

Synergistic effects of PRIMA-1^{Met} (APR-246) and 5-azacitidine in TP53-mutated myelodysplastic syndromes and acute myeloid leukemia

Nabih Maslah,^{1,2,3} Norman Salomao,³ Louis Drevon,³ Emmanuelle Verger,^{1,3} Nicolas Partouche,⁴ Pierre Ly,¹ Philippe Aubin,¹ Nadia Naoui,¹ Marie-Helene Schlageter,^{1,3} Cecile Bally,⁵ Elsa Miekoutima,⁵ Ramy Rahmé,⁵ Jacqueline Lehmann-Che,^{2,6} Lionel Ades,^{2,3,5} Pierre Fenaux,^{2,3,5} Bruno Cassinat^{1,3#} and Stephane Giraudier^{1,2,3#}

¹APHP, Service de Biologie Cellulaire, Hôpital Saint-Louis, Paris; ²Faculté de Médecine Université Paris Diderot Paris 7, Paris; ³INSERM UMR-S 1131, Hôpital Saint-Louis, Paris; ⁴Faculté de Médecine Paris 12-UPEC, Hôpital Henri Mondor, APHP, Créteil; ⁵APHP, Service d'Hématologie Senior, Hôpital Saint-Louis, Paris and ⁶Unité d'Oncologie Moléculaire, Hôpital Saint-Louis, APHP, Paris, France

#BC and SG contributed equally as co-senior authors

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Correspondence: STEPHANE GIRAUDIER - stephane.giraudier@aphp.fr

Supplementary methods

Transcriptomic assay

SKM1 cells were cultured for 18 h in the presence of APR, AZA, or APR + AZA, respectively at the IC₁₀ and the IC₅₀, each condition being carried out in triplicate. Total RNAs were extracted using a Qiacube® device (Qiagen), quantified using a NanoDROPTM instrument (100 ng/mL) and qualified in a bioanalyzer (Agilent). The gene expression profiling was performed with HuGene 2.0 arrays (Affymetrix) at the Cochin Institute genomic platform (Paris). The data were then analyzed using Ingenuity Pathway Analysis (IPA), taking into account a fold change (FC) > 1.2 for comparisons with APR and a FC > 2 for AZA, as well as a p < 0.05. These analyses were pursued with Database for Annotation, Visualization and Integrated Discovery (DAVID) and Gene Set Enrichment Analysis (GSEA) using the following parameters for signification: p < 0.05 for DAVID, a false discovery rate (FDR) < 0.25, and a normalized enrichment score (NES) > 1. In order to find synergistically regulated pathways by the combination of drugs, we selected genes with an absolute value of the differential expression expressed as a fold change (FC) of the APR + AZA combination versus no treatment greater than the sum of the fold changes of each drug on its own: (FC (APR + AZA) - (FC (APR) + FC (AZA))) > 1 or < -1).

Xenotransplantations

Six-week-old female NSG mice, purchased from Charles River Laboratories, were intravenously injected with 10⁷ SKM1 cells transduced by a LV-Gluc-GFP, as previously described³². After injection of luciferin (35 mg/g) the tumor volume was evaluated by measurement of the bioluminescence on an IVIS® Spectrum *in vivo* imaging system (PerkinElmer) in radiance (p/sec/cm²/sr). Treatment was started as soon as the bioluminescence reading exceeded 10⁶ p/sec/cm²/sr. Low-dose APR (35 mg/kg, IV), AZA (5 mg/kg, IP), or a combination of AZA and APR were administered daily for 7 days and tumor volumes were measured until day-22 post-SKM1-Luc injection. The mice were euthanized at day-25 after the start of the treatment.

