1 for 120 h and the cells were then analyzed by flow cytometry. The frequency of apoptotic cells was determined by estimating the cells in the sub-G_1 compartment (lower left quadrant). The apoptotic index (sub-G_1 population as a fraction of sub-G_1 + G_1 populations) is shown in the right inset. (B) Immunostaining of nuclei from P39 and Mutz-3 cell lines and cells from AML patient n. 1 treated with 100 nM KU for 24 h. Frequency of cells displaying γH2AX-P foci(%) (black bars) or rad51 foci(%) (diagonal bars), detected by immunofluorescence following KU addition. More than 300 nuclei were counted per experiment, n=3. (C-F) Soft agar clonogenic assays were used to determine cell survival in the P39 cell line following treatment with a PARP inhibitor alone or in combination with MS275 and/or 5' aza 2' dCR. (C) Exposure to 10 nM KU with varying concentrations of 5' aza 2' dCR was continuous for 12-14 days where 5' aza 2' dCR was added 48 h prior to KU (circles), 24 h prior to KU (dashed line) or simultaneously (gray line, diamonds). (D) Exposure to 250 nM 5' aza 2' dCR with varying concentrations of KU was continuous for 12-14 days where 5' aza 2' dCR was added 48 h prior to KU (circles), 24 h prior to KU (dashed line) or simultaneously (gray line, diamonds). (E) Exposure to 250 nM 5' aza 2' dCR and 10 nM KU with varying concentrations of MS275 was continuous for 12-14 days where 5' aza 2' dCR was added 48 h prior to KU+MS275 (diamonds), 24 h prior to KU+MS275 (triangles), simultaneously with KU+MS275 (squares) and with KU+MS275 only (dashed line). (F) Exposure to 250 nM 5' aza 2' dCR and 100 nM MS275 with varying concentrations of KU was continuous for 12-14 days where 5' aza 2' dCR was added 48 h prior to KU+MS275 (diamonds), 24 h prior to KU+MS275 (triangles), simultaneously with KU+MS275 (squares) and with KU+MS275 only (dashed line).



Online Supplementary Figure S2. The effect of PARP inhibitors on leukemic cell lines. Cell survival was assessed by a soft agar clonogenic assay. The cells were exposed to PARP inhibitors continuously for 12-14 days. (A) KU was added at various concentrations to human leukemic cell lines, Me-1, Raji, NB4, OC-1 .HL60, OCI-AML3, Kasumi-1, U937, K562 and normal peripheral blood lymphocytes (PBL). (B) The effect of non-cytotoxic concentrations of PARP inhibitors in combination with 5' aza 2' dCR. Cells were cultured in 10 nM KU and varying concentrations of 5' aza 2' dCR in P39, HL60 and K562 cells. Survival with KU + 5' aza 2' dCR is shown by the dashed line, and survival with 5' aza 2' dCR alone is shown with the solid line. (C) The effect of non-cytotoxic concentrations of 5' aza 2' dCR in combination with PARP inhibitors. Cells were cultured with 250 nM 5' aza 2' dCR and varying concentrations of KU in P39, HL60 and Mutz-3 cells. (D) The effect of non-cytotoxic concentrations of MS275 and/or 5' aza 2' dCR on PARP inhibitor-induced cytotoxicity of leukemic cells. Exposure to 100 nM MS275 and 250 nM 5'-aza-2'-dCR with varying concentrations of KU in P39, Mutz-3 and HL60 cells. KU + MS275 (triangles), 5' aza 2' dCR + KU + MS275 (diamonds), and KU alone (squares).



Online Supplementary Figure S3 Combination index (CI) plots for KU, MS275 and 5' aza 2' dCR in combination. Cells from AML patient n. 2 were treated with (A) increasing concentrations of 5' aza 2' dCR + 5 nM KU, (B) increasing concentrations of MS275 + 5 nM KU or (C) increasing concentrations of KU + 50 nM MS275. Trypan blue exclusion assays were used to determine cell survival. The fractional effect is the range of increasing concentrations, where the lowest concentration is 1% and the highest is 100%.

Online Supplementary Table S1. World Health Organization (WHO), French-American-British (FAB) classification of AML patients investigated in the study.

Patient	WHO FAB classification
1	M5a
2	TLD-AML
3	M6
4	M1
5	CML → AML
6	M3
7	M5
8	M2
9	M5
10	M3
11	M4
12	M3

TLD - tri-lineage dysplasia; CML → AML: chronic myeloid leukemic blast crisis transformation to AML.