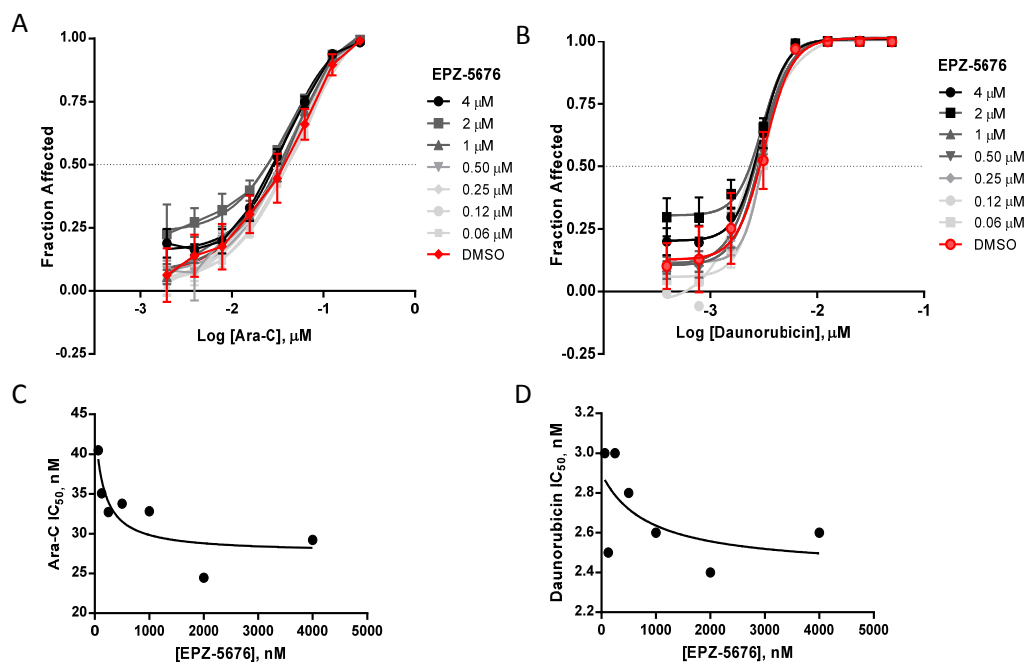


DOT1L Inhibitor EPZ-5676 Displays Synergistic Antiproliferative Activity in Combination with Standard of Care Drugs and Hypomethylating Agents in *MLL*-Rearranged Leukemia Cells

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Supplemental Figures

Supplemental Figure 1: EPZ-5676 does not enhance the anti-proliferative effect of SOC drugs in the non-*MLL* rearranged SKM-1 cells