	SKM1			K562			THP-1			KG1a			HL60		
	APR (M)	AZA (M)	CI	APR (M)	AZA (M)	CI	APR (M)	AZA (M)	CI	APR (M)	AZA (M)	CI	APR (M)	AZA (M)	CI
IC10 APR / IC10 AZA	0,11.10 ⁻⁶	0,10.10 ⁻⁶	4,13	0,33.10 ⁻⁶	0,40.10 ⁻⁶	6,64	0,25.10 ⁻⁶	0,10.10 ⁻⁶	3,85	0,40.10 ⁻⁶	0,25.10 ⁻⁶	2,79	1.10 ⁻⁶	1.10 ⁻⁶	1,79
IC10 APR / IC50 AZA	0,11.10 ⁻⁶	0,54.10 ⁻⁶	0,47	0,33.10 ⁻⁶	1,3.10 ⁻⁶	0,70	0,25.10 ⁻⁶	1,3.10 ⁻⁶	0,63	0,40.10 ⁻⁶	1,6.10 ⁻⁶	0,56	1.10 ⁻⁶	4,5.10 ⁻⁶	0,85
IC50 APR / IC10 AZA	0,8.10 ⁻⁶	0,10.10 ⁻⁶	0,86	1,5.10 ⁻⁶	0,40.10 ⁻⁶	0,85	1,6.10 ⁻⁶	0,10.10 ⁻⁶	0,91	2,5.10 ⁻⁶	0,25.10 ⁻⁶	0,71	2,5.10 ⁻⁶	1.10 ⁻⁶	0,75
IC50 APR / IC50 AZA	0,8.10 ⁻⁶	0,54.10 ⁻⁶	0,26	1,5.10 ⁻⁶	1,3.10 ⁻⁶	0,32	1,6.10 ⁻⁶	1,3.10 ⁻⁶	0,37	2,5.10 ⁻⁶	1,6.10 ⁻⁶	0,43	2,5.10 ⁻⁶	4,5.10 ⁻⁶	0,31

Supplementary Table 1: The combination index (CI) of APR and AZA calculated for the SKM1, K562, KG1a, THP-1, and HL60 AML cell lines.

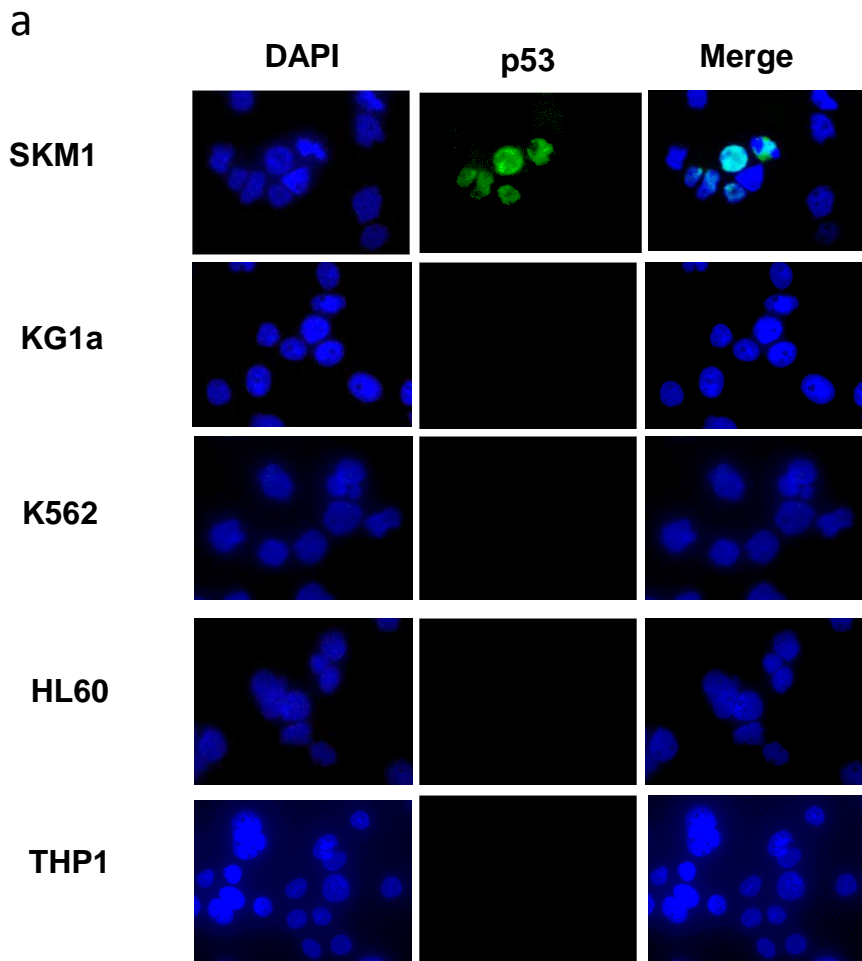
Ten percent (IC₁₀) and fifty percent (IC₅₀) inhibitory concentrations of each drug for the various cell lines (expressed in molarity, M), followed by the combination index (CI) calculated according to Chou-Talalay's method of constant ratio of APR and AZA. A CI > 1 indicates no synergism, a CI = 1 indicates an additive effect, 0.3 < CI < 0.9 indicates synergism and a CI < 0.3 is indicative of a strong synergistic effect.

Supplementary Table 2 (total)

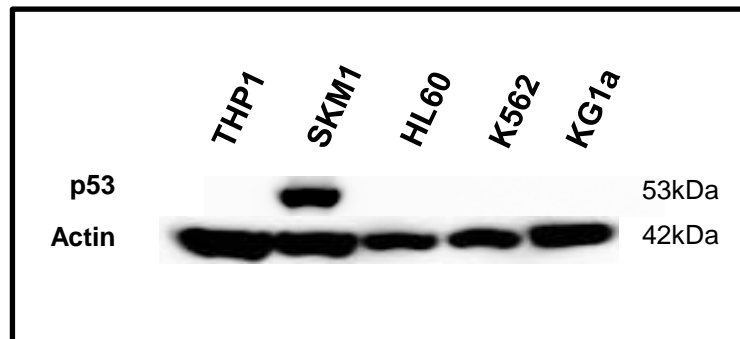
Supplementary Table 2. Transcriptomic data of SKM1 cells treated with APR, AZA or APR+AZA comparing different conditions of treatment.

Supplementary Table 3 (Synergy)

Supplementary Table 3. Table showing differential gene expression expressed as a fold change (FC) of the APR + AZA combination versus no treatment greater than the sum of the fold changes of each drug on its own: $(FC (APR + AZA) - (FC (APR) + FC (AZA))) > 1$ or < -1 .