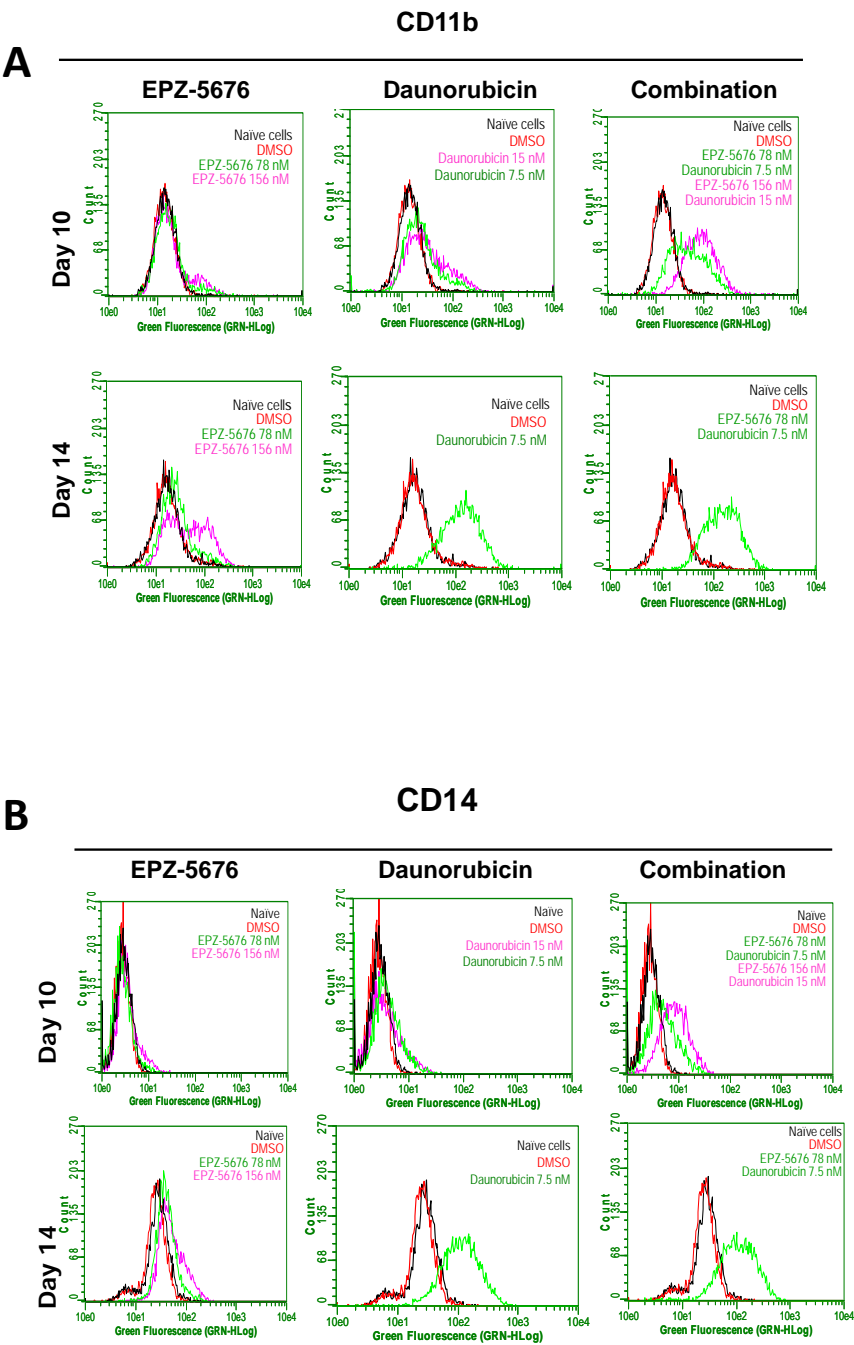


SKM-1 cells were treated with EPZ-5676 and SOC drugs according to the co-treatment model described under *Materials and Methods*. All dose response plots were generated in Graphpad Prism and curves fit to a four-parameter model with variable slope. EPZ-5676 was tested SOC from 0.06 to 4 μM concentration in 2-fold dilutions. A) No significant potency shift was observed when EPZ-5676 was combined with Ara-C in the SKM-1 cell line. B) No significant potency shift was observed when EPZ-5676 was combined with daunorubicin in the SKM-1 cell line. C) IC₅₀ values of Ara-C as single agent and in the presence of increasing concentrations of EPZ-5676 in the SKM-1 cell line were plotted and the α constant was calculated from the fitted curve as described in the Supplemental text. The maximum fold shift (calculated as $1/\alpha$) was 1.6. D) IC₅₀ values of daunorubicin as single agent and in the presence of increasing concentrations of EPZ-5676 in the SKM-1 cell line were plotted and the constant was calculated from the fitted curve as described in the Supplemental text. The maximum fold shift (calculated as $1/\alpha$) was 1.2.