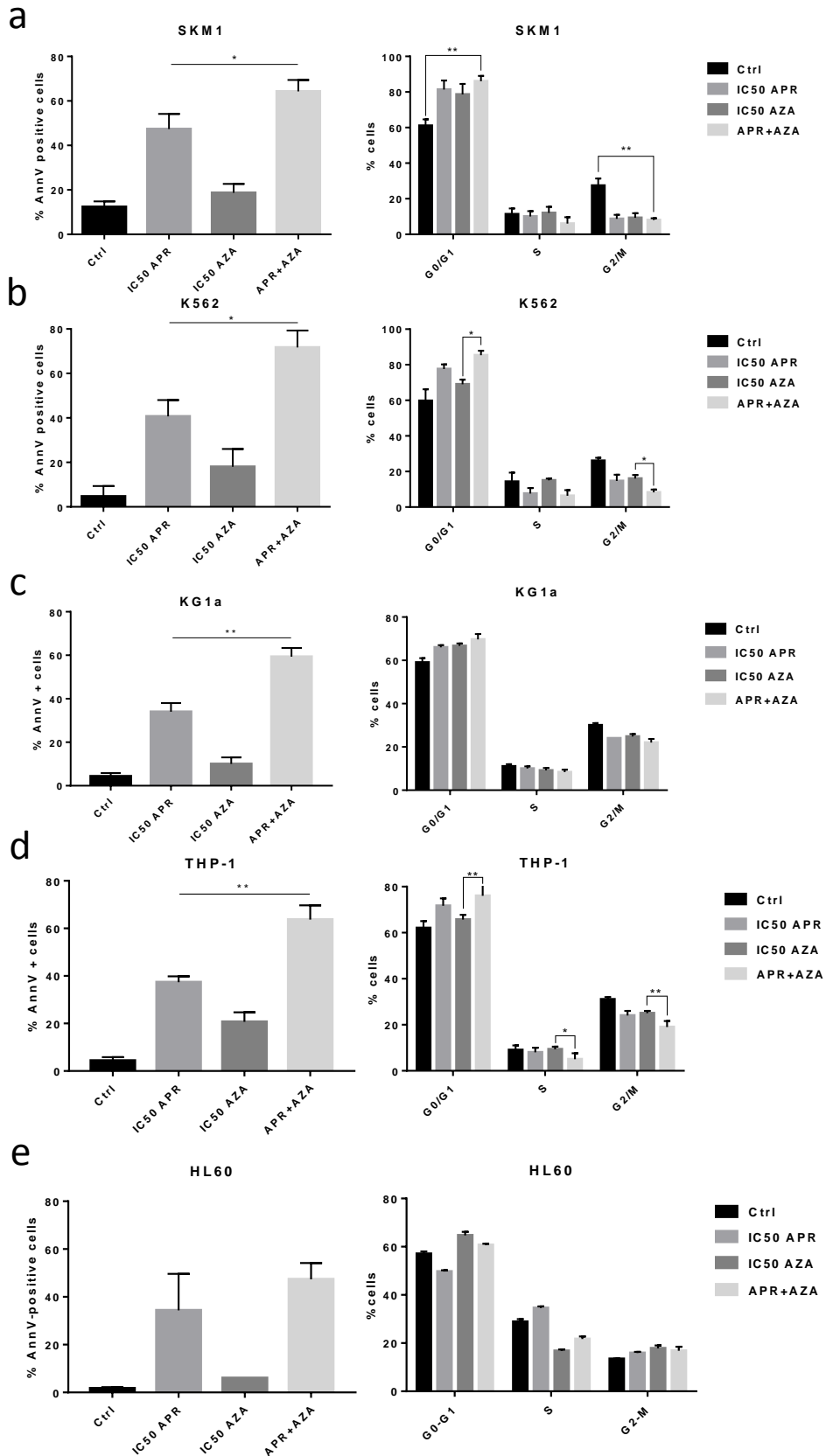


b

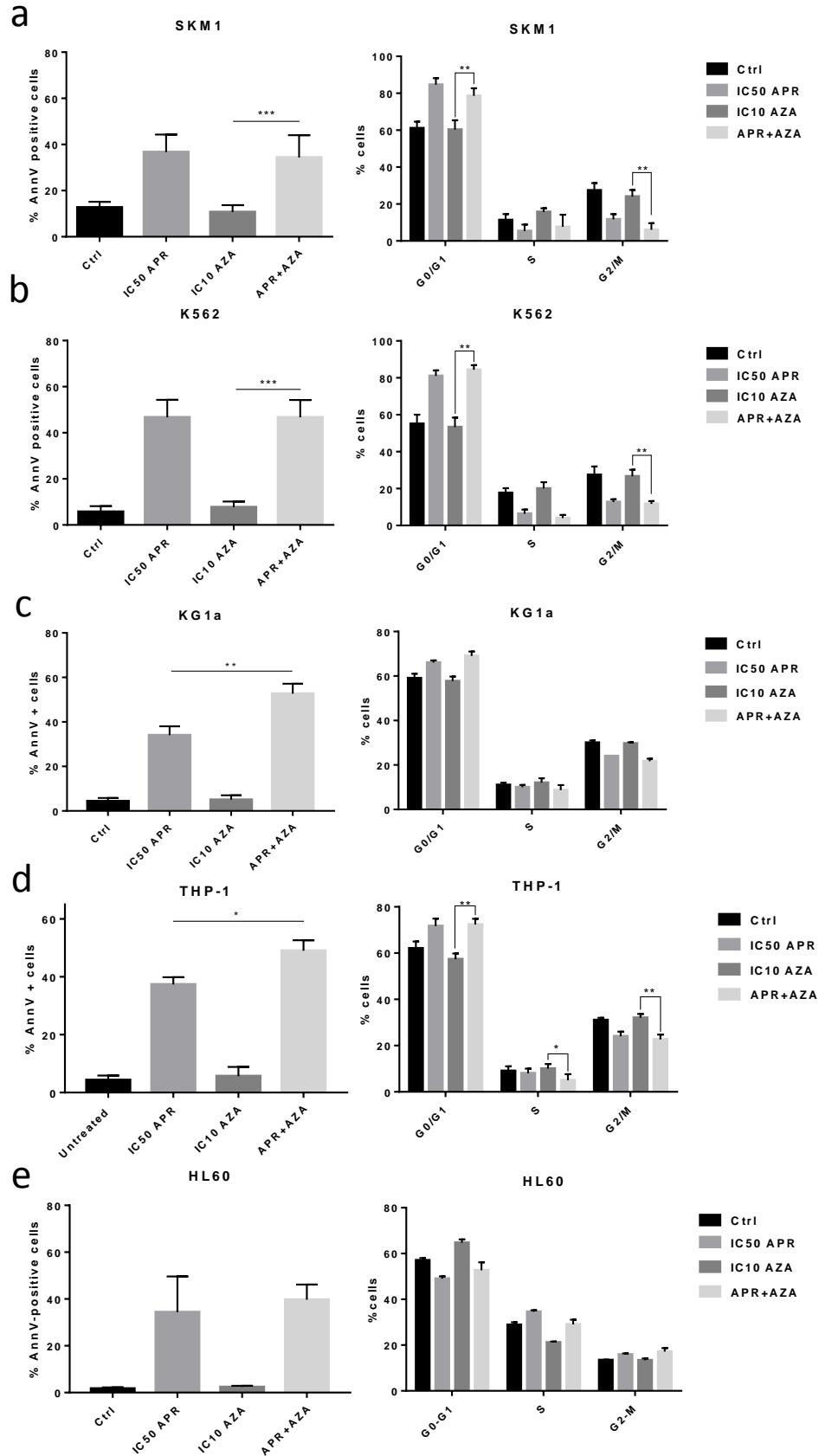


Supplementary Figure 1. p53 expression in AML/MDS cell lines.