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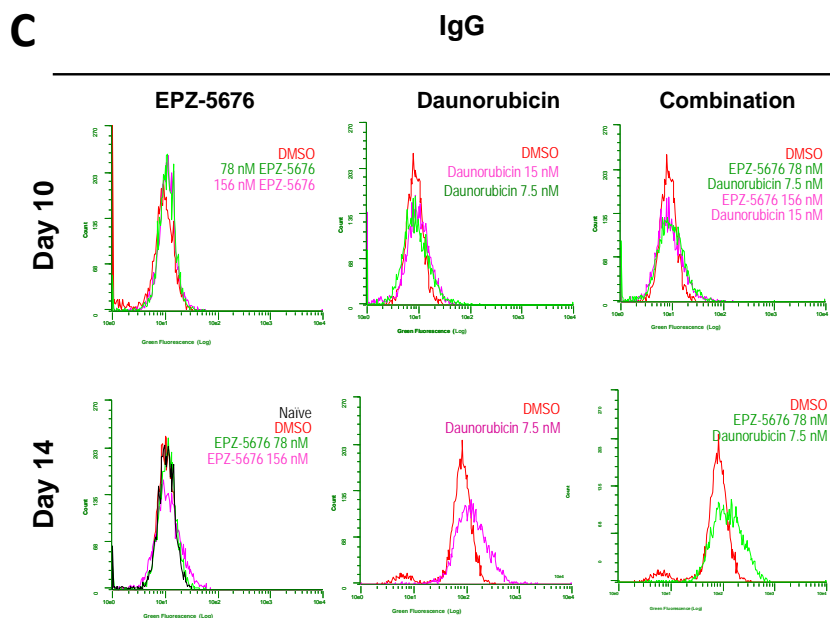
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Supplemental Figure 2: EPZ-5676 and daunorubicin as single agents and in combination promote time and concentration dependent up-regulation of the differentiation markers CD11b and CD14 in *MLL*-rearranged cells



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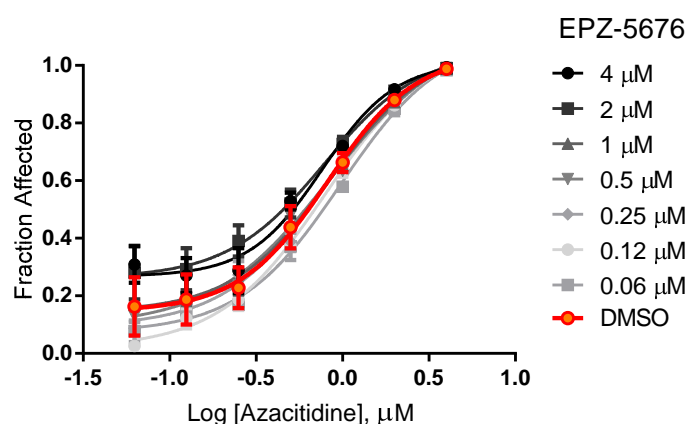
Supplemental Figure 2: MOLM-13 cells were treated as described under *Materials and Methods* for mechanism of cell death studies. EPZ-5676 and daunorubicin as single agents and in combination promote time and concentration dependent up-regulation of the differentiation markers CD11b and CD14. Histograms in black represent data from naïve cells, Red histograms represent data from DMSO treated cells. Histograms in magenta and green represent the indicated concentrations of single agent or combination of the two agents. Results are representative of two biological experiments. A) Flow cytometry analysis for cell surface expression of CD11b shows time and dose dependent up-regulation of the marker in cells treated with EPZ-5676 and daunorubicin as single agents and in combination. B) Flow cytometry analysis for cell surface expression of CD14 shows time and dose dependent up-regulation of the marker in cells treated with EPZ-5676 and daunorubicin as single agents and in combination. C) Flow cytometry analysis for cell surface expression of IgG isotype control. Similar results were obtained in two independent experiments.