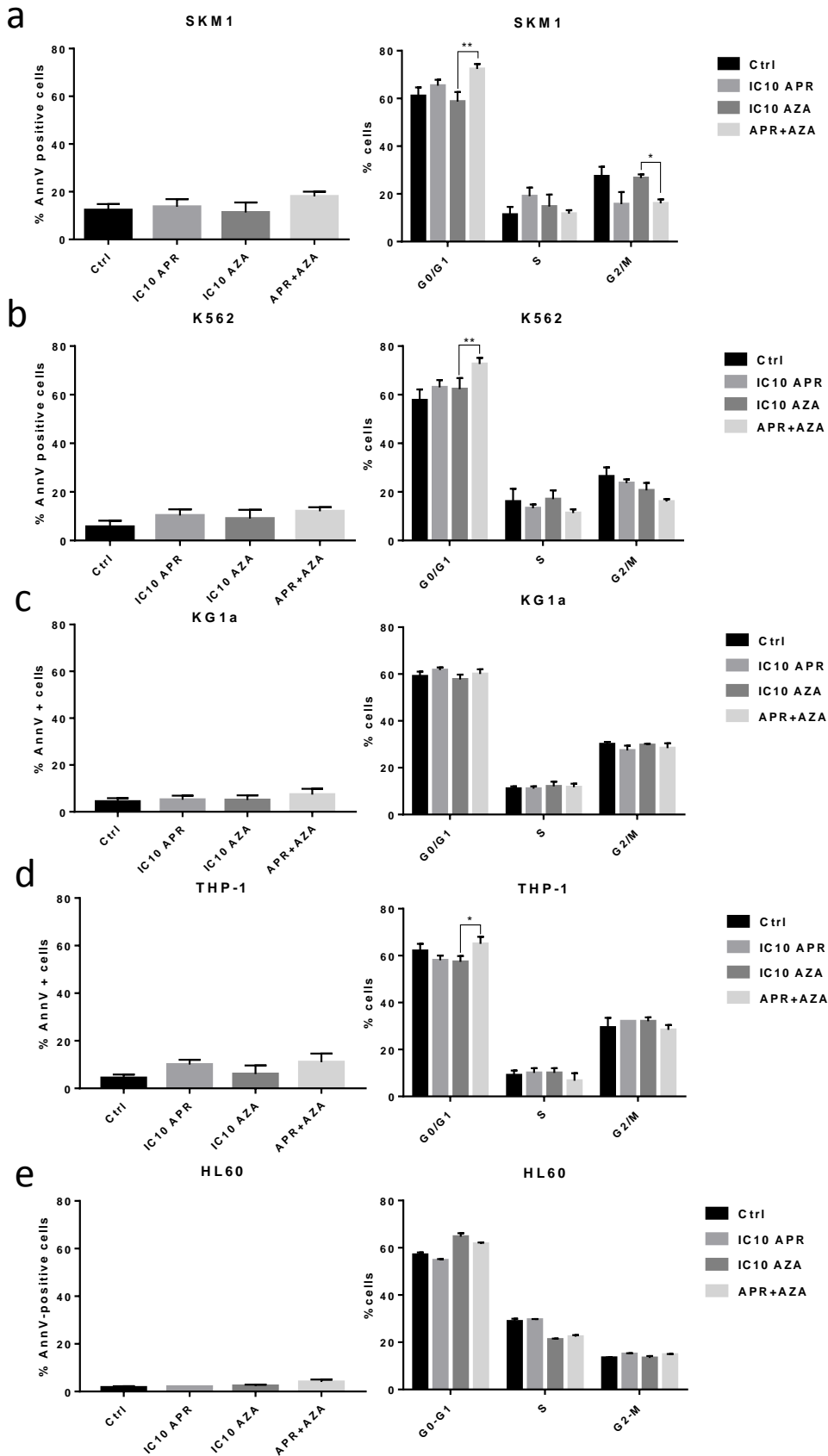
(a) Immunofluorescence and (b) Western blot showing p53 expression in SKM1 cell line exhibiting a *TP53* point mutation (p.R248Q) and in four other cell lines showing nonsense mutations (KG1a, K562, HL60, and THP-1)