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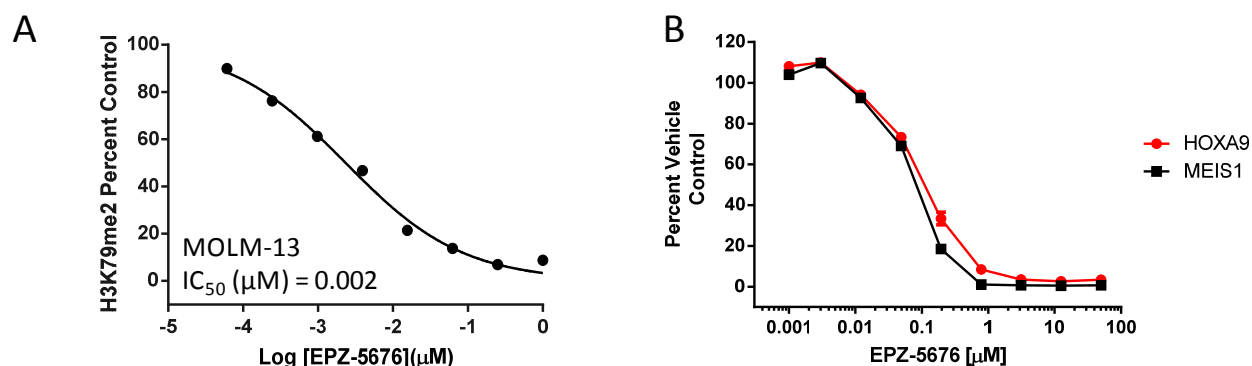
Supplemental Figure 3: EPZ-5676 does not enhance the anti-proliferative effect of azacitidine in the non-*MLL* rearranged SKM-1 Cells

SKM-1 cells were treated with EPZ-5676 and azacitidine as single agents and in combination according to the co-treatment model described under *Materials and Methods*. SKM-1 cells dose response curve analysis showed that the IC_{50} value for azacitidine as single agent was $0.65\mu M$ and for EPZ-5676 was greater than $4\mu M$. The maximum fold shift in the potency of azacitidine was 1.2 when combined with EPZ-5676 at $4\mu M$.



Supplemental Figure 4. EPZ-5676 inhibits global H3K79 methylation and *MLL*-rearranged target genes

MOLM-13 cells were treated in the presence of 0.2% DMSO or increasing concentrations of EPZ-5676. Cells were processed and analyzed for modulation of global H3K79me2 and *MLL*-r target genes *HOXA9* and *MEIS1* as previously described (Daigle et al., 2013). A) Concentration dependent inhibition of H3K79 methylation in MOLM-13 cells following a 4 day EPZ-5676 treatment as measured by quantitative ELISA for H3K79me2. B) Quantitative real-time PCR analysis of *MLL*-r target genes *HOXA9* and *MEIS1* in MOLM-13 following 7 days of treatment with a dose response of EPZ-5676. Relative mRNA expression levels are plotted as a percentage of those in vehicle-control treated cells.

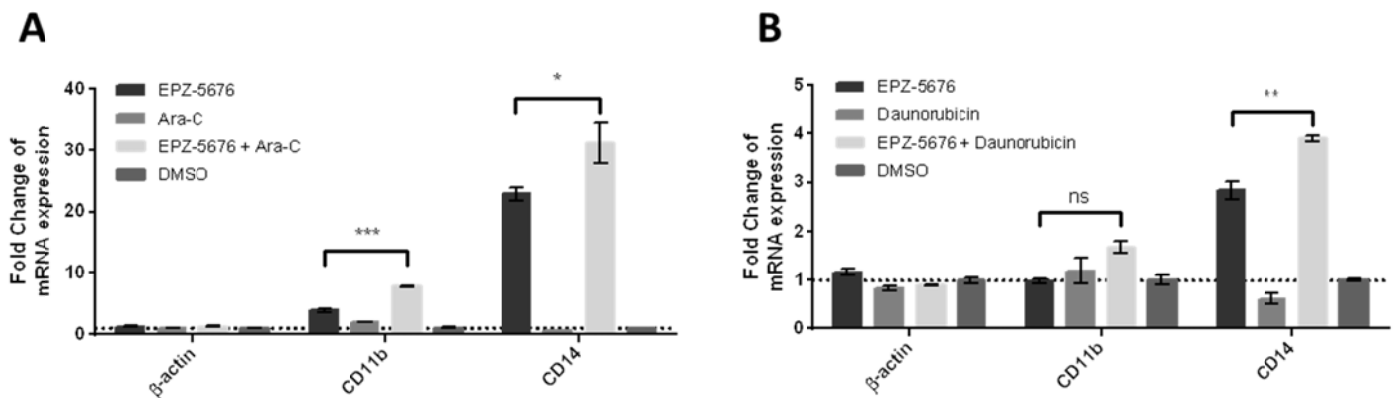


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Supplemental Figure 5. EPZ-5676 induces upregulation of mRNA expression of CD11b and CD14 as single agent and in combination with SOC

Molm-13 cells were treated for 10 days with single agents EPZ-5676, Ara-C and Daunorubicin or in combination as described in the *Methods* section. A) Gene expression analysis of CD11b and CD14 revealed an increase of mRNA levels of both genes upon treatment with EPZ-5676 alone (78 nM) that was enhanced with the combination of EPZ-5676 and Ara-C (78 nM EPZ-5676 + 31 nM Ara-C). B) Gene expression analysis of CD11b and CD14 revealed an increase of mRNA levels of CD14 upon treatment with EPZ-5676 (40M) as single agent that was augmented when EPZ-5676 was combined with Daunorubicin (40 nM EPZ-5676 + 3.8 nM Daunorubicin). No significant mRNA level increase of CD11b was observed with the combination of EPZ-5676 and Daunorubicin. Value for statistical analysis are a mean of duplicates +/- SD. One-way ANOVA plus Tukey post-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$



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Supplemental Table 1: Summary of combination studies of EPZ-5676 with AML and ALL standard of care drugs and chromatin modifying agents (D_m and CI values included)

Compounds were tested in combination with EPZ-5676 according to the co-treatment model described under *Materials and Methods*. The anti-proliferative effect of the combinations in the MOLM-13 and MV4-11 cell lines was evaluated using the Calcsyn software. The D_m value signifies the degree of potency of single agent activity, such as IC_{50} , as calculated by the Calcsyn Software. In these studies the lowest CI value obtained with a corresponding Fa greater than 0.5 was used to assess the degree of synergism or antagonism as described in the *Data Analysis* section. For the SKM-1 cell line the potency shift of the compound tested in the presence of EPZ-5676 was calculated from the dose response curves as described in the *Data Analysis* section and used to define the combinatorial effect.