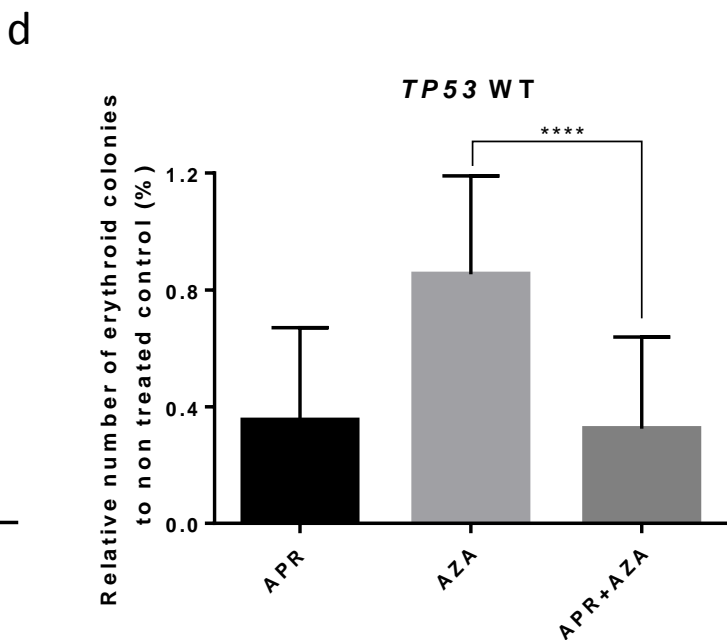
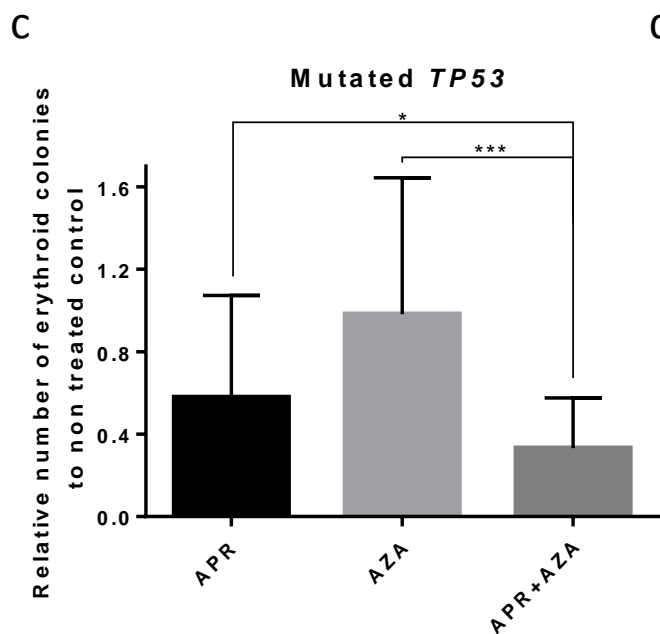
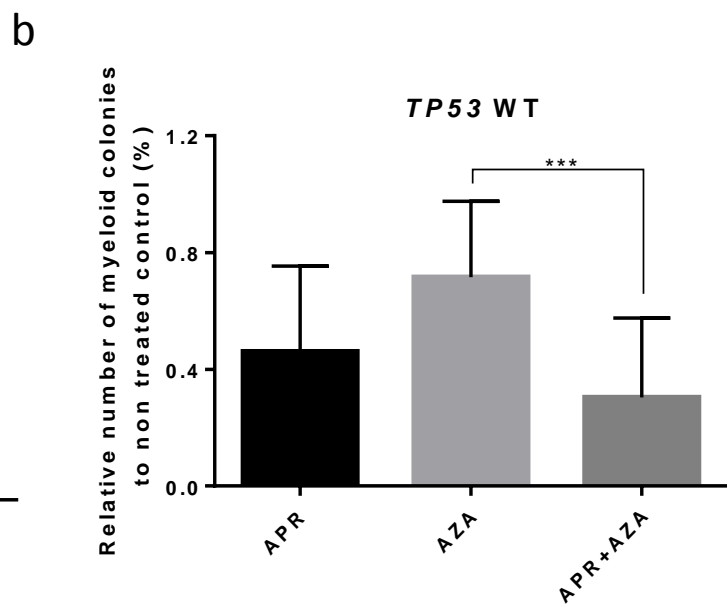
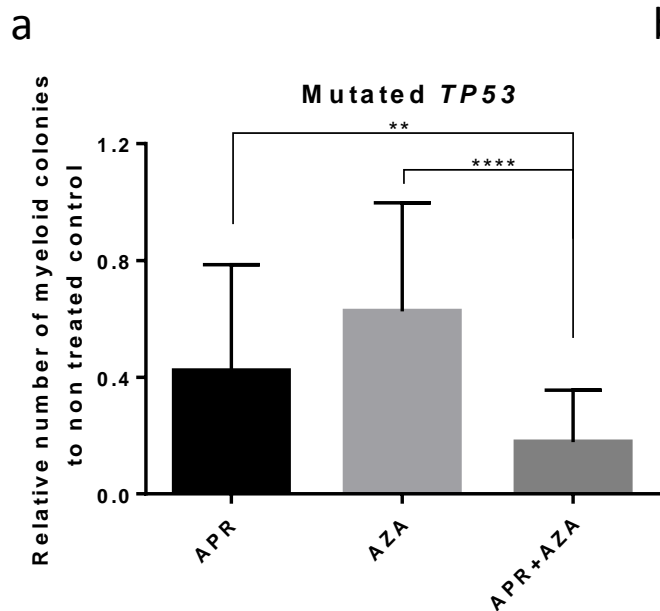
Supplementary Figure 2. The combination of APR and AZA promotes a G0/G1 blockade and apoptosis. Percentages of Annexin V-positive cells at day 3 post-treatment with IC50 APR, IC50 AZA or the combination of the two drugs at the same concentration and proportions of cells in G0/G1, S or G2/M phase two days after IC50 APR, IC50 AZA isolated treatment or the combo APR+AZA in (a) SKM1, (b) K562, (c) KG1a, (d) THP-1 and (e) HL60 cell line. *p < 0,05, **p < 0,01.