	MOLM-13 (MLL-AF9)			MV4-11 (MLL-AF4)			SKM-1 (non-MLL rearranged)		
Rationale	Parameter	Compound (nM)	Result	Parameter	Compound (nM)	Result	Parameter	Compound (nM)	Result
AML standard of care		Ara-C	Strong Synergy		Ara-C	Strong Synergy		Ara-C	No Effect ^d
	D_m	14.3		D_m	130.6		IC_{50}	52.2	
	CI	0.23		CI	0.18				
		Daunorubicin	Synergy		Daunorubicin	Strong Synergy		Daunorubicin	No Effect ^d
	D_m	2.1		D_m	2		IC_{50}	3.1	
	CI	0.56		CI	0.20				
DNA methyltransferase inhibitors		Azacitidine	Strong Synergy		Azacitidine	Synergy		Azacitidine	No Effect ^d
	D_m	577.6		D_m	1497.3		IC_{50}	2046.0	
	CI	0.28		CI	0.38				
		Decitabine	Synergy		Decitabine	Synergy		Decitabine	No Effect ^d
	D_m	9.0		D_m	28.7		IC_{50}	16.3	
	CI	0.65		CI	0.64				
Histone Deacetylase inhibitors		Vorinostat	Additive / Synergy		Vorinostat	Antagonistic		Vorinostat	N/D
	D_m	142.9		D_m	358.9		IC_{50}	314.0	
	CI	0.51		CI	2.075				
		Panobinostat	Synergy		Panobinostat	Antagonistic		Panobinostat	N/D
	D_m	1.31		D_m	1.7		IC_{50}		
	CI	0.61		CI	1.49				
Demethylase inhibitors		Tranylcypromine	Strong Synergy		Tranylcypromine	Synergy		Tranylcypromine	No Effect ^d
	D_m	7030.0		D_m	2165.7		IC_{50}	935.0	
	CI	0.26		CI	0.37				
		LSD1 inhibitor II	Nearly Additive		LSD1 inhibitor II	Synergy		LSD1 inhibitor II	Enhancement
	D_m	4114.7		D_m	6323.4		IC_{50}	10472.0	
	CI	1.18		CI	0.33				
Bromodomain inhibitors		IBET-151	Synergy		IBET-151	Strong Synergy		IBET-151	No effect ^b
	D_m	335.5		D_m	56.0		IC_{50}		
	CI	0.39		CI	0.29				
		JQ1	Additive		JQ1	Additive		JQ1	No Effect ^d
	D_m	36.0		D_m	24.0		IC_{50}	4.9	
	CI	1.34		CI	0.86				
ALL standard of care		Mitoxantrone	Synergy		Mitoxantrone	Synergy		Mitoxantrone	No Effect ^d
	D_m	0.40		D_m	0.062		IC_{50}	0.43	
	CI	0.42		CI	0.38				
		Methotrexate	Additive		Methotrexate	Additive ^c		Methotrexate	No Effect ^d
	D_m	10.9		D_m	14.4		IC_{50}	11	
	CI	1.19		CI	1.83				
		Mafosfamide	Strong Synergy		Mafosfamide	Strong Synergy		Mafosfamide	No Effect ^d
	D_m	250.1		D_m	111.6		IC_{50}	976.0	
	CI	0.13		CI	0.13				
		Prednisolone	Antagonistic ^b		Prednisolone	Antagonistic ^b		Prednisolone	Enhancement ^b
	D_m	N/D		D_m	N/D		IC_{50}	>10000	
	CI	N/D		CI	N/D				
		Vincristine	Additive		Vincristine	Additive		Vincristine	No Effect ^d
	D_m	0.31		D_m	0.32		IC_{50}	0.0040	
	CI	0.99		CI	1.28				

^aNo enhancement was observed based on analysis of the IC_{50} shift of the test compound in the presence of EPZ-5676.

Analysis of IC_{50} shifts is described under *Materials and Methods*

^b IC_{50} of test compound not achieved

^cMethotrexate showed antagonistic effect in combination with EPZ-5676 at some constant ratios

^dEnhancement or leftward shift in IC_{50} was observed at concentrations of EPZ-5676 of 2000 nM and above

N/D: not determined

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Supplemental text

When the anti-proliferative activity of a compound is affected by a second compound (A) of distinct mechanism of action, the effect of varying concentration of A ([A]) on the IC_{50} of the first compound can be described by the following relationship (1).

$$IC_{50}^{[A]} = \frac{[A] + K_A}{\frac{K_A}{IC_{50}^0} + \frac{[A]}{\alpha IC_{50}^0}} \quad (S1)$$

where [A] and K_A are the concentration of compound A and its single agent anti-proliferative IC_{50} for the cell line under study (which may not be experimentally determinable), respectively. $IC_{50}^{[A]}$ is the IC_{50} of the test compound in combination with [A] and IC_{50}^0 is the IC_{50} of the test compound alone (i.e., at [A] = 0). The term α is a constant that indicates the degree of potency enhancement effected by compound A and is given by the following ratio.

$$\alpha = \frac{IC_{50}^{\infty}}{IC_{50}^0} \quad (S2)$$

where IC_{50}^{∞} is the limit of the IC_{50} value of the test compound expected at infinite concentration of compound A. Thus, the reciprocal of α gives the maximum fold-shift in IC_{50} effected by compound A.