Supplementary Figure 3. The combination of APR and AZA promotes a G0/G1 blockade and apoptosis. Percentages of Annexin V-positive cells at day 3 post-treatment with IC50 APR, IC10 AZA or the combination of the two drugs at the same concentration and proportion of cells in G0/G1, S or G2/M phase two days after IC50 APR, IC10 AZA isolated treatment or the combo APR+AZA in (a) SKM1, (b) K562, (c) KG1a, (d) THP-1 and (e) HL60 cell line. * $p < 0,05$, ** $p < 0,01$.

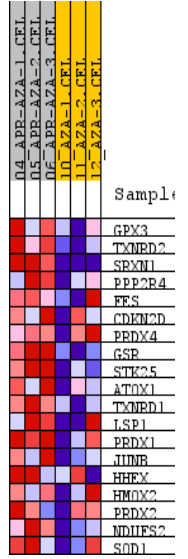
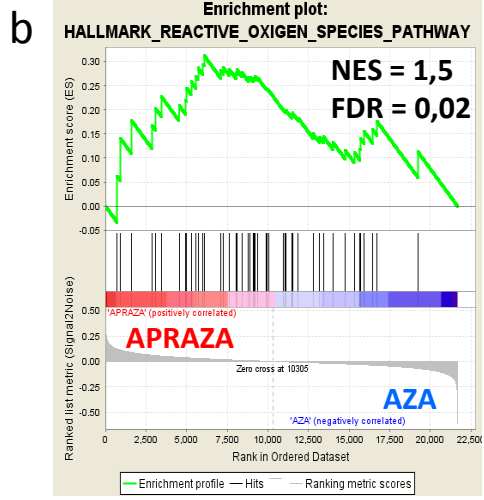
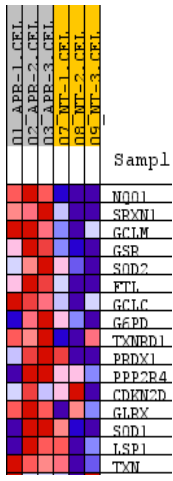
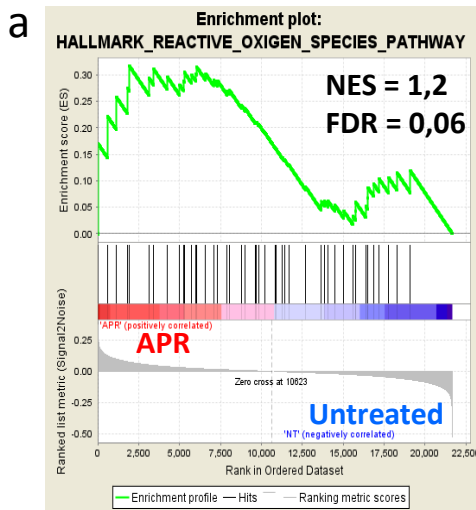


Supplementary Figure 4. The combination of APR and AZA promotes a G0/G1 blockade and apoptosis. Percentages of Annexin V-positive cells at day 3 post-treatment with IC10 APR, IC10 AZA or the combination of the two drugs at the same concentrations and proportion of cells in G0/G1, S or G2/M phase two days after IC10 APR, IC10 AZA isolated treatment or the combo APR+AZA in (a) SKM1, (b) K562, (c) KG1a, (d) THP-1 and (e) HL60 cell line. * $p < 0,05$, ** $p < 0,01$.



Supplementary Figure 5. APR is active on primary cells of *TP53*-mutated MDS samples.

Relative numbers of myeloid colonies to untreated control in (a) *TP53* mutated and (b) *TP53* wild type (WT) MDS samples treated with APR, AZA or the combination APR+AZA in semi-solid medium (methylcellulose). Relative numbers of erythroid colonies to untreated control in (c) *TP53* mutated and (d) *TP53* wild type (WT) MDS samples treated with APR, AZA, or the combo APR+AZA. * $p < 0,05$ ** $p < 0,01$ *** $p < 0,001$ **** $p < 0,0001$.



Supplementary Figure 6. Gene enrichment plots and associated heatmap of ROS pathway. **(a)** SKM1 treated cells with IC 10 APR 18 hours compared to untreated cells and **(b)** SKM1 cells treated with the combo APR IC10 +AZA IC 50 (APRAZA) 18 hours versus AZA alone (AZA background). NES: Normalized Enrichment Score, FDR: False Discovery Rate